Langdon R. Elsworth
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Editors

Fertilizers

Properties, Applications and Effects

NOVA

FERTILIZERS: PROPERTIES, APPLICATIONS AND EFFECTS

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Contents

Preface

Fertilizers are compounds given to plants to promote growth; they are usually applied either through the soil, for uptake by plant roots, or by foliar feeding, for uptake through leaves. Fertilizers can be organic (composed of organic matter), or inorganic (made of simple, inorganic chemicals or minerals). They can be naturally occurring compounds such as peat or mineral deposits, or manufactured through natural processes (such as composting) or chemical processes (such as the Haber process).

Fertilizers typically provide, in varying proportions, the three major plant nutrients (nitrogen, phosphorus, and potassium), the secondary plant nutrients (calcium, sulfur, magnesium), and sometimes trace elements (or micronutrients) with a role in plant nutrition: boron, chlorine, manganese, iron, zinc, copper, and molybdenum. This new book presents recent and important research from around the globe.

Chapter I - Degradation of soil fertility under long term application of inorganic fertilizers is an increasingly serious problem damaging sustainability of modern agriculture. Ecological fertilization is proposed to solve the problems caused by the current fertilization in modern intensive agricultural systems. A rice-ryegrass rotation system established in southern China is a good example of the ecological fertilization practice. The integrative ecological benefits of the rotation system, particularly in aspects concerning to growth and yield responses of paddy rice have been investigated since 1990s. It was observed that the yields of the subsequent paddy rice in many cases were increased for at least 10% in the rotation system when comparing to those in conventional rice cropping. Physical, chemical and biological properties of the paddy soil cropped ryegrass in winter were markedly improved. The evidences of the improvements were observed in availabilities of nutrients, soil enzymes activities, soil microbial features, slow-releases of nutrients etc.

Agricultural nitrogen (N) and phosphorus (P) are main contributors of non-point source pollution to water bodies and often lead to water eutrophication. In the rice - ryegrass rotation system, compound fertilizers are still applied in both ryegrass and rice cropping processes. We valuated the environment influences of the applied N and P in the rice - ryegrass rotation as a model of the ecological fertilization based on the investigation for the patterns of N and P outflows from the rotation system.

Chapter II - Soybean is the world’s most important source of protein and accounts for nearly 70% of the world protein meal consumption. This has led to the production of soybean in a wide range of environmental conditions across a huge geographic expanse. Soybean has a distinct advantage over non-leguminous crops through its ability to acquire N via symbiotic N-fixation. However, other nutrients are critically important to optimize soybean production, both through direct effects on growth and development as well as through their influences on soybean biological N fixation. In this review the authors highlight the fertility requirements and considerations for soybean production and examine the relationships between soybean mineral nutrition and biotic factors such as selected disease, insects, and nematodes.

Chapter III - The amount of fisheries waste generated in Korea is expected to increase with a steady increase in population to enjoy taste of slices of raw fish. The fisheries waste is reduced and reutilized through the fish meal production. The process, which uses fish wastes such as heads, bones or other residues, is the commonest used in the Korean industries. The first step of the fish-meal manufacturing processes is the compression and crushing of the raw material, which is then cooked with steam, and the liquid effluent is filtered off in a filter press. The liquid stream contains oils and a high content of organic suspended solids. After oil separation, the fish-meal wastewater (FMW) is generated and shipped to wastewater treatment place. FMW has been customarily disposed of by dumping into the sea, since direct discharge of FMW can cause serious environmental problems. Besides, bad smell, which is produced during fish-meal manufacturing processes, causes civil petition. Stricter regulations for this problem also come into force every year in Korea. Therefore, there is an urge to seek for an effective treatment to remove the organic load from the FMW; otherwise the fish meal factories will be forced to shut down.

Biological treatment technologies of fish-processing wastewater have been studied to improve effluent quality (Battistoni and Fava, 1995; Park et al., 2001). The common feature of the wastewaters from fish processing is their diluted protein content, which after concentration by a suitable method would enable the recovery and reuse of this valuable raw material, either by direct recycling to the process or subsequent use in animal feed, human food, seasoning, etc. (Afonso and Borquez, 2002). It has been also reported that the organic wastes contain compounds, which are capable of promoting plant growth (Day and Katterman, 1992), and seafood processing wastewaters do not contain known toxic or carcinogenic materials unlike other types of municipal and industrial effluents (Afonso and Borquez, 2002). Although these studies imply that FMW could be a valuable resource for agriculture, potential utilization of this fish wastes has been limited because of its bad smell (Martin, 1999). There is an increasing need to find ecologically acceptable alternatives to overcome this problem.

Aerobic biodegradation has been widely used in treatment of wastewaters, and recently references to the use of meso- and thermophilic microorganisms have become increasingly frequent (Cibis et al., 2006). During the biodegradation, the organic matter is biodegraded mainly through exothermic aerobic reactions, producing carbon dioxide, water, mineral salts, and a stable and humified organic material (Ferrer et al., 2001). There have been few reports that presented the reutilization of biodegraded waste products as liquid-fertilizer: a waste product of alcoholic fermentation of sugar beet (Agaur and Kadioglu, 1992), diluted manure streams after biological treatment (Kalyuzhnyi et al., 1999), and biodegraded fish-meal wastewater in our previous studies (Kim et al, 2007; Kim and Lee, 2008). Therefore, aerobic biodegradation is considered to be the most suitable alternative to treat FMW and realize a market for such a waste as a fertilizer.

The growth of plants and their quality are mainly a function of the quantity of fertilizer and water. So it is very important to improve the utilization of water resources and fertilizer nutrients. The influence of organic matter on soil biological and physical fertility is well known. Organic matter affects crop growth and yield either directly by supplying nutrients or indirectly by modifying soil physical properties such as stability of aggregates and porosity that can improve the root environment and stimulate plant growth (Darwish et al., 1995). Incorporation of organic matter has been shown to improve soil properties such as aggregation, water-holding capacity, hydraulic conductivity, bulk density, the degree of compaction, fertility and resistance to water and wind erosion (Carter and Stewart, 1996; Franzluebbers, 2002; Zebarth et al., 1999). Combined use of organic and inorganic sources of nutrients is essential to maintain soil health and to augment the efficiency of nutrients (Lian, 1994). Three primary nutrients in fertilizers are nitrogen, phosphate, and potassium. According to Perrenoud’s report (1990), most authors agree that N generally increases crop susceptibility to pests and diseases, and P and K tend to improve plant health. It has been reported that tomato is a heavy feeder of NPK (Hebbar et al., 2004) and total nitrogen content is high in leaves in plants having a high occurrence of bitter fruits (Kano et al., 2001). Phosphorus is one of the most essential macronutrients (N, P, K, Ca, Mg, S) required for the growth of plants, and the deficiency of phosphorus will restrict plant growth in soil (Son et al., 2006). However, the excessive fertilization with chemically synthesized phosphate fertilizers has caused severe accumulation of insoluble phosphate compounds in farming soil (Omar, 1998), which gradually deteriorates the quality as well as the pH of soil. Different fertilization treatments of a long-term field experiment can cause soil macronutrients and their available concentrations to change, which in turn affects soil micronutrient (Cu, Fe, Mn, Zn) levels. Application of appropriate rates of N, P and K fertilizers has been reported to be able to increase soil Cu, Zn and Mn availabilities and the concentrations of Cu, Zn, Fe and Mn in wheat (Li, et al., 2007). It has been also reported that higher rates of fertilizers suppress microbial respiration (Thirukkumaran and Parkinson, 2000) and dehydrogenase activity (Simek et al., 1999). Recently, greater emphasis has been placed on the proper handling and application of agricultural fertilizers in order to increase crop yield, reduce costs and minimize environmental pollution (Allaire and Parent, 2004; Tomaszewska and Jarosiewicz, 2006).

Hydroponics is a plant culture technique, which enables plant growth in a nutrient solution with the mechanical support of inert substrata (Nhut et al., 2006). Hydroponic culture systems provide a convenient means of studying plant uptake of nutrients free of confounding and uncontrollable changes in soil nutrient supply to the roots. Thus, it is fit for test of fertilizing ability of liquid fertilizers. The technique was developed from experiments carried out to determine what substances make plants grow and plant composition (Howard, 1993). Water culture was one of the earliest methods of hydroponics used both in laboratory experiments and in commercial crop production. Nowadays, hydroponics is considered as a promising technique not only for plant physiology experiments but also for commercial production (Nhut et al., 2004; Resh, 1993). The technique has been also adapted to many situations, from field and indoor greenhouse culture to highly specialized culture in atomic submarines to grow fresh vegetables for the crew (Nhut et al., 2004). Hydroponics provides numerous advantages: no need for soil sterilization, high yields, good quality, precise and complete control of nutrition and diseases, shorter length of cultivation time, safety for the environment and special utility in non-arable regions. Application of this culture technique can be considered as an alternative approach for large-scale production of some desired and valuable crops.

Prevention of slowing down deteriorative processes is required after a liquid fertilizer was produced by aerobic biodegradation in order to maintain its quality during the period of circulation in market. Generally, the lower the pH, the less the chance that microbes will grow and cause spoilage. It has been known that organic acids can lower the pH and have a bacteriostatic effect (Zhuang et al., 1996), although a number of other methods have also reported for microbial control (Agarwal et al., 1986; Curran et al., 1990; Stratham and Bremner, 1989). A means of a more long-term preservation of the liquid fertilizer is required for a higher additional value.

In this study, a large-scale biodegradation was successfully carried out for three days in a 1-ton reactor using the original FMW, and properties of the biodegraded FMW, such as phytotoxicity, amino-acid composition and its change during long-term preservation, concentrations of major and noxious components, and fertilizing ability on hydroponic culture plants were determined to examine the suitability of the biodegraded FMW as a fertilizer.

Chapter IV - Organic as well as mineral fertilizers were used for centuries to improve quantity and quality of forage produced on permanent grassland. In many regions, application of organic fertilizers on farm land resulted in creation or in enlargement of oligotrophic plant communities highly valuated by nature conservation today. Industrial production of synthetic fertilizers started in the middle of the 19th century and since that time, long-term fertilizer experiments were established. Ten grassland experiments at least 40 years old are still running in Austria, Czech Republic, Germany, Great Britain, Poland and Slovakia.

The present paper demonstrates that short and long-term effects of fertilizer application on plant species composition differ substantially. In contrast, short-term experiments cannot to be used to predict long-term effects. On oligotrophic soils, highly productive species supported by short-term N fertilizer application can completely disappear under long-term N application whilst other nutrients such as P become limiting. Under P limiting conditions, species characteristic for low productive grasslands (sedges, short grasses and some orchids) can survive even under long-term N application. It is more likely that enhanced P soil content causes species loss than N enrichment.

Residual effect of fertilizer application differs substantially among individual types of grassland and nutrients applied. Decades long after-effects of Ca and P application were revealed in alpine grasslands under extreme soil and weather conditions decelerating mineralization of organic matter. In extreme cases, resilience of the plant community after long-term fertilizer application can take more than several decades. Changes in plant species composition may even be irreversible. At lower altitudes with less extreme soil and climatic conditions, residual effect of fertilizer application is generally substantially shorter.

From the comparison of long-term vs short-term nutritional effects, it was concluded that long-term fertilizer experiments are irreplaceable as many existing models and predictions can be validated only by means of long-term manipulation of plant communities and their continuous observation and documentation. In conclusion, the authors give examples of how to apply forward-looking grassland research on existing long-term experiments and explain the extraordinary value that is provided by plant-soil-environment equilibrium.

Chapter V - The recycling and utilization of animal excreta with slurry is an integral element of dairy farming on grassland. Slurry is the most important source of fertilizer nutrients on animal farms, its application, however, is still not satisfactorily solved. The major concern is that part of the applied nutrients are inefficiently utilized for plant growth due to a misbalance of local growth conditions within a grassland field and nutrients on offer. The authors therefore hypothesize that precision fertilizer management can help to improve nutrient use efficiency by reducing loss through site-specific management.

There is evidence that improper use of nutrients on agricultural fields potentially increases the amount of nutrients released to the environment. The need to improve nutrient handling by applying up-to-date agricultural technology is therefore described. Experimentalists and farmers are faced with the problem of how to determine within-field heterogeneity to which they can respond by using recently developed precision application techniques. Such heterogeneity pertains to soil as well as to crop characteristics. Hence, it is discussed if mapping procedures and sensors can be utilized to detect heterogeneity.

A slurry application technique is described and a layout of system components is presented based on current research. The impact of such technique can yet not be anticipated due to lack of experimentation. Therefore, a simulation model has been applied to estimate the effect to reduced losses through precision fertilizer application on nutrient budget and losses within a typical dairy farm on grassland in temperate climates.

Chapter VI - The application of conservative agricultural practices, as shallow tillage, crop rotations and organic amendments, could usefully sustain plant performances and increase soil fertility, thus playing an important role in the sustainable agriculture. Therefore, the aim of this research was to study the possibility to reduce the agronomic inputs, investigating the effects of different soil tillage, crop rotations and nitrogen (N) fertilization strategies on a rain-fed durum wheat crop. To accomplish this goal, a two-year field trial (2002 and 2004) was carried out in a typical Mediterranean environment (Apulia Region, Southern Italy), determining wheat yields and quality, N uptake, N utilization, plant N status and the most important soil properties.

In a split split-plot experimental design with three replications, two tillage depths, conventional (40-45 cm depth) and shallow (10-15 cm) were laid out in the main plots. Sub-plots were three two-year crop rotations, industrial tomato-durum wheat (TW), sugar beet-durum wheat (SBW) and sunflower-durum wheat (SW). Sub sub-plots (40 m^2 each) were the following N fertilization strategies which supplied durum wheat 100 kg N ha^{-1}: 1. mineral (Nmin); 2. organic (Ncomp), with Municipal Solid Waste (MSW) compost; 3. mixed (Nmix), with MSW compost and mineral N; 4. organic-mineral (Nslow), with a slow N release fertilizer. These treatments were compared with an unfertilized control (Contr).

The results showed no significant difference between the trial years in wheat yields and, between the soil tillage depths, poor variation in wheat grain production, protein content, grain and straw N uptake, thus showing the possibility to reduce the arable layer without negatively affect the durum wheat performances.

With reference to the crop rotations, grain yields significantly decreased in the following sequence: SBW, TW and SW, with 5.05, 4.74 and 4.52 t ha^{-1}, respectively, while grain protein contents sequence was: SW, SBW and TW (12.0, 11.2 and 10.6 %).

Among the N treatments, the most interesting responses were almost always obtained with Nmix, that showed not only the significantly highest grain yields (5.29 t ha^{-1}), but also protein content similar to that of Nmin treatment. The Ncomp achieved, in comparison with Nmin and Nmix treatments, lower grain yields, protein contents and total N uptake.

On the whole, these findings highlighted that only the partial substitution of the mineral fertilization with the organic one is a sustainable technique, to obtain good wheat performances and reduce the environmental risks due to the high levels of mineral fertilizers application. Furthermore, another useful advice for the farmers could be the determination of pre sowing mineral N, the application of MSW compost before wheat sowing and the continue monitoring of plants N status during cropping cycles.

Chapter VII - Rainfed rice, which does not have any constructed irrigation facilities, occupies one third of all rice culture area in the world, and is generally characterized by low productivity. Northeast Thailand is a representative rainfed rice producing region. Rice yield there is as low as 1.7 t ha^{-1} on average, due to drought as well as low soil fertility. In order to improve productivity, we conducted an investigation of farmer's fields and improvement trials in experimental fields from 1995 to 2004. This manuscript summarizes the results in relation to fertilizer application.

Application rates of chemical fertilizer ranged from 0 to 65 kg N ha^{-1} among farmers, with an average of 24 kg N ha^{-1}. The agronomic efficiency of nitrogen (yield increase per unit applied nitrogen) was less than 20 kg kg^{-1}. Although the effect of N fertilizer was generally small, the effect was apparent in the field where rice biomass was small due to late transplanting or infertile soil. The simulation model we developed indicates that optimizing N fertilizer application could improve yield. The field experiment revealed that the N recovery efficiency (kg N absorbed / kg N applied) was 35% under frequent split application of 30 kg N ha^{-1} and increased up to 55% under 150 kg N ha^{-1}. Slow release fertilizer distinctly increased the N recovery efficiency (71%). Wood chip manure and green manure both increased yield, but only slightly. Incorporating pond sediments into fields increased rice yield over three seasons by increasing the N recovery efficiency. These results suggested that the proper use of N fertilizer and improving its recovery efficiency is the key to increasing the yield of rainfed rice in Northeast Thailand.

Chapter VIII - In agricultural systems, excess application of N-fertilizer may result in NO_3^- displacement to deeper soil layers that may eventually end up in the groundwater. Increases in nitrate concentrations above the drinking water quality standards of the World Health Organization (50 mg L^{-1}) is a common concerning in most agricultural areas. Along the Mediterranean coast of Spain, where the cultivation of citrus fruits predominates, a severe increase in contamination by leaching of the nitrate-ion has been observed within the last two decades. Nowadays, efforts are being directed to understand the large number of processes in which N is involved in the plant-soil system, in order to reduce N rate and N losses, which may result in surface and ground water pollution, maintaining crop productivity. Thus, several trials relying on ^{15}N techniques are being conducted to investigate the improvement of nitrogen uptake efficiency (NUE) of citrus trees. The use of ^{15}N tracer opens the possibility to follow and quantify this plant nutrient in different compartments of the system under study. This article gathers the results of several studies based on ^{15}N techniques, carried out by the authors, in order to reevaluate current fertilization programs, organized according to different management practices: (1) Irrigation system: the dose of N and water commonly applied to commercial citrus orchards (up to 240 kgN·ha^{-1}yr^{-1} and 5000 m^{3}·ha^{-1}yr^{-1}) can be markedly reduced (roughly 15%) with the use of drip irrigated systems. (2) Time of N application: the N recovered by plants is 20% higher for summer N applications than for spring N when applied in flood irrigation trees. In drip irrigation, the highest NUE is obtained when N rate is applied following a monthly distribution in accordance with a seasonal absorption curve of N in which the maximum rates are supplied during summer. (3) N form and use of nitrification inhibitors (NI): nitrate-N fertilizers are absorbed more efficiently than ammonium-N by citrus plants, however ammonium fertilizers are recommended during the rainfall period. The addition of NI to ammonium-N fertilizers increases NUE (16%), resulting in lower N-NO$_3^-$ content in the soil (10%) and in water drainage (36%).(4) Split N application: several split N applications result in greater fertilizer use efficiency and smaller accumulations of residual nitrates in the soil. (5) Soil type: N uptake efficiency is slightly lower in loamy than in sandy soils when N is applied both as nitrate and ammonium form; on the contrary, N retained in the organic and mineral fractions is higher in loamy soils that could be used in the next growing cycle. In addition to nitrogen and irrigation management improvement, plant tissue, soil and water N content must be considered in order to adjust N dose to plant demand, since rates exceeding N needs result in lower NUE.

Chapter IX - The use of an electronic tongue is proposed for remote monitoring of the nutrient solution composition produced by a horticultural closed soilless system. This new approach in chemical analysis consists of an array of non-specific sensors coupled with a multivariate calibration tool. For the studied case, the proposed system was formed by a sensor array of 10 potentiometric sensors based on polymeric (PVC) membranes with cross selectivity and one sensor based on Ag/AgCl for chloride ion. The subsequent cross-response processing was based on a multilayer artificial neural network (ANN) model. The primary data combined the potentials supplied by the sensor array plus acquired temperature, in order to correct its effect. With the optimized model, the concentration levels of ammonium, potassium and nitrate fertilizer ions, and the undesired saline sodium and chloride ions were monitored directly in the recirculated nutrient solution for more than two weeks. The approach appears as a feasible method for the on-line assessment of nutrients and undesired compounds in fertigation solutions, where temperature and drift effects could be compensated. The implemented radio transmission worked robustly during all the experiment, thus demonstrating the viability of the proposed system for automated remote applications.

Chapter X - A collection of papers, selected from a natural zeolite conference in 1982, was published in 1984 under the heading “Zeo-Agriculture: Use of Natural Zeolites in Agriculture and Aquaculture” edited by Wilson G. Pond and Frederick A.Mumpton. This publication, for the first time, focused attention on the agricultural potential of zeolitic rocks and demonstrated the application of these materials over a broad area of plant and animal sciences. This work by associating mineralogical and biological research was a precursor to what is now become known as “Geomicrobiology” and as such has lead to new discoveries that will be of great benefit to agronomy. It is shown, in this chapter, that the interaction of zeolite mineral surfaces with the microbial activity of waste organic material results in a soil amendment that introduces both available carbon and nitrogen into damaged and degraded soils that are lacking in biodiversity. During the decomposition of the organic phase ammonia is released and quickly adsorbed by the zeolite mineral. This reaction promotes the formation of a large population of nitrifying bacteria which oxidize ammonium ions from the surface of the zeolite crystals to produce first nitrite and finally nitrate ions, which enter the soil pore-water. This process is continuous in that adsorbed ammonium ions are oxidized and replaced by further ammonium ions until their source is exhausted. In this respect the presence of the zeolite phase acts to buffer the system against the loss of ammonia by volatilization and aqueous leaching. Further more the enzyme reactions involved in the nitrification produce protons which react with the soil to liberate cations and provide plant nutrients in a form that can be taken up from the soil solution. Non-metals such as phosphorous and sulphur as well as trace metal elements, that are essential to plants, are present in adequate concentrations from the decomposing organic waste and reactions with inorganic soil matter. Plant growth experiments clearly demonstrate the efficacy of the organo-zeolitic-soil system in both clean and contaminated cases. By introducing organic waste the explosion of the microbial landscape, that occurs as a result of the application of the biological fertilizer, helps satisfy the carbon demand imposed by a greatly expanded population of heterotrophic microorganisms. This can overcome the problem imposed by the heavy use of inorganic fertilizers as the loss of carbon from the soil is limited by the incorporation of organic material. It therefore appears that the loss of soil biodiversity, and consequent degeneration of soil structure that results from the over use of synthetic chemical fertilizers can be to a greater or lesser extent, depending on climatic conditions and access to suitable materials, reversed by this geomicrobial approach to soil fertility.

Chapter XI - Phosphogypsum is a waste by-product of the phosphate fertilizer industry, which is usually disposed in the environment because of its restricted use in industrial applications. The main environmental concerns associated with phosphogypsum are those related to the presence of natural radionuclides from the U-238 decay series e.g. increased radiation dose rates close to the stack, generation of radioactive dust particles, radon exhalation, migration of radionuclides into neighbouring water reservoirs and soil contamination. Environmental impact assessment studies related to phospogypsum disposal, indicate that usually direct gamma radiation originating from the stacks and inhalation of radioactive phosphogypsum dust don’t pose any serious health risk to people working on phosphogypsum stacks. On the other hand, radon emanation is one of the greatest health concerns related to phosphogypsum, because inhalation of increased levels of radon and its progeny may pose a health risk to people working on or living close to stacks. Physico-chemical conditions existing in stack fluids and leachates are of major importance and determine radionuclide migration in the environment. Among the radionuclides present in phoshogypsum U, Po-210 and Pb-210 show increased mobility and enrichment in the aquatic and terrestrial environment. Regarding Ra-226, the largest source of radioactivity in phosphogypsum, newer investigations show that the Ra-226 concentration in stack fluids is moderate and its release form stacks to terrestrial environments insignificant. Nevertheless, further research is required to improve the understanding of the radionuclide geochemistry occurring within, and beneath, phosphogypsum stacks.

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Chapter I

Ecological Fertilization: An Example for Paddy Rice Performed as a Crop Rotation System in Southern China

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Abstract

Degradation of soil fertility under long term application of inorganic fertilizers is an increasingly serious problem damaging sustainability of modern agriculture. Ecological fertilization is proposed to solve the problems caused by the current fertilization in modern intensive agricultural systems. A rice-ryegrass rotation system established in southern China is a good example of the ecological fertilization practice. The integrative ecological benefits of the rotation system, particularly in aspects concerning to growth and yield responses of paddy rice have been investigated since 1990s. It was observed that the yields of the subsequent paddy rice in many cases were increased for at least 10% in the rotation system when comparing to those in conventional rice cropping. Physical, chemical and biological properties of the paddy soil cropped ryegrass in winter were markedly improved. The evidences of the improvements were observed in availabilities of nutrients, soil enzymes activities, soil microbial features, slow-releases of nutrients etc.

Agricultural nitrogen (N) and phosphorus (P) are main contributors of non-point source pollution to water bodies and often lead to water eutrophication. In the rice - ryegrass rotation system, compound fertilizers are still applied in both ryegrass and rice cropping processes. We valuated the environment influences of the applied N and P in the rice - ryegrass rotation as a model of the ecological fertilization based on the investigation for the patterns of N and P outflows from the rotation system.

1. Ecological Fertilization: Concept, Principle and Necessity

The practice of agricultural production, which has provided us with numerous products on which we could maintain our living and further development, has always been the most fundamental one in the human history. The developmental history of the world agriculture indicates that the world agriculture has gone through three main stages, namely, traditional agriculture stage which mainly depended on the human and animal labor force, modern agriculture stage characterized by the great application of inorganic fertilizers and pesticides in the practice and the ecological agriculture stage which pays more attention to sustainability.

1.1 Traditional management of soil fertility in paddy of China

All through the ages, China has always been a country with large population. The great pressure brought by this expanding population made the Chinese attach vital importance to the land utilizing efficiency. We can glimpse the social pressure generated by food shortage through an ancient encyclopedia wrote two-thousand-year ago, named *Lü Shi Chun Qiu* (*Lü's Annals*), which recorded a regular that a given ploughland should afford a minimum food supplies for the people's needs (Li, 1981). What a marvelous accomplishment it was that the paddy soil has been kept fertile for thousands of years in such an intensive cultivation. When F.H. King, an American agronomist paid a visit to China at the beginning of the 20th century, he was astonished and highly honored it as a miracle in world agricultural history in his *Farmers of Forty Centuries* (King, 1911).

Such wonder mostly owes to a Chinese traditional agro-system called Intensive Cultivation, which was even considered by some scholars as a hinge that combined the nature and society of China as well as a fate of Chinese social development which differed from the Western (Xi, 1984). As a Chinese accustomed saying goes widely among the farmers, Intensive Cultivation means that we should be devoted to cultivate and manage our paddy field as careful as caring for our babies.

How to maintain the fertility of soil is the key difficulty of cultivate. Two thousand years ago, the Chinese encyclopedia, *Lü Shi Chun Qiu* (*Lü's Annals*) had pointed out that the utilization of land should be associated with proper protection. It said that people could use organic fertilizers to supply and maintain the fertility of soil, then making the ploughland 'always productive' (Chinese Academy of Agricutural Sciences, 1986). The earliest record of the fertilization technology to paddy field can be found in *Chen Fu Nong Shu*, a Chinese agricultural encyclopedia in Song Dynasty, which indicated that fertilization must be associated with the soil conditions of paddy (Chinese Academy of Agricutural Sciences, 1986). And the viewpoint of using farmyard manure to keep soil fertility is the essence of Chinese agriculture. Farmyard manure is the traditional organic source for maintaining and improving the chemical, physical and biological properties of soil (R. Riffaldi *et al.*, 1998). Many papers had reported that frequent addition of farmyard manure could increase soil microbial biomass carbon, which could lead to a positive effect on both soil aggregation and macro porosity (McGill et al., 1986).

Though this way of traditional agricultural production generated less stress on environment, and embodied the principle of harmonious coexistence of human and nature, it could not meet the need of fast growing population, ampliative scale of agricultural production and high demand of economy etc. Therefore, since 1970s, traditional management of soil fertility in paddy of China has been changed gradually.

1.2 Degradation of the soil fertility as result of modern intensive agricultural system

From the 1930s, the appearance and application of inorganic fertilizers and pesticides symbolized the arrival of modern agriculture era (Wang, 2003). The modern system involves the plenty use of chemical fertilizers, herbicides, pesticides, fungicides and plant growth regulators. It is also accompanied with the increasing use of agricultural mechanization, which has enabled a substantial increase in production. China, as a developing country with a huge population, its agriculture gradually transformed from traditional to modernized in order to fulfill the need of the population. A survey made by FAO showed that China produced 31,986,707 t of fertilizer in 2002, accounting for 22% of the whole world production (FAO, 2004). Modern agriculture is favored by its high efficiency, but it has its own disadvantages as the following : (1) Degradation of soil fertility. The chemical fertilizer has greatly increased the production, however, the crops may generate a reliance on it, which is not expected previously (Carroll *et al*., 1990). Long-term and vast application of chemical fertilizers would make this productivity-increasing effect weaker and weaker. Moreover, due to the intensive utilization and lack of soil fertility maintenance and improvement measures in the past, organic matters content of the paddy soil has declined in most areas of China. For example, in the Heilongjiang plain of north China, soil organic matter content of Chernozems, the dominant soil type that is well known for its high organic matter content, has decreased from 60-80 g kg^{-1} 30 years ago to currently 30-40g kg^{-1} (Xu *et al*., 1998). (2) Chemical contamination, for example, the pollution of groundwater and surface water. In the paddy farming practice, when any N compound is applied to a submerged field, it is easy to loss through leaching, denitrification, volatilization and runoff, thus contaminates the water (Ghosh, 1998). And even worse, long-term rice cultivation and fertility development results in an increase in mobility of the P added as fertilizers to water and can cause regional eutrophication (Li, 2006). (3) Degradation of agricultural products quality. The great amount of N fertilizers applied in paddy can bring on the results of high level of nitrate, deteriorating taste and reduce shelf life of the rice (Xu & Wang, 2003). Moreover, excessive application of chemicals often induce serious residues on food (Carroll *et al*., 1990). (5) Loss of biodiversity. Instead of the tradition mainly using the native varieties, modern intensive agricultural system just produce few crops in a given district. Such a change induced great loss of biodiversity in agriculture ecosystem, which may give birth to a lot of hidden difficulties (Wu, 2004).

1.3 Ecological fertilization for maintaining sustainability of paddy rice production

Due to the disadvantage and limitation of modern agriculture, it is urgent to develop a brand new kind of agriculture which could not only maintain the sustainability of production, but also environmental-friendly. This kind of agriculture is roughly called ecological agriculture.

Stephen defined eco-agriculture as a subject that designs and manages the ecological system of sustainable agriculture (Gliessman Stephen R. Agroecology; ecological processes in sustainable agriculture. CRC Press, 1997). Luo (2001) asserted that ecological agriculture denies the disadvantages of modern agriculture and is based on the principle of harmonious coexistence of human and nature. It attaches more importance on the biodiversity on which ecological system is built, and aims to assure the food safety and maintain the eco-environmental benefits via increasing the inner circulation and reducing the application of chemical fertilizers and pesticides so as to lessen the stress to natural resource and public environment.

A series of Chinese Ecological Agriculture (CEA) development projects and programs at different administrative levels, such as state, provincial, counties, and townships, etc. have been carried out across China since the 1980s (State Environmental Protection Bureau, 1991; Office of the Leading Group on National Eco-Agriculture Construction, 1996). Up to 1999, the demonstration area of the CEA project was more than 7 million hm^2. Except the governmental programs, there were another 8 million hm^2 belonging to non-governmental programs (Wang, 1999). Ecological agriculture in Jiangsu Province exhibited a typical example of the CEA. The ecological agricultural system emphasizes multi-cropping with N-fixing crops, efficient animal production, integrated use of organic manure and inorganic fertilizers, re-utilization and recycling of nutrients contained in organic materials and related side occupations (Wu *et al*, Ecological agriculture within a densely populated area in China,1989).

In 1999, *via* a 10 years experiment, Yang *et al.* establish an Italian ryegrass-rice rotation (IRR) system in southern China. In winter the system provides the farmers with lots of ryegrass which can be used as green forage for livestock while in summer produces rice. The system has been proved to be a very efficient way to improve yield and maintain the ecological balance. It has estimated that this system can increase the rice yield by about 10% which is significant in rice production. The system also combines crop production with animal production, and it is expected that the system would start a sustainable agricultural system which joins elements of soil, crop, herbage and livestock together. Besides, the system itself distinguished with other rotation systems for that it is a system rotated by gramineous plants-ryegrass and rice. In general, the rotation system is constructed by leguminous and gramineous plants. Leguminous plants are famous for its capability of nitrogen fixing thus used widely in rotation systems, however, to our surprise, the ryegrass-rice rotation system-a rotation system between gramineous plants gains much more yield than rotation systems between leguminous plants which is a topic worth discussing. It has been found that the ryegrass-rice rotation system increased the nitrogen contain in the soil which subsequently increased the rice yield in summer. The soil was ecologically fertilized in the series of experiments. Farmers living in south China benefit a lot due to this ecological fertilization process.

Ecological fertilization can be deemed as an agronomic approach to increase soil fertility and improve crop production by using and enhancing the effects between organisms each other and the interaction between the organisms and soil environment.

Ecological fertilization embodies the principles of ecological economics, makes use of the interaction between the organisms and soil environment to improve the quality of crops as well as to increase the crop yield. It coexists harmoniously with the environment and can be applied widely in such a world that pays more and more attention to her sustainability.

2. Establishment and Development of a New Crop Rotation System for Paddy Rice In Southern China as an Example of Ecological Fertilization

Along with the changes of agricultural system in the recent 20 years in China, traditional cultivating style has been greatly altered. After the modern intensive agriculture methods laying on non-scientific use of agricultural chemicals are oppugned, agriculture sustainability has been treated as a crucial aspect concerning to the human being development. In the rice cropping area in southern China, the importance of crop rotation in paddy is reestimated, and a Italian ryegrass-rice rotation (IRR) system was been newly established as the result. This rice-forage- animal multi-farming method was found to associate with soil fertility maintaining effectiveness as an typical example of the ecological fertilization.

2.1 Objections and dwindle of traditional crop rotations for paddy rice in southern China

Traditional agricultural systems of China are considered to be one of the most sustainable agricultural systems, because cultivations for the same crops at the same farms have been operated for several thousand years before 1980s. Crop rotation must be a crucial point for maintaining the sustainability as well as use of organic fertilizer, intercropping, green manure application, etc. *Li Ji · Yue Ling*, a Chinese ancient book as a Confucian sutra recorded that Chinese farmer used wild plants to improve soil fertility from Zhou Dynasty (1134 B.C.-247 B.C.) (Gu and Peng, 1956) According to Cao (1989), green manures have been applied since Han Dynasty (206 B.C. - A.D. 220) in paddy rice cropping areas in southern China, and wild plants was used at that time. Selected plant species have been cultivated as green manure since Wei Dynasty (A.D. 222-225). From Ming Dynasty (A.D. 1368-1644), typical crop rotations in the areas were associated with the green manure application. Chemical fertilizer was firstly used from 1904, and only 185 thousands ton were applied in whole China. Before 1980s, organic fertilizers made up of 60-70% of total volume of fertilizers in China. In Zhejiang province, for example, inorganic fertilizers applied in paddy were averagely 1.3 ton hm^{-2}, while the organic fertilizers amounted to more than 3 ton hm^{-2} (Cao, 1989). (Cao

(1989) History of fertilization for paddy rice cropping. Agricultural History of China No.1, 83-89)

For paddy rice cropping, green manure had ever played important role on fertility maintenance, and about 80% of the total area green manure cropped in China had been used for rice cropping before 1980s. There are annually two cultivations of paddy rice in most areas of southern China, and before 1980s, green manure crop was immediately cropped following each paddy rice harvest, especially in winter after the harvest of later rice. The main green manure crops were *Sesbania cannanina* (Retz.) Poir. (summer cropping) and *Astragalus sinicus* L. (winter cropping), both are annual or biennial legume.

S. cannabina, which originally comes of tropics of eastern hemisphere, has very high growth rate in the summer in southern China, and was intercropped with early rice as green manure for later rice cropping. *A. sinicus* is a native legume in southern China and has been used as green manure in winter paddy for a long time. Its growing season is in winter, from October to March, about 5 months. By using the two legume plants, “early rice-sesbania-later rice-astragalus” had ever constructed the mostly integrated crop rotation in paddy of southern China. However, because of inconvenience in the use of the sesbania, the practice was not much popularized as astragalus did. For astragalus, more than 8 millions hm^2 winter paddies were cropped in southern China, and it rated more than 50% of the total area of paddy in the region in middle 1970s. Thus, a lot of winter paddies were used for green manure production under the “early rice - later rice - astragalus” rotation before 1980s.

The application of the green manures favored the fertility maintenance of paddy soil and increased rice production. The use of the sesbania was proved to increase later rice production and content of soil organic matter for about 5-10% and about 4%, respectively (Investigating Group of YLGX, 1975). It was evaluated that application of 500 kg fresh sesbania was comparable to the effectiveness of 12-14 kg ammonium sulfate (NH_3SO_4) for later rice (Jin, 1976). But it was also found that the intercropping of early rice and sesbania leaded to yield decrement of early rice for 4-8%. The fact debased the total benefit of sesbania to rice production and only 3-6 % increment in total yield of rice could be obtained in many cases (Investigating Group of YLGX, 1975). On the other hand, for using sesbania, hardship for rice farming was greatly elevated and the intercropped sesbania made inconvenience during early rice harvest. As the result, practice for using sesbania was not much popularized.

Astragalus as green manure of paddy rice was much more popular than sesbania. In 1949, there were about 2.27 millions hm^2 astragalus distributed mainly in the middle and low reaches of Yangtse River. It was increased to more than 7.67 millions hm^2 in 1970s and the cultivation areas extended to whole southern China (Cao, 1989). Comparing to the sesbania, the astragalus winter cropping had more effects on soil improvement and yield increment of subsequent rice. It was proved that yield of paddy rice and content of soil organic matter increased by 15.47% and 7.23%, respectively, after a three-year continuous winter cropping of astragalus (Ye et al., 1993). In Jiangsu Province, a 9 years continuous astragalus cultivation resulted an increase of soil organic matter from 1.21% to 2.14%. It was found that soil bulk density decreased by 0.07, 0.12 and 0.14 g cm^3 in the 1st, 2nd and 3rd year after astragalus cultivations, respectively, and soil porosity increased correspondingly by 2.48, 4.97 and 8.00%, respectively, accompanying with respective 6.84, 14.85 and 18.96% increments of water stable aggregates (Chen et al., 1989).

However, there was a great elevation in use of inorganic fertilizer after 1980s. In early 1960s in China, only 8 kg hm^{-2} nitrogen and 2 kg hm^{-2} phosphorus for crop production were from inorganic fertilizer (Zhang et al., 2004), while in the period from 1961 to 1999, total amount of nitrogen applied in China increased by 43.8 folds, and the nitrogen fertilized per hectare for paddy rice production has now reached up to 180 kg, about 75% higher than the world average (Peng et al., 2002) (Peng et al., 2002). In southern China, an 89.2 kg hm^{-2} increment of inorganic fertilizer resulted in a 973.3kg hm^{-2} increment of cereal yield in 1980s (Liu et al., 2007). Because of the convenience and laborsaving in use of chemical fertilizer, Chinese farmers are gradually giving up the traditional fertilizing style, and using fewer and fewer organic fertilizers, including green manure. Proportion of nutrients from organic fertilizer in total nutrients supply was 99.9%, 91.0%, 80.7%, 66.4%, 47.1%, 43.7%, 37.7% and 31.4% in 1949, 1957, 1965, 1970, 1980, 1985, 1990 and 2000, respectively (Zhang, 2001), and according to State Environmental Production Administration of China (SEPAC, 2005), the proportion was reduced to 25%. In 1976, area of green manure crops in China was more than 13 millions hm^2, but it was now reduced to about 4-5 millions hm^2 (Huang et al., 2006).

The another reason that leaded in the decrement of cultivation of astragalus was because the cultivation of astragalus and the incorporating its tissues into paddy soil increased greatly content of nitrogen in the soil and ripeness of early rice was be put off (Chen et al., 1989), and ripeness of late rice was subsequently deferred. As the result, production of the late rice would be easily suffered by cold snap frequently occurring in late autumn.

2.2 Development of Italian ryegrass - rice rotation system

Attributing to the reasons above-mentioned as well as the low price of cereal in Chinese market, the traditional crop rotation system could not bring enough income to balance their labor output for farmers. Thus, the farmers became to have less and less concern for the use of paddy in winter, and large area of the paddy has been winter-idled since middle 1980s. There were at least 10 millions hm^2 paddies that were idled in winter in southern China in 1990. Based on the background that commercial economy was quickly developing even in rural area of China, reconstructing the cropping system to earn both ecological and economic benefits was needed.

Considering that temperature in winter of the southern China is suitable for growth of most temperate plants, and shortages of green fodder restricted seriously the development of the local livestock industry in the area, a rice- forage-animal multi-farming system was suggested in 1989, and a study on Italian ryegrass- rice rotation (IRR) system in the area have been continuously carried out since the year (Yang et al., 1994; Yang et al., 1995a; Yang et al., 1995b; Yang, 1996; Yang et al., 1997a; Xin et al., 1998a; Xin et al., 1998b; Yang et al., 1999; Xin et al., 2000; Xin et al., 2002; Xin et al., 2004a; Xin et al., 2004b). The efforts led to the establishment of a new crop rotation system in the whole rice cropping area of southern China. According to the practices of the rotation system since 1992, it was found that the new agronomic approach not only laid a foundation of herbivorous animal production, but also improved the local agricultural structure and increased economic and ecological benefits of the agriculture industry, and directly raised income of farmers.

2.3 Integrative benefits of the IRR system

2.3.1 Forage productivity of the IRR system

In 3 field experiments carried out in Guangdong province, China, average forage yield was 12.9 ton hm^{-2} in dry matter under intensive cultivating condition (Yang et al., 1994), and it was 7.3 ton hm^{-2} under zero tillage (Yang et al., 1995b) (Table 2.1). Contents of average crude protein of the Italian ryegrass (IRG) were rather high, ranged from 12.4% to 25.8%, relating to cultivating intensity.

Table 2.1 Yields (ton DM hm^{-2}) and contents of crude protein (CP, %) of Italian ryegrass winter cropped under different cultivating conditions in paddy in Guangdong, China.

	Experiment A		Experiment B		Experiment C	
Cultivating condition	Normal seeding with plowing		Overseeding before rice harvest		Drilling without plowing	
Cultivating period	Oct. 17, 1990-Mar. 6, 1991		Oct. 29, 1991-Mar. 29, 1992		Nov. 24, 1991-Mar. 29, 1992	
Climate condition	Continuous drought from Nov. to Dec.		Continuous drought from Nov. to Jan.		Continuous drought from Nov. to Jan.	
	Yield	CP	Yield	CP	Yield	CP
Average	12.9	24.12	7.3	17.32	6.5	19.13

The results proved that the IRG could afford higher forage productivity even under inferior climate and soil condition. The method of overseeding before rice harvest (Experiment B) is useful because germination of the seed of IRG sown into the rice population were profited from the higher soil moisture and the efficacious use of rainfall in late autumn. Although the forage quality in the overseeding was decreased slightly when compared to that in drilling without plowing (Experiment C), average yield of IRG in the former was similar to that in the later (no significance at $p<0.05$ level). Therefore, the overseeding before rice harvest is treated as the most laborsaving and appropriate cultivating method for the IRR system. Nowadays, farmers are using the method for cropping IRG in winter paddy in a wide area of southern China.

The fertility of paddy soil in southern China was proved to degrade markedly in the IRR studies. It was found that if there were no application of chemical fertilizer, yield of IRG was extremely low, only 15% of that when 1125 kg hm^{-2} compound fertilizer (N:P:K=15:15:15) were applied, indicating that soil nutrients can not be maintained and cycled in the soil or the soil nutrient balance was destroyed. Because chemical farming methods have been carried out for a long time and less and less organic fertilizers was applied in China, the soil retrogressing process widely occurred there is mainly symbolized by the decrease of content of soil organic matter (Wang et al., 2007). Winter cropping of IRG shall be a good solution to the problem because the large amount of residues of IRG (0.60-1.15 kgDM m^{-2}), which will be incorporated into soil, is the original material of soil organic matter.

2.3.2 Growth and yield response of subsequent paddy rice to the ryegrass cropping and incorporation

Because both rice and IRG are gramineous plant, effects of IRG winter cropping to soil fertility and production of subsequent rice are strongly concerned by farmers, agricultural technicians and agricultural managing department of local government. For getting information to clarify the query, a series of experiments have been conducted since 1991. In both experiments in 1991 and 1994, yields of early rice in IRG cropped paddy was 0.99 and 0.78 kg m^{-2}, respectively, 14% and 13% higher than those in winter idled paddy, and the increments were significant ($p<0.05$) (Yang, 1996). Similar result was found in late rice, indicating that the effects of the IRG cropping and incorporation can improve annual productivity of paddy soil for the two growth seasons of the paddy rice.

The growth and yield improvements are evidently associated with changes of soil properties which presented the effectiveness of the ecological fertilization in the IRR system and will be discussed in the next Chapter. The most noticeable differences of paddy rice growth in between the IRG cropped and winter idled paddies were observed in available tillering and seed filling. The enhanced tiller availability and fruit satiation performed by the IRG winter cropping and incorporation is not likely only from the effects of common soil nutrients but also from the effects of some phytohormone-like substances which will be described in the Chapter 4. Fertilization for the IRG production is one of crucial factor leading to yield elevation of subsequent paddy rice, because the growth of IRG depends on the fertilization dramatically (Table 2.2).

Table 2.2 Yields of subsequent rice under different fertilizing treatments in the IRG cropped period

Treatments	Early rice (kg hm^{-2})	Late rice (kg hm^{-2})	Total (kg hm^{-2})	Increment (%)
CK	5660	4910	10570	0.0
A	5515	4888	10403	-1.6
B	6072	5588	11660	10.3
C	6018	5674	11692	10.6

Notes: CK - winter idled; A - IRG winter cropped with zero fertilizer; B - IRG winter cropped with 1125 kg hm^{-2} urea fertilizing; C - IRG winter cropped with 1125 kg hm^{-2} compound fertilizer (N:P:K=15:15:15) applying; Increment - percentage of the yield increment in the treatments comparing to the CK.

2.3.3 Other benefits of the IRR system

(1) Controlling paddy weeds and pests

Because of the frequent harvest of the IRG in winter, paddy weeds have usually no chance to fruit. On the other hand, high density of IRG population can also restrain growth of the weeds. Therefore, IRG winter cropping is effective in the control of paddy weeds.

It was found that densities of the insects as natural enemy of pests were obviously increased in the paddy IRG winter cropped as comparing with that winter idled (Table 2.3). The results have excited the bio-control researchers who are bothered for a long time by the problem how to help the natural enemies to overwinter safely in South China where the idled paddies are mostly bare in winter. The researchers are collecting more evidences to prove that the IRG population provided a safe overwintering habitat for the natural enemies in where the IRR system was established.

Table 2.3 Population densities of insects in paddies IRG winter cropped and winter idled (×1000 heads hm^{-2}).

	Araneid	Predaceous insects	Parasitic insects	Pests	Others
IRG cropping	660	105	270	360	1710
Winter idling	165	15	30	105	120

(2) Saving fodder grain

Because the forage quality of IRG in the IRR system is proved to be rather high (Table 2-1, the contents of crude protein were more than 15% in most cases), higher productions of animals, such as cattle, goose, grass carp, rabbit etc., and even pig were gained in farmer's practices, accompanied with a great save of fodder grain (Table 2.4).

Table 2.4 Some examples about fodder grain save by feeding IRG

	Fodder grain save
For feeding pig	Save concentrated feed for 20%.
For feeding goat	Save grain feed cost for 120 yuan $head^{-1}$ y^{-1}, and increase milk production for 20%.
For feeding grass carp	50kg fresh IRG equal 6kg maize powder.
For feeding goose	Grain feed are not needed at all.

(3) Reducing abandonment of non-point pollutants from agriculture

As above-mentioned, the ecological fertilization based on the IRR system can elevate soil fertility, and control paddy weeds and pests effectively. So the practices of the IRR system for an enough long period can certainly result in reductions of the use of chemical fertilizers, pesticides and herbicides in rice production. Combining with animal production, for example pig and grass carp, both are fed using IRG, farmers can use manure of pig to feed grass carp, and irrigate paddy for both IRG and rice production using the pool water fed the grass carp. Thus, nutrients for crops and animals will be adequately combined into a cycle, and the agricultural wastes, as non-point pollutants, will be intensively reduced. It has been proved that outflow of N and P in paddy soil, as pollutants once they run into surface water, can be restrained by the IRG winter cropping, which will be demonstrated in the Chapter 4. These benefits draw a picture which reveals that the IRR system is an environmental friendly agricultural system.

(4) Increasing stability of regional agriculture industry

In China, agriculture is a non-preponderant industry although it has a large scale, because the current business system cannot support agricultural products entering market smoothly. Thus, multiform farming systems are necessary for individual farmers. The establishment of the IRR system has promoted the change from traditional single-crop farming to a series of crop-animal multiple systems and results in the increase of stability of regional agriculture industry (Yang *et al.*, 1997b). In many provinces in South China, IRR system has been treaded as an important farming system, and the areas of paddy IRG cropped in winter are continuously increasing. Before 1995 in Guangdong, Sichuan, Jiangxi and Guangxi provinces of China, the historically accumulative paddy area operated under the IRR was about 400 hundreds hm^2, and in 1999, the paddy area performed annually under the IRR system in the 4 provinces was about 930 hundreds hm^2, while it was recently increased to more than 2000 hundreds hm^2.

3. The Impacts of the IRG Winter Cropping and Incorporation on Paddy Soil Properties

The quality of a soil is central to determining the sustainability and productivity of the above-ground communities (Doran et al., 1994). Thus, it is imperative to develop and implement management strategies that maintain the quality of soil such as practices that conserve organic matter and maintain/enhance soil fertility and productivity. Cover crops grow during periods when the soil might otherwise be fallow. In the IRR system, the IRG is a cover crop with special usages as fodder. In the growth season of the IRG, it plays the role of cover crop, and is harvested for feeding animals, while its residues will be returned into soil as green manure before subsequent rice is transplanted. The effects of the IRG as cover crop and green manure crop on soil properties and production of the subsequent rice are therefore one of crucial determinant for assessing the feasibility of the IRR system.

In recent years the importance of cover crops in crop production is increasing due to concern for improving soil quality and reducing chemical inputs. Dabney et al. (2001) reviewed the advantages of using cover crops to improve soil properties, including reduce soil erosion, increase residue cover, increase water infiltration into soil, increase soil organic carbon, improve soil physical properties, improve field capacity, recycle nutrients, weed control, increase populations of beneficial insects, reduce some diseases, and increase mycorrhizal infection of crops. However, A few studies also reported some negative effects of cover crops on agricultural soils (Vyn et al., 1999; Karlen and Doran, 1991; Martinez and

Guiraud, 1990; Francis et al., 1998; Wyland et al., 1995). Despite these results, the consensus is that cover crops are able to improve soil properties and environmental qualities if with proper selection, use, and management.

It is generally agreed that non-leguminous crops are less capable than leguminous crops of increasing soil N availability and crop productivity. The IRR system is a novel rotation system between two graminaceous plants practiced only in southeastern China, which we know very little about its ecological impacts. Graminaceous crops are often fertilizer-demanding, therefore rotations between two graminaceous plants are not commonly adopted, so that the paddy soil response to the IRG winter cropping and incorporation is crucial to our understanding on the IRR system.

3.1 Physical and chemical properties

The earliest finding about the improvement of soil properties by the IRG winter cropping was made by Yang (1996), who found that there were 21, 4, 29, 3, 11, 26 and 57 % increment in contents of organic matter, total N, P, and K and available N, P and K, respectively, in the soil IRG winter cropped when comparing to the winter idled soil. Contents of soil N, P, K at subsequent rice cropping stage were also higher in the IRG winter cropped paddy than in the winter idled one, and the difference in total N was significant ($p<0.05$) (Yang et al., 1997a). The availability of soil nitrogen in the whole growth period of the rice was promoted by the IRG cropping. Detailed analysis on soil nutrient balance showed that, there was an unknown input of soil nitrogen besides the application of fertilizers in the growth period of IRG (Yang et al., 1997b). Similar results were observed by Xin et al. (1998a) who found that the IRG winter cropping increased the contents of total N, P and K in the soil for 26%, 32% and 14%, respectively over the growth period, while in the winter idled control plots, soil total N and P decreased by 13% and 4%, and total K increased by 10% (Figure 3.1).

Xin et al. (1998a) also found that the IRG winter cropping improved the availability of N, P and K in the soil. In the IRG winter cropping treatment, the contents of available N, P and K were elevated by 67%, 33% and 78%, while in the control plots, the contents of available N and P in the soil was decreased by 20% and 13%, and available K was increased by 10%. The comparison between the IRG cropped and the idled cases revealed a large increment in nutrient availability as responding to the IRG cropping (Figure 3.1). These results showed that the N, P and K applied in organic and inorganic forms in the IRG winter cropping period can be kept in soil, and many of them shall be associated with the extensive fibrous root system of the IRG, so that surface runoff and leaching of the nutrients may be effectively prevented.

Recent studies indicated that graminaceous cover crop species could improve the Fe-nutrition of fruit trees grown on calcareous soils by enhancing Fe-availability (Rombola and Tagliavini 2006; Cesco et al., 2006). Xin (1998c) also found that the IRG winter cropping enhanced the availability of Ca, Mg, Mn, Fe in soil. In the winter idled plots, concentrations of exchangeable Ca, Mg, active Mn and Fe decreased by 18.8%, 45.9%, 49.4% and 29.8%, respectively, in the period between harvesting late rice and transplanting early rice. The concentrations of exchangeable Ca, Mg, active Mn and Fe were respectively 32.7, 44.9, 11.1 and 7.5% higher in the soil of the IRG cropped paddy than in the winter idled paddy (Figure 3.1), and the differences in the exchangeable Ca and Mg were statistically significant (*p*<0.01). The IRG winter cropping could alter soil pH and soil oxidation reduction potential through secreting organic acids and other materials, which may be the reasons that the availabilities of the nutritional cations in soil can be increased (Xin et al. 1998c).

IRG own developed fibrous root, and their amounts in the soil surface layer (0-10 cm) could reach up to 1148 g DM m^{-2} (Yang et al., 1995a). Soil organic matter will drive from these biomass after the residue and the root tissues are incorporated into paddy soil, and the soil physical properties, such as soil bulk density, number of soil pores, soil aeration and permeability, will be improved. It was found that the IRG winter cropping and the residue incorporation resulted in a 66% increment in content of soil organic matter in rice cropping stage, while in the idled plots, it decreased by 14% (Xin, 1998c). The IRG winter cropping and incorporating also lowered the bulk density of the soil by 9.4% in the rice cropping stage, while in the winter idled plots, it was decreased only by 4% (Xin et al., 2004b).

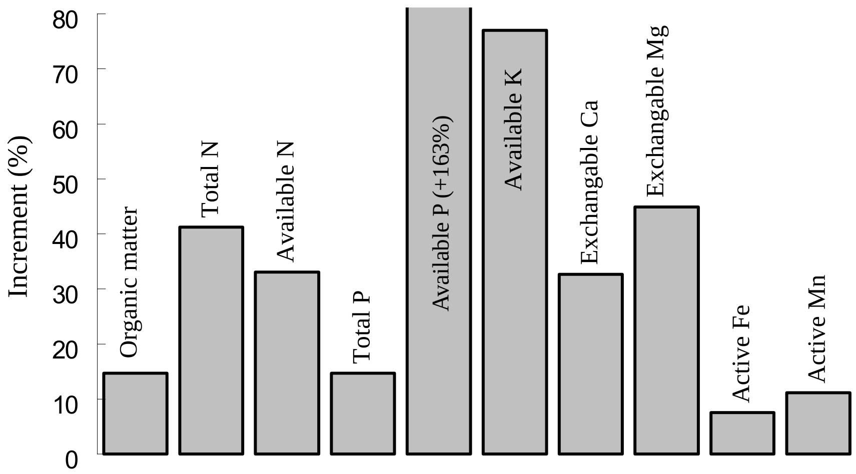

Figure 3.1 Increments in some soil properties under the IRG winter cropped condition comparing to those under winter idled

Similar results were obtained in a long-term study (Wang et al., 2005), in which, the soil bulk density in a conventional “early rice - late rice – fallow” cultivation was higher than those in several “rice - grasses” rotation systems, and the total porosity and the non-capillary porosity of the soil in the later were both improved when comparing to the former. Wang (2004) reported that orchard soil properties were improved after cultivating IRG, especially nutrients availabilities. In this study, the contents of organic matter, available N, available P and exchangeable K in surface soil (0-20cm) IRG cropped were respectively 45.7-64.3%, 46.5-66.0%, 1.1-6.5 folds and 1.7-4.2 folds higher than those no plant cultivated. Li and Zhang (2000), Chang et al. (2005) and Zhu et al. (2007) also observed the soil improvement and some of the researchers proved the consequent yield increment of subsequent rice (Li and Zhang, 2000; Zhu et al., 2007).

The facts above-mentioned indicate that a proper crop rotation and the effective use of residues of cover crop by incorporating them into soil is an affirmative approach to improve soil physical and chemical properties. The performance exceeds the function of traditional fertilization with inorganic fertilizers, which can only supply soil nutrients. In the IRR system, the improvement of soil properties is integrative and roots mainly in the interactions between crops each other and between crops and soil environment, and thus is a good example of the ecological fertilization.

3.2 Biological and biochemical properties

Soil biological properties are closely related to the chemical environment in the soil and are important in controlling soil chemical and physical properties (Brye et al., 2004). Soil microorganisms play a crucial role in maintaining soil quality due to their action in nutrient cycling through the decomposition of organic matter and nutrient storage (Turco et al., 1994).

Enzymes catalyze all biochemical reactions and are an integral part of nutrient cycling in soil. Soil enzymes are believed to be primarily of microbial origin (Ladd, 1978) but also originate from plants and animals (Tabatabai, 1994). They are usually associated with viable proliferating cells, but enzymes can be excreted from a living cell or be released into soil solution from dead cells (Tabatabai, 1994). The free enzymes complex with humic colloids and may be stabilized on clay surfaces and organic matter (Boyd and Mortland, 1990). Soil enzyme activity is one of the basic natures of the soil, and could be treated as the indicator of the intensity and trends of all the biochemistry processes processing in soil. Soil enzyme activities are mainly affected by cultivation style as well as soil humidity, temperature, bulk density, pH value, contents of nutrients, fertilization and irrigation, etc. (Zhou, 1987). Cover crops would increase enzyme activities compared to systems receiving reduced C inputs (winter fallow) (Bolton et al., 1985; Martens et al., 1992; Goyal et al., 1993).

The ecological fertilization, as one of the functions of the IRR system, improved soil biological properties. Activities of soil hydrogen peroxidase, dehydrogenase, protease, phosphotase, urease and convertase under the IRG winter cropoing were 19.8, 36.1, 17.4, 41.7, 14.7 and 11.3 % higher, respectively, than those under the winter idled (Figure 3.2) (Xin, 1998c). The increments of the soil enzymes were proved to be relevant to the rhizosphere activity of the IRG. Activities of dehydrogenase, protease, phosphotase and urease in rhizosphere soil were 31.7, 36.5, 70.6 and 17.8% higher than those in non-rhizosphere soil. These results indicate that the IRG winter cropping can greatly elevate the soil bioactivity which may concerns to soil microbe activities, substances transformation, as well as the utilization of nutriment elements, and consequently associates with the growth improvement of the subsequent rice.

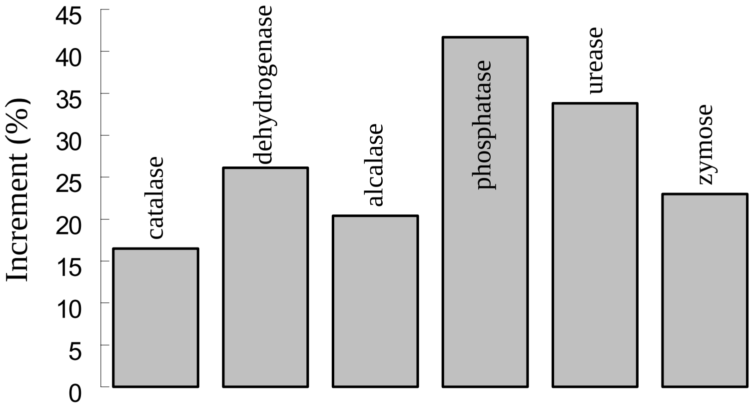

Figure 3.2 Increments of activities of several soil enzymes under the IRG winter cropped condition comparing to those under idles

Although soil microorganisms accounts for only 1 to 3% of organic C and 2 to 6% of organic N in soil (Jenkinson, 1987), it plays a key role in soil organic matter and nutrient dynamics by acting as both a sink (during immobilization) and as a source (mineralization) of plant nutrients (Kumar and Goh, 2000). It was proved that using cover crop could alter microbial biomass (Linn and Doran 1984; Wagner et al. 1995; Kirchner et al. 2003; Zablotowicz et al. 1998), as well as microbial community structure (Lupwayi et al. 1998; Feng et al. 2003).

In the IRR system, the IRG winter cropping leaded to a significant increase in the soil microbial biomass ($p<0.01$) and the effect was not only observed at the periods of planting IRG, but also at the subsequent rice growing season (Xin et al., 1998b). But in the winter idled paddy, the effect was not recognized. It was found that there were 1.2 folds and 3.4 folds increments in densities of bacteria and actinomycete, respectively, and 44% decrease in fungi density in the surface soil of the IRG cropped paddy comparing to those of winter idled paddy Xin et al. (Xin, 2004b). The huge increments in densities of bacteria (1200%) and actinomycete (900%) in the rhizosphere soil of the IRG comparing to the non-rhizosphere soil (Xin, 2004b) give good explanation for the response of microbial community in the surface soil, and the enhanced actinomycete community may associated with the health of the soil ecosystem, which may be characterized by the response of fungi.

It is considered that the ecological fertilization accompanying with the IRR system could accelerate the cycling of the soil nutrients via the melioration of soil microbes. The enhanced ecological performance is impossibly actualizing with the chemical methods for soil management which relies on mainly inorganic fertilizers.

3.3 Rhizosphere activities

Rhizosphere is the microhabitat which is several millimeters surrounding the plant roots. In this microhabitat, the physical, chemical as well as biological conditions have direct impacts on the move and transformation of water and nutrients from soil to root, availability of soil nutrients, physiological activity of plant roots, survival and propagation of beneficial and harmful microbes, accumulation and degradation of pollutants (Liu et al., 1997). With the extending of the root system in soil, rhizosphere microorganism usually distribute by gradients, and their quantities are significantly higher than non-rhizosphere soil, this phenomenon is called rhizosphere effect (Katznelson, 1946).

Yang et al. (1997b) found that, inoculating the microbes collected from paddy rice rhizosphere into the culture solution could significantly improve the growth and biomass accumulation of the hydroponic IRG. This implies that the microbes take part in the process of IRG substances production, and the growth promotion of rice and the improvement of soil properties in the IRR system may also be relevant to the effects of the rhizosphere microbes.

The pH value and electric conductivity in rhizosphere soil of IRG differ from those in the non- rhizosphere soil, especially in the electric conductivity (Xin et al., 2004b). Concentrations of polysaccharides and most organic acids, as well as the activities of many enzymes in the IRG rhizosphere soil are also higher than those in the non-rhizosphere soil (Xin et al., 2004b). These well explained the enhanced availabilities of nutrients in the IRG cropped paddy soils.

Seedlings of paddy rice grown in the rhizosphere soil of IRG revealed a better growth than that in the non-rhizosphere soil (Figure 3.3). Under the middle fertilizing level, plant height, leaf area, dry matter weight of shoots and roots, root quantity and root length of the rice seedlings grown in the rhizosphere soil were respectively 5.3, 5.1, 4.7, 14.5, 12.5 and 6.4% higher than those in the non-rhizosphere soil. The content of leaf chlorophyll and root activity was also 7.3 and 15% higher, respectively (Xin et al., 2002).

Associating these results with the improvements in availability of soil nutrients and soil microbial community in the rhizosphere soil of IRG, the effects of the ecological fertilization actualized in the IRR system are embodied in the microhabitat surrounding the root of IRG, which is fine and with large bulk surface area.

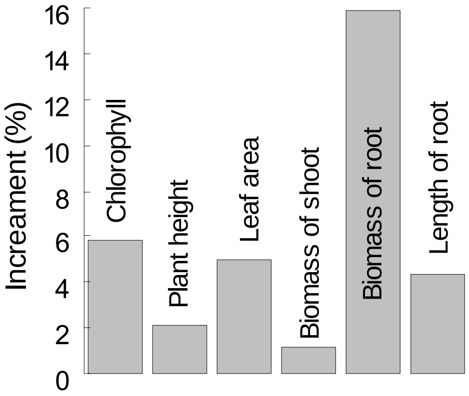

Figure 3.3 Increments (%) of characters relevant to growth of rice seedling grown in the IRG rhizosphere soil comparing with those grown in non-rhizosphere soil.

3.4 Slow-release like effect for the nutrients fixing in the IRG residues

Many of the studies on the nutrients releasing from residues of cover crops focused on the leguminous cover crops. Some studies showed that the pattern and timing of decomposition of residues in the soil depends on the residue quality, particularly C/N ratio, soluble C, lignin, polyphenol contents, soil type, temperature, soil moisture content and timing and method of incorporation. (e.g.Vanlauwe et al.,1997; Cadisch and Giller, 1997). Decomposition of organic residues involves two phases, an initial rapid breakdown of readily decomposable plant components and a slower decay phase (Swift et al., 1979).

Xin et al. (2004a) reported that the biodegradation of IRG residue resulted in a 74.9% decomposition in the whole growth stage of early rice, and the mineralization rates of C, N, Mn and Mg were lower than 80%. There seemed to be a slow-release effect in the process of IRG residue decomposition. The release rate of all the nutrients in IRG residue reached 92%-99% at the harvest time of late rice. It is estimated that the root system of IRG can separately release 865.42, 26.92, 1.75, 23.83, 2.74, 0.08, 0.16 and 0.05 g kg^{-1} of organic matter, N, P, K, Fe, Mn, Mg and Ca, respectively, to the 0-20 cm soil layer. In the IRR system, the IRG fixes the nutrients in winter, which is a rainless season and thus the fertilization in the period may be much environment-friendly because the applied nutrients have less chance to runoff and leach and shall have high utilizing rate by the IRG. After the residues of the IRG being incorporated into paddy soil, release of the nutrient in the residue are much slower than those in the inorganic fertilizers. Ye et al. (1998) found that microbial biomass N in the soil IRG incorporated maintained at the level higher than that before the incorporation for 60 days, and the mineralized N reached the highest level and the higher level was kept for 120 days till the end of the experiment. While for the N in urea, 100% of the N will be normally hydrolyzed in a rather short time, only 3-7 days (Xi, 1994).

A synchronized release of nutrients in fertilizer with crop demand is expected (Myers et al., 1994), and slow-release inorganic fertilizers have been developed since 1950s. So, in the IRR system, the slow-release effect of the nutrient fixed in the IRG residue represented another merit of the ecological fertilization, which connects to fertilizer efficiency, soil management, production cost and environment protection.

4. Evaluation of environmental influence of the ryegrass winter cropping

To make high efficiency of fertilizer in agricultural practices to reduce the disturbance of the overflowed nutrients upon natural ecosystems, particularly the natural water environment, is an important target of the ecological fertilization. The market of inorganic fertilizers has been dramatically expanded with the development of agricultural industry (Zhu, 1990). According to the statistic data, the domestic market of fertilizer in China has increased from 25.903 million tons in 1990 to 44.116 million tons in 2003, and the market of nitrogen fertilizer raised from 16.384 million tons in 1990 to 21.499 million tons in 2003 (China Statistical Yearbook, 2004).

Although the increased application of fertilizer in agriculture has enhanced the productivity of crops, the efficiency of the fertilizing is fairly low due to inappropriate application (in terms of both amount and methods) of fertilizer. Based on the survey in various locations in China, the availabilities of the fertilizers in China are as low as 30-35% for N fertilizers, 10-20% for P fertilizers, and 35-50% for K fertilizers (Sun et al., 2001). The low fertilizer utilizations have brought on a huge input of the nutrients into natural environment via pathways like runoff, leaching, denitrification, adsorption and erosion. Statistics showed that the national average of fertilizer leaching and runoff amounts to 40% of the total fertilizers application (Wang, 2001). Excessive use of fertilizer has caused various contaminations in over 1/5 of arable lands in China (Zhao, 1994), led to huge economic loss, and tipped off the fragile balance in agricultural ecosystems (Qu, 1999). Non-point source pollution by agricultural practice and fertilizer application has become an important research topic in the international ecological community.

As the IRR system has gradually gained its popularity, the studies on its environmental influence, especially on N, P cycles through the system, become eminently important. The boosting up of the nutrients utilization in the IRR system via the improved nutrient cycle in the rice-ryegrass-soil system will assuredly declare the establishment of the ecological fertilization embodying in the IRR system.

4.1 Effects of agricultural nitrogen and phosphorus on pollution and eutrophication of water bodies

Non-point source pollution stands in the contrary site against point source pollution when viewed with the angle of in type. It refers to the larger scope, results from the forces of rainfall-runoff, the dissolved or solid pollutants go through from non-specific locations into adjacent receiving water bodies (such as rivers, streams, lakes, artificial reservoirs and gulf). Besides, when the pollutants are excessive accumulated in water, eutrophication would occur. Agricultural non-point source pollution, owing to the broad range it affects, the great amount of pollutants output, and the strong effect to the adjacent water bodies, has been considered to be the major source of water pollution.

Negative effects of agricultural activities on surface and ground water quality have been a topic of concern in many parts of the world for several decades. The agricultural pollutants come mainly from the improper utilization of fertilizer and pesticide, especially the huge amount of nitrogen and phosphate fertilizer. The loss of N, P fertilizer in agriculture is mainly driven by the process of precipitation-runoff process, what is more, the main body of the loss happens during the storm runoff event. (Beegle et al., 2000; Behrendt and Opitz, 2000). Moreover, the inappropriate land utilization and improper farmland management cause the erosion, will accelerate loss of N, P in the soil (Mander, 2000). An increased nutrient loss from arable land due to the nutrient surplus in agricultural systems is suggested as the leading reason for the water quality deterioration of the lake in China (Fan et al., 1997; Jin et al.1999).

The consequences following the great amount loss of agricultural N, P are the increasingly severe water pollution problems (Wang, 2001). Data has shown that most of the lakes in China have undergone the change from oligotrophi state to the eutrophication state since the twentieth century (Zhang et al., 2002; Lu et al., 2002). For example, for Tai Lake and Dianchi Lake, the agricultural non-point source pollutants has composed of 57, 38 and16% of the water pollutants NH4+-N, TP and COD, respectively (Wu, 2001).

Wang et al. (1996) found that NH_4^+-N in paddy soil is more easily absorbed by soil particles than NO_3^--N, and the NH_4^+-N could be hold in soil more steadily, while the NO_3^--N could easily filtrate into the beneath of profile, rendering that the NO_3^--N is easily entering groundwater via leaching. Wang et al. (1997) have also proved that the major form of N leaching in paddy soil is NO_3^--N.

4.2 Nitrogen and phosphorus application when the ryegrass winter cropping in paddy

Surface runoff, leaching drainage, subsurface runoff are the major pathways of soil phosphorus loss. Since it is putative that P harbors a higher affinity with soil, it is recognized that there are few conditions when the element P moves as downward vertical migration (Heckrath et al., 1995; Sims et al., 1998). Soil corruptions caused by surface runoff are thought to be the chief pathway through which P flows from soil to accepting water body. In fact, the transfer of P in interflow in soil could happen in areas where are overdue-fertilized. Zhang et al. (2001) concluded in a simulated paddy assay that the concentration of P transferred in interflow in paddy soil could be higher than previously realized, leading the soil P to leach into groundwater.

In the IRR system, fertilization is performed in two stages including the IRG growing season in winter and the paddy rice growing season from spring to summer. IRG is much responsive to fertilizer and fertilizer tolerable, so that heavy fertilization is easily practiced for harvesting more green fodder. Fortunately, the IRG has high ability to accumulate nitrogen, and the concentration of N could be as high as 3.5% in its aboveground (Yang et al., 1994). It was found that N input (31.8 g m^{-2}) throughout fertilization was 28% less than the N output (59.8 g m^{-2}) from harvesting green fodder, while a positive balance (+22.0%) in soil P was observed (Xin et al., 1998b). As above-mentioned, fertilizing in the IRG growing season is much safe for environment protection, and part of the nutrients fixed in the IRG tissues will release into soil with a slow-release like process after the tissues were incorporated into the paddy soil. Associating with the soil improvement in many aspects in the IRR system as above mentioned, it is believed that the fertilizers applied in the rice growing season, which may highly risks the environment pollution due to the runoff and leaching of the applied inorganic nutrients in the rainy season, could be reduced after a long term practice under the IRR system.

4.3 Control of outflows of nitrogen and phosphorus from the soil in the IRR system

Soil left bare after tillage operations is prone to erosion, and induces the run-off and leaching of nitrogen, phosphorus and pesticides into surface and ground water (Jürg Hiltbrunner et al., 2007). According to Xin et al. (2000), when the application of compound fertilizer goes to 750-1500kg hm^{-2}, the productivity of the IRG increases in a dosage dependent manner, which may cause the oversupply of the nutrients, particularly N and P, and harm soil and surrounding environments. Hence, it is count for much to assess the environment influence of the practice of the IRR system.

Li et al. (2005**)** investigated the environmental burden under the fertilizing level of 375 and 750kg hm^{-2} for IRG winter cropping. The result showed that most of the N and P in soil were absorbed and fixed by the IRG tissues. Because the contents of N and P and the yield of the IRG were increased accompanying with the increase of the fertilizing rate, the volume of the fixed N and P by the IRG was directly proportional to the fertilizing rate. In the winter idled paddy, the N accumulated in weeds was 3.289 g m^{-2}, while in the IRG cropped paddies received each 375 and 750 kg hm^{-2} inorganic fertilizer (N:P:K=15:15:15), the N fixed in the IRG tissues were as high as 9.120 and 12.920 g m^{-2}, respectively. The N losses through infiltration in the idled paddy were 2.70 g m^{-2}, even significantly higher than those in the IRG cropped fields (1.37 and 1.69 g m^{-2} under the 375 and 750 kg hm^{-2} fertilizing, respectively). The N fixed by the weeds or the IRG were 1.2, 6.7 and 7.6 folds of those leached N in the idled paddy and the two IRG copped paddies, respectively (Li, 2005). Comparing to the N, much more effective P fixation was observed. It was found that the P fixed by the weeds or the IRG in the idled paddy and the two IRG copped paddies were 40, 144 and 198 folds of those leached (Li, 2005).

The N and P losses through infiltration leaching take up quite small proportion of the total N and P in soil and the IRG plant, and it was found that the losses were negatively proportional to the fertilizing rate, indicating that the promoted IRG growth by the fertilizing are favorable to fix the N and P in the soil-plant system. After rainy season came, the loss of NO_3-N, NH_4^+-N and P increased as the fertilizing rates rose, while the proportions of the lost N and P to the total N and P in soil and Plant were decreased as the fertilizing rates rose. The loss of N was mainly in form NO_3-N, being consistent with the previous studies (Wang et al., 1996; Wang et al., 1997).

According to Li (2005), although no fertilizer was applied in the winter idled paddy, the losses of NO_3^--N, NH_4^+-N and P through soil infiltration were found to still be 26.18, 0.83, and 0.028 kg hm^{-2}, respectively. The NO_3^--N, NH_4^+-N and P losses in the IRG winter cropped paddy received 375 kg hm^{-2} inorganic fertilizer (N:P:K=15:15:15) were 13.24, 0.43 and 0.021 kg hm^{-2}, significantly lower than those in the winter idled paddy. Similarly, when the fertilizing rate increased to 750 kg hm^{-1}, N loss in case the IRG cropped (NO_3^--N 16.18 kg hm^{-2}; NH_4^+-N 0.70 kg hm^{-2}) was significantly lower than that under fallow, while the P loss (0.029 kg hm^{-2}) slightly increased for 4.4%, but the difference was not significant (Li, 2005). These results revealed that the IRG coverage in the fallow season of rice can reduce the losses of N and P, which is helpful to cut down the influence of agricultural pollutants to surface and ground waters. Kurtz et al., (1946; 1952) indicated that the continuous soil cover provided by the living mulch/cover crops is a good strategy for reducing soil erosion. Macdonald et al. (2005) also reported that reductions in N leaching exceeded 90% when comparing cereal cropping systems with and without cover crops. In fact, among the various practices that reduce soil erosion and leaching of such nutrients as $NO3^-$-N, winter cover cropping has gained more acceptance by growers as it has been proved to be more effective in reducing soil erosion, N leaching and contamination of water (Meisinger et al. 1991; Francis et al. 1994; Thorup-Kristensen et al., 2003).

It is obvious that the IRR system is an environment-friendly agricultural approach in the utilization of inorganic fertilizers. The safe nutrients supply in winter lets the IRG has chance to fix the N, P and K and other nutrients in its tissues, and then leaves in paddy soil in form of residues. The IRG residues release the nutrients fixed in their growing season in winter when they decompose gradually, and thus the releasing process is slower, so that more nutrients can be effectively absorbed by the subsequent rice. On the other hand, the IRG provides soil cover and nutrients carrier in winter, which greatly decreased the runoff and leaching of the soil N and P. Therefore, the addition of the IRG in the traditional rice production in southern China, which provides land cover and carrier or filter of soil nutrients, constituted the environment-friendly mechanism of the IRR system as a typical style of the ecological fertilization. The practice is considered to be contributive to the protections of surface and ground waters.

5. Conclusion

The ecological fertilization is not a physical or chemical simplex process, but a typical biologically leaded complex performance. Crop rotation, cover crop and green manure crop applications, biological N fixation using legume or non-leguminous plants, methods to enhance the functional microorganisms in soil, such as AMF, rhizobium, photosynthetic bacteria, actinomycete etc., are considered to have potential to achieve the effectiveness of the ecological fertilization. The ecological fertilizing techniques can not only supply the soil nutrients, but also improve soil physical, chemical and biological properties, such as (1) increasing availability of the nutrients, (2) forming the proper soil aeration, permeability and water retainability by elevating content of soil organic matter, water-stable aggregates and soil porosity, (3) enhancing the activity of soil ecosystem which can be indicated by the improvement of soil microbial community and the increments of the soil enzymes, and (4) declining the overflow of N and P as pollutants of natural environment. The ecological fertilization may be contributive to the bio-controlling for harmful pests although the proofs have not yet be sufficiently investigated. The IRR system which has been widely practiced in the rice cropping areas in southern China is found to be endued with most advantages of the ecological fertilization. It is convinced that the soil fertility management under the ecological fertilization is the most crucial characteristic of ecological agriculture.

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Chapter II

Soybean Mineral Nutrition and Biotic Relationships

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Abstract

Soybean is the world's most important source of protein and accounts for nearly 70% of the world protein meal consumption. This has led to the production of soybean in a wide range of environmental conditions across a huge geographic expanse. Soybean has a distinct advantage over non-leguminous crops through its ability to acquire N via symbiotic N-fixation. However, other nutrients are critically important to optimize soybean production, both through direct effects on growth and development as well as through their influences on soybean biological N fixation. In this review we highlight the fertility requirements and considerations for soybean production and examine the relationships between soybean mineral nutrition and biotic factors such as selected disease, insects, and nematodes.

Introduction

Soybean has been grown in Asia for thousands of years; however it has only been in the last 70 years that soybean has risen to be the world's most important source of protein and vegetable oil. In 2006, soybean accounted for 68 % of the world's protein meal consumption and 29 % of the world's vegetable oil consumption. Soybean has clearly become a staple

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crop of the modern world. Additionally, there are many other uses for soybean ranging from ink to glue and increasingly as a biofuel (biodiesel). Worldwide hectarage of soybean is roughly estimated at 94 million ha. More than 70 million ha are in large-scale commercial production in the Western Hemisphere from about latitude 56° N in Canada to about latitude 30° N in the southern USA and then from just south of the equator in northern Brazil to about latitude 38° S in Argentina. Together the nations of the Western Hemisphere accounted for greater than 86% of the total worldwide soybean production (top producers: United States 38%, Brazil 25%, Argentina 19%, Canada and Paraguay each at 2%).

World-wide soybean is grown in a tremendous range of environments including different soil types, cultural practices, temperature norms, rainfall and irrigation, and varied disease and insect pressures. All of these factors and many more can singly and in varied combinations affect soybean fertility management. Soybean production in the tremendous range of environments encountered is made possible by the availability of adapted cultivars and tailored management practices. Covering all possibilities in one chapter would be a monumental if not impossible task. However, there are common principles, practices and considerations that are universal (or nearly so) and are highlighted in this chapter.

Soybean fertility and nutrient management has been well studied and documented. Reviews in the first two editions (1973 and 1987) of the American Society of Agronomy monograph “Soybeans: Improvements, Production, and Uses”, delve into great detail about soybean mineral nutrition (deMooy et al., 1973b; Mengel et al., 1987) and are an excellent starting point for a detailed understanding of soybean fertility. Today, most soybean producing states in the USA have soybean fertility recommendations readily available online. Most are excellent resources applicable to local conditions and problems. In many cases local extension agents, state soybean specialists and/or consultants can provide specific recommendations. However, to make accurate site-specific recommendations, a soil test is imperative.

Plants require elemental nutrients in various amounts although the amounts vary greatly with species, genotype, soil, and environmental factors (Table 1). Essential elements are generally defined as either a macronutrient or micronutrient based on the relative amount required by the plant (Table 1). Although, micronutrients are required in very small amounts, lack of any one nutrient can adversely impact production. The processes and rates of natural accumulation of nutrients in the soil (mineralization, organic matter decomposition, etc.) are complex and highly dependent on soil type and local conditions. For grain crops, as seed is produced and removed from the field, nutrients are lost every season. This loss must be replaced naturally or with fertilizers in order to maintain production.

Table 2 shows an estimate of nutrient removal from a 50 bu ac^{-1} (3359.5 kg ha^{-1}) soybean crop. In most cases, the removal of at least some nutrients is faster than replenishment by natural processes. In such situations nutrient reserves are exhausted and soils become deficient. Obviously, the higher the yield environment, the greater the demand for all nutrients. This can be especially important for the macronutrients nitrogen (N), phosphorus (P) and potassium (K) as they are needed in the greatest amounts. However, yields can be limited or even severely restricted via deficiencies in any of the other nutrients. Understanding the yield potential of an environment coupled with the nutrients required to achieve maximum yield is necessary in maintaining a fertility program. There is no economic advantage in fertilizing for a 3000 kg ha^{-1} or greater yield if other environmental/production factors restrict yield to a 1500 kg ha^{-1} potential (i.e. low rainfall and no irrigation). Conversely, fields consistently producing higher yields, require special attention to fertility due to the higher nutrient removal rates year after year. In some cases, over-abundance of mineral elements can be toxic and result in lower yields (e.g. Mn and Al toxicity).

Table 1. Nutrient concentrations considered adequate in plants and the range of concentrations found in crop plants. (Derived from Tables 3.3 and 3.4 of Epstein and Bloom, 2005).

Nutrient	Symbol	Dry Matter Concentration[†‡]		Range of Concentrations[†¶]
		μmol g^{-1}	mg kg^{-1}	mg kg^{-1}
Micronutirents				
Nickel	Ni	0.001	0.05	0.05-5
Molybdenum	Mo	0.001	0.1	0.1-10
Cobalt††	Co	0.002	0.1	0.05-10
Copper	Cu	0.1	6	2-50
Zinc	Zn	0.3	20	10-250
Sodium[‡‡]	Na	0.4	10	10-80,000
Manganese	Mn	1	50	10-600
Boron	B	2	20	0.2-800
Iron	Fe	2	100	20-600
Chlorine	Cl	3	100	10-80,000
Macronutirents				
Silicon[‡‡]	Si	30	1,000	1,000-100,000
Sulfur	S	30	1,000	1,000-15,000
Phosphorus	P	60	2,000	1,500-5,000
Magnesium	Mg	80	2,000	500-10,000
Calcium	Ca	125	5,000	1,000-60,000
Potassium	K	250	10,000	8,000-80,000
Nitrogen	N	1000	15,000	5,000-60,000
Oxygen	O	30,000	450,000	
Carbon	C	40,000	450,000	
Hydrogen	H	60,000	60,000	

† Values are based shoot material of mainly crop plants.

‡ Values represent the threshold concentration over which limitations are not likely experienced.

[¶] Range of values commonly found in crop plants on a dry weight basis. However, values vary greatly with species, genotype, soil, and environmental factors.

††Considered essential in biological nitrogen fixation.

[‡‡] Currently not considered an essential nutrient.

Managing soil fertility is critical to optimize soybean production and to maximize economic return. In this chapter we will focus on soybean specific information and provide an update on current soybean fertility management and research. We will examine the major nutrients in turn and then consider selected micronutrients. Next we will discuss fertility issues related to disease, insect and nematode pressure. Lastly, we will conclude by highlighting fertility issues for future research.

Table 2. Recently reported estimated nutrient content of soybean seed based on a 50 bu ac^{-1} (3359.5 kg ha^{-1}) seed yield[†].

Nutrient	Zublena (1991)	Franzen and Gerwing (1997)	Wiebold and Scharf (2000)	Rehm (2001)	Murrell (2005)	Average	Seed Concentration
	kg ha^{-1}						g kg^{-1}
Nitrogen (N)	210.6	210.6	235.2	235.2	212.8	220.9	65.7
Potassium (K_2O)	82.9	73.9	70.6	84.0	72.8	76.8	22.8
Phosphorus (P_2O_5)	45.9	49.3	22.4	50.4	47.0	43.0	12.8
Sulfur (S)	25.8	5.6	11.2	11.2		13.4	4.0
Calcium (Ca)	21.3	10.1	11.2	11.2	11.8	13.1	3.8
Magnesium (Mg)	11.2	10.1	12.3	12.9	10.1	11.3	3.3
Copper (Cu)	0.056					0.056	0.017
Manganese (Mn)	0.067					0.067	0.020
Zinc (Zn)	0.056					0.056	0.017

[†] Sources may have summarized previously reported research.

Soil Sampling and Soil Test Results

With well over 90 million ha worldwide, soybean production faces the full range of fertility problems. The first step in any soil fertility consideration is knowing the current status of nutrients on a field-to-field basis. Most commonly this information is gained through soil testing at a dedicated soil testing laboratory. Numerous public and private soil testing laboratories exist and they, along with extension agents and crop consultants, can assist with interpretation and recommendations. Nonetheless, it is critical that soil sampling for soil testing be conducted properly and that the results of the test are understood. In conjunction with testing, site-specific knowledge of the cropping and soil fertility history can be important in considering recommendations. This can include information such as crop rotation schemes, the application of chemicals such as agricultural lime that require years to fully modify soil properties and the banding of fertilizer which can affect soil sampling strategies. Details of soil testing and interpretation are well described elsewhere (Hergert, 2000a; Hoeft and Peck, 2002) and numerous web-based resources exist.

Soil pH

Soil pH is one of the most important considerations for soybean fertility for maximum production. Over a range of soil pH values nutrient availability can vary greatly. Nutrients may be essential at low amounts but toxic at higher levels. Additionally, for soybean, soil pH can influence nodulation and N_2-fixation. Figure 1 shows nutrient availability as related to a range of soil pH values (Hoeft and Peck, 2002). Clearly, nutrient availability is affected differentially by soil pH. Additionally, different crops have different optimum pH ranges and for soybean, a soil pH ranging between 6.0 and 6.5 is considered optimum (Vitosh et al., 1995). Vitosh et al. (1995) suggested that soil pH should be corrected when the pH of the sampling zone falls 0.2 or 0.3 pH units below the recommended level.

Soils naturally become more acidic with time and degree of weathering (Varco, 1999). Applications of lime are usually used to raise the soil pH. Ferguson et al. (2006) suggested that applications of lime are likely to be profitable on soils with a pH of 5.8 or less (0 to 20 cm depth) or on subsoils with a pH of 6.0 or less (to a depth of 60 cm or more). They also suggested that as lime is relatively insoluble, it may take from four to seven years for full payback. The amount of lime to apply to change the soil pH is affected by the sampling depth, lime particle size (i.e. fineness of grind), soil type (mineral or organic) and incorporation depth. Vitosh et al. (1995) provided resources for taking these factors into account in calculating lime applications. Additionally, Heatherly and Elmore (2004) suggested that as variation occurs naturally within and among soil series, site specific applications of lime based on spatial variability may be appropriate.

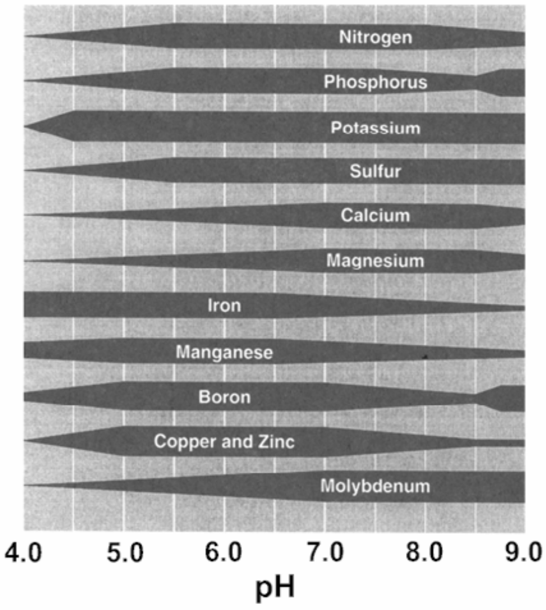

Figure 1. Plant nutrient availability as related to soil pH. Bar thickness indicates nutrient availability. (From Hoeft and Peck, 2002).

Nitrogen

Nitrogen is usually the most limiting nutrient in crop production, partly because large amounts are required by plants and partly because of its lack of durability in the soil environment. As indicated in Tables 1 and 2, N is the nutrient required in the greatest quantity for growth, and for soybean, the nutrient in the greatest quantity in the seed. Soybean seed has a high N content because it is approximately 40% protein (compared to corn at approximately 11% protein) and protein is approximately 16% N. Most commercial fertilizer N is produced through the Haber process of converting N_2 to ammonia ($N_2 + 3H_2$ --> $2NH_3$). However, this is an energy intensive and expensive process. The great advantage of soybean and one of the basic reasons for its dominance as the world’s source of protein, is its ability to assimilate N from the atmosphere through a symbiotic relationship with a bacteria (*Bradyrhizobium japonicum*) in a process termed dinitrogen (N_2) fixation.

The process of N_2-fixation is well-described elsewhere (see Postgate, 1998 for example). Briefly, it is the process by which atmospheric N_2 is reduced to NH_4^+ by the bacteroid form of *Bradyrhizobium* in the gall-like nodules that form on soybean roots following bacterial infection and colonization. These nodules provide a controlled, low-oxygen environment critical for the function of the nitrogenase enzyme complex responsible for nitrogen fixation. Additionally, the N_2-fixation process also creates a unique nutrient requirement not normally found in plants. Although not an essential element of plants, the nitrogen fixing symbionts in the nodules of soybean plants have an absolute requirement for cobalt (Co) as part of an enzyme complex, cobalamin, which functions in the bacteriods (Epstein and Bloom, 2005).

Virtually no information exists regarding Co-limited N_2-fixation in agricultural production of soybean.

Estimates vary but soybean obtains from 50 to 75 % of the required N from N_2-fixation (Hardarson et al., 1984; Bergersen et al., 1985; Matheny and Hunt, 1983; Unkovitch and Pate, 2000) and the rest from N in the soil (NH_4^+ and NO_3^-). However, the N_2-fixation process is expensive for the soybean plant with estimates usually ranging from about 8.0 to 12.0 g of carbohydrate required for each g of N_2 fixed (see review by Phillips, 1980 and Serraj et al., 1999). Nonetheless, it is the N_2-fixation process that provides the soybean plant with the necessary N to economically produce a high-protein content seed. Inoculation of soybean with *Bradyrhizobium* is generally not required for fields that have recently been cultivated with soybean and did not exhibit N deficiency symptoms. However, if a field is new to soybean production, inoculation is most likely required.

Numerous research studies have been conducted either supplementing N_2-fixation or attempting to supplant N_2-fixation with N fertilizer. Usually these research efforts are addressing two fundamental questions. The first is whether “starter” N is economically beneficial for early soybean vegetative growth until nodules form and become active. The second question is whether N_2-fixation provides enough N for maximum production or if supplemental N is required. Starter N studies are usually conducted with relatively low rates of N (15-35 kg N ha^{-1}) and have had decidedly mixed results. Most studies indicate that there is little or no response to low rates of starter N (Johnson, 1987; Varco, 1999; Hoeft et al., 2000; Heatherly et al., 2003; Scharf and Wiebold, 2003). However, there are conditions such as poorly drained soils, low organic matter soils, low residual soil nitrate, very low soil pH, or in situations where N is temporarily immobilized by soil microorganisms (i.e. decomposition of wheat straw) in which starter N may be beneficial (Johnson, 1987; Lamb et al., 1990; Whitney, 1997; Ferguson et al., 2000). In these problem soils, larger amounts of N (56-112 kg ha^{-1}) were used. Additionally, a study by Osborne and Riedell (2006) in which none of the above conditions seem to apply, found starter N as low as 16 kg N ha^{-1} resulted in a yield increase of 6% in 2 of 3 years, although they question whether this increase in yield was sufficient to offset the fertilizer cost. There are potential dangers in applying a starter N. Too high of a rate can potentially impede or delay nodulation and reduce nodule function (Harper and Gibson, 1984; Gibson and Harper, 1985). In general, if there is not a history of soil problems in the field, application of a starter N is an unnecessary expense (Varco 1999; Hoeft et al., 2000).

As with starter N, reports of the benefits of supplemental N are mixed. Additionally, the use of supplemental N is complex and confounded by timing considerations, rates and application methodologies. Barker and Sawyer (2005) applied two rates of N (45 and 90 kg N ha^{-1}) at beginning pod growth (R3; Fehr et al., 1971) in a two year, five-location study in Iowa. They found no positive effects on grain yield or grain quality and concluded that growers should not consider soil applications of fertilizer N during early reproductive stages. A similar two-year study in Minnesota with soil applied N at a rate of 84 kg ha^{-1} on plots at 12 locations reached the same conclusion (Schmitt et al., 2001). In Virginia, Freeborn et al. (2001) applied N to the soil at growth stages R3 or R5 at rates of 0, 14, 28, 56, 84, 112, or 168 kg ha^{-1} but saw no response. In contrast, some studies applying N after R3 have shown positive results (Wesley et al., 1998; Gascho, 1993; Afza et al., 1987). Additionally, N applied at planting using rates of from 60 to > 500 kg ha^{-1} has shown significant increases in yield (Sorensen and Penas, 1978; Purcell and King, 1996, Taylor et al., 2005; Ray et al., 2006). These studies also showed that yield increases from N applied at planting were associated with increased seed number m^{-2} whereas most studies with late-season applications of N showed no yield effect and no effect on seed number. Nitrogen applied after pod set would be too late to affect the processes determining seed number and therefore may not affect yield (except in soils with N problems). Ray et al. (2006) concluded that N from N_2-fixation may be limiting early in the season for maximum yield potential and that late-season applications of N may not overcome this limitation. Nonetheless, there are studies as cited above that do show a positive response to N applications after pod set. These conflicting results serve to highlight the complexity of making fertilizer recommendations given the tremendous range of site-specific factors that can affect fertility. In general we conclude that unless a field has a history of N_2-fixation problems or other special considerations (highly acidic soils for example) it is not likely to be economical to apply supplemental in-season N.

Phosphorus

As indicated in Table 2, P is removed in soybean seed in quantities surpassed only by K and N. Phosphorus is not considered a mobile element in the soil (Sander and Penas, 2000) but is mobile in the plant as P is translocated from vegetative plant parts to the developing seed (Karlen et al., 1982). Phosphorus is used in numerous molecular and biochemical plant processes, particularly in energy acquisition, storage and utilization (Epstein and Bloom, 2005). Phosphoric compounds are key constituents of DNA, RNA, and membranes. Nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine triphosphate (ATP) are key energy carriers and transducers in photosynthesis and the Calvin-Benson cycle. ATP is sometimes referred to as the “energy currency” of life (Epstein and Bloom, 2005) which also highlights the importance of P.

In soybean, P-deficiencies can have whole plant effects such as delayed flowering and maturity (Marschner, 1995; Sinclair, 1993) and may also be manifested as stunted growth and small leaflets (Sinclair, 1993). Phosphorus has also been shown to promote the number and weight of nodules and to increase pod number (Jones et al., 1977). Ferguson et al. (2006) recommended that soil P concentrations above 12 mg kg^{-1} (Bray-P1 test) would not result in a seed yield increase (Figure 2). Other reports indicate that when soil P concentrations are above 20 mg kg^{-1} soybean seed yield increases are not likely (deMooy et al., 1973a; Mallarino et al., 1991, Webb et al., 1992; Borges and Mallarino, 2003).

In a test at 14 locations in the Midwest USA using ridge tillage, Borges and Mallarino (2003) found no distinct advantage to deep-band placement P over broadcast P. Similar conclusions were reached by other studies for no-tillage soybeans (Buah et al., 2000; Borges and Mallarino, 2000). In contrast, Bullen et al. (1983) did find soybean seed yield increases in banded-P over broadcast-P. This inconsistency again serves to highlight possible site-

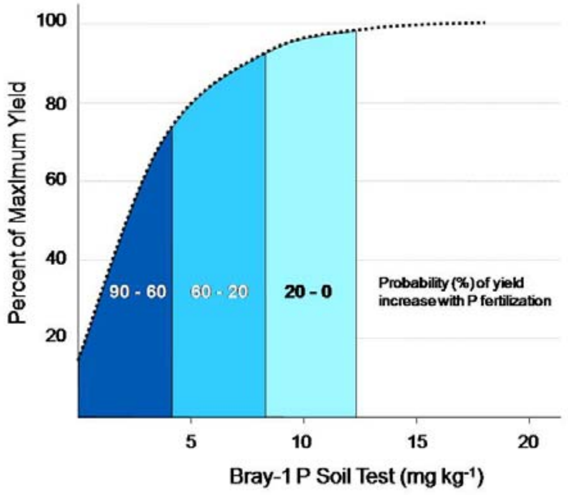

Figure 2. Probability of soybean yield increase at various values of soil P as determined by the Bray P-1 soil test. (From Ferguson et al., 2006.)

specific variation, for example Heatherly and Elmore (2004) recommended band application of P if soil P values are low. Furthermore, as Borges and Mallarino (2000) indicated, deep-banding P has the potential benefit of reducing surface water contamination. Phosphorus research also indicates that smaller, annual applications of P are more effective than a biennial (two-yearly) application in both conventional (deMooy et al., 1973a) and no-tillage soybean (Buah et al., 2000). Smaller applications also have the potential to reduce surface water P-contamination.

Potassium

After N, K is removed in the highest quantities in soybean seed (Table 2). Potassium is not an integral constituent of any metabolite but serves to activate numerous enzymes, serves as a counter ion and is the major cationic inorganic cellular osmoticum (Epstein and Bloom, 2005). Potassium deficiency in soybean initially appears as irregular yellowing and mottling around leaf margins of older leaves which then progresses to necrosis and downward cupping of leaf margins (Frank, 2000). Potassium is readily translocated from older to younger tissue and therefore deficiency symptoms begin in lower, older leaves and then move up the plant (Frank, 2000).

Soil K is dependent on the type and content of minerals and clay and is not associated to any great extent with soil organic matter (Frank, 2000). Plant roots obtain most of their K by diffusion from the soil solution which contains about 1 to 2% of the total K in the soil (Frank, 2000). Yield increases from K fertilization are only likely in soils below 90 to 130 mg K kg^{-1} as determined by the ammonium acetate method (Borges and Mallarino, 2000). In no-tillage soybean, placement studies have found from none or small yield benefit (Buah et al., 2000;

Borges and Mallarino, 2000; Yin and Vyn, 2002) to significant yield increases (Yin and Vyn, 2003) with banded K applications compared to broadcast applications. Similarly, in soybean ridge tillage systems inconsistent yield benefits were found for banded K compared to broadcast K. Likely K-fertilization methodology is determined by the economics of application methodology and environmental considerations.

Interestingly, various studies have reported K-fertilization effects on protein and oil concentrations. Yin and Vyn (2003) found that banded K increased seed oil concentration over broadcast K and both band and broadcast K decreased protein concentration, with banded K reducing protein concentration more than broadcast K. Similar K fertilization effects on protein and oil have also been reported by others (Gaydou and Arrivets, 1983; Sale and Campbell, 1986). However, the effects on protein and oil were relatively minor and the overall yield increase as a result of K fertilization was probably more important. Nonetheless, this does indicate the influence of K on seed protein and oil synthesis pathways. This is not surprising since, among other things, K is known to be important for N transport to the site of proteins synthesis in seeds and for charge balance of acidic amino acids which are often abundant in seed storage proteins (Blevins, 1985).

Sulfur, Calcium and Magnesium

Sulfur (S), calcium (Ca) and magnesium (Mg) are sometimes referred to as “secondary” macronutrients because requirements are substantially less that N, P, K but requirements are still hugely more than for the micronutrients. All three elements are required in about the same quantities in soybean seed (Table 2). Sulfur is an integral component of carbon compounds and other biochemical entities, particularly certain amino acids (i.e cysteine and methionine) and coenzymes (Epstein and Bloom, 2005). Calcium is involved in the regulation of many enzymes, is structurally associated with cell walls and plays a major role in environmental signal transduction associated with abiotic and biotic stress (Epstein and Bloom, 2005). Magnesium is a component of chlorophyll and activates more enzymes than does any other element (Epstein and Bloom, 2005). All three elements in the form of SO_4^{2-}, Ca^{2+}, and Mg^{2+} serve as counter ions (Epstein and Bloom, 2005).

Plant residues and soil organic matter contain the vast majority of S in the soil (Ferguson et al., 2006). Soybean S-deficiency symptoms appear as a light green to yellowish color similar to the yellowing of leaves observed in N-deficient plants, but the yellowing occurs in newer growth rather than older leaves (Varco, 1999; Hergert, 2000b). Yield responses have been seen in soils with available S levels of 4 mg kg^{-1} or less (Kamprath and Jones, 1986). Most S-deficiencies occur in sandy soils, but adding S to sandy soil will not necessarily increase yield (Hergert, 2000b). Organic matter content is an important consideration in evaluating S-deficiencies. Soil tests for S may not be accurate except on sandy soils (Hergert, 2000b) and plant tissue analysis as a diagnostic tool is recommended (Vitosh et al., 1995). Hitsuda et al. (2004) suggest diagnosing S-deficiency in soybean seed as a more accurate, faster, and easier methodology. The seed S analysis results would be used as a guideline for the next season’s fertility program for S. Note that if elemental S fertilizer is used, it should be applied at least two months before planting to allow mineralization to the plant-available sulfate form to occur; otherwise a sulfate fertilizer should be used (Vitosh et al., 1995). Irrigation of soybean is an increasingly common practice, especially for maximum soybean production. In some areas irrigation water is an important source of S in the form of sulfate-S (Hergert, 2000b). However, this is highly dependent on local conditions.

Unlike S, standard soil tests provide reliable measures for Ca and Mg availability. Commonly reported sufficient levels for crop growth are exchangeable Ca > 200 mg kg^{-1} and exchangeable Mg > 50 mg kg^{-1} (Vitosh et al., 1995). Soybean has a higher requirement for Ca than corn or sorghum and a constant supply is needed throughout the growing season because Ca is not readily translocated within the plant (Marschner, 1995). Additionally, Ca and Mg uptake can be greatly reduced by excessive K fertilizers (Vitosh et al., 1995).

Micronutrients

Micronutrients are required in extremely small quantities (Table 1). Nonetheless, when the nutrient concentration drops below a certain optimal level, plant growth and/or development can be negatively impacted. In fact, micronutrient deficiencies can have dramatic effects on crop plants and severely reduce yield. Conversely, high concentrations of micronutrients can be toxic to plants. Micronutrients are essential constituents of enzymes and other metabolic entities (Cu, Fe, Mn, Mo, Ni, and Zn), can activate or control enzymatic activity (Cl, Cu, Fe, Mn, and Zn), serve as a cellular osmotica or counter-ion (Cl^-) and in the case of B, be structurally associated with the cell wall (Epstein and Bloom, 2005). As summarized in Table 3, micronutrients are involved in a wide range of critical biochemical and physiological plant processes.

Specific micronutrient deficiencies can occur either because of inadequate quantities in the soil or unavailability due to soil properties (very sandy soils, low organic matter soils, out-of-range pH, excess lime or salts, poor drainage, and/or soil compaction). In some cases, micronutrient deficiencies on problem soils can be overcome by planting tolerant soybean cultivars. Using tolerant cultivars is usually the best option, for example, Goos and Johnson (2000) tested three methods of reducing Fe-deficiency chlorosis in soybean and concuded the most practical control measure was cultivar selection. Nonetheless, foliar applications of some micronutrients (notably Fe) may be required to overcome immediate problems.

Deficiency symptoms are well characterized in production literature and on state extension web sites, most often accompanied by excellent photographic documentation (for examples see Hoeft et al., 2000; Sawyer, 2004; Potash and Phosphate Institute, 2007). In the USA, Fe and Mn deficiencies are the most common soybean micronutrient deficiencies (Adams et al., 2000a). As with S, in some cases soil analysis is not sufficient for generating fertilizer recommendations and tissue analysis must be conducted. Some success in using non-distructive fluorescence and reflectance measurements has been demonstrated for Mn, Zn, Fe, and Cu (Adams et al., 2000a and b) although traditional plant tissue analysis remains the standard.

Table 3. Primary biochemical/physiological functions of micronutrients.

Nutrient	Primary Functional Area	Key Enzymes/Complexes
Boron (B)	Cross-link two molecules of a cell-wall polysaccharide (pectin) Other roles unclear	Cell wall structure
Chlorine (Cl)	Enzyme cofactor Oxygen evolution Cellular osmoticum	Photosystem II Activate tonoplast proton pumps Guard cells of stomata
Cobalt (Co)	Biological nitrogen fixation specific to bacteriods	cobalamin
Copper (Cu)	Enzyme cofactor Protein component Electron transport Detoxify oxidants	Photosystem I Mitochondrial electron transport Superoxide dismutase
Iron (Fe)	Enzyme cofactor Protein component Detoxify oxidants Electron transport Nitrogen metabolism Sulfur metabolism	Mitochondrial electron transport Photosystem I Cytochrome Nitrogenase
Manganese (Mn)	Enzyme cofactor Protein component (two) Oxygen evolution Detoxify oxidants	Photosystem II Superoxide dismutase Allantoate amidohydrolase
Molybdenum (Mo)	Nitrogen metabolism Protein component	Nitrate reductase Nitrogenase Xanthine oxidase/dehydrogenase
Nickel (Ni)	Nitrogen metabolism Protein component (one enzyme)	Urease Hydrogenase
Sodium (Na)	Essential in some C4 Photosynthesis (NAD-malic enzyme type) Cellular osmoticum	role unclear
Zinc (Zn)	Required at the active site of many enzymes Enzyme cofactor Protein component (many)	DNA transcription (Zinc fingers) >80 Zn containing proteins

It is not only deficiencies that can affect micronutrient status in soybean. For example, high Mn concentrations can interfere with the uptake of Fe and induce Fe-chlorosis symptoms (Roomizadeh and Karimian, 1996). Additionally, if micronutrient applications are recommended based on soil tests, the irrigation water should be tested before applying micronutrients as adequate levels may be applied in the irrigation water (Penas and Ferguson, 2000). Often soybean is grown in rotation with corn and in many situations a well-fertilized corn crop provides all the nutrients needed for soybean production and this may be especially true for micronutrients. Care must be taken with application of micronutrients because what is optimal for one crop may be toxic to another crop. For example, Penas and Ferguson (2000) state that the optimum level of B for sugar beets may depress the yield of oats. Certainly, with many micronutrients there may be a narrow range that defines deficiency and toxicity. Additionally, in heavily leached, acidic tropical soils, metals such as Al^{3+} and Mn^{2+} can be problematic for soybean production.

Influence of Nutrient Status and Fertilization on Pests and Diseases

More than 100 soybean pathogens of varying importance to particular production regions have been identified (Hartman et al., 1999). While information available for some of these diseases is extensive, comparatively little is known with regard to their relationships with soybean mineral nutrition. Studies examining the influence of fertilization and mineral nutrition on plant growth and yield by far outnumber those exploring the effects on pests and diseases. However, it is clear that the nutritional status of a plant influences its interaction with other organisms. Mineral nutrients not only influence the chemical composition of a plant but also affect its morphology and anatomy and in turn raise or reduce the sensitivity to pest and/or pathogen attack (Marschner, 1995). In general, adequate nutrition resulting in vigorous plants reduces the severity of the impact of pest or disease attack. However, the dynamics of plant-pest and plant-disease interactions are often very specific and need to be examined on a case by case basis.

The impact of a fertilizer application on plant-pathogen/pest interactions may be due to its influence on the overall plant nutritional status, the function of individual mineral nutrients in plant metabolism, through a direct effect on the pathogen/pest, or may be a function of indirect effects such as through the impact on soil microbial dynamics. The outcome will also depend on the timing of application relative to plant and pathogen developmental stages as well as the stage of disease development. In addition, the method of application with regard to placement (e.g. broadcast, band, foliar etc.) will modulate its impact on disease or pest development. A number of these aspects will be highlighted in the following paragraphs. The information given in this section is not intended to be comprehensive but rather to illustrate the importance of mineral nutrients with regard to plant-pathogen/pest interactions. At the same time, it is clear how little information is available on the effects of soybean nutritional status and fertilization on pest and disease dynamics, particularly in regard to the mechanisms underlying the documented observations.

Phytophthora Rot (*Phytophtora sojae* (Mart.) Sacc. f. sp. *glycines*)

Phytophthora root and stem rot is a serious problem in many soybean-producing regions around the world. For instance, for the period of 1996 through 1998, Phytophthora was the second most important biotic stress factor limiting soybean yield in the northern soybean producing states of the USA (Wrather et al., 2001a and b). Worldwide annual losses to soybean caused by *P. sojae* have been estimated at $1-2 billion annually (Tyler, 2007). Disease resistance genes, at least 14 of which have been identified, are important targets for breeders and are effective disease management tools (Grau et al., 2004; Tyler, 2007). Cultivars differing in tolerance to the disease in a race-independent manner also exist. The losses caused by *P. sojae* are influenced by numerous factors including genotype susceptibility and environmental factors such as soils that are prone to water logging. Management practices including tillage, improved drainage, crop rotation and timing of planting are important components of an integrated approach to control the disease and should go hand in hand with the selection of resistant or tolerant cultivars (Schmitthenner, 1999).

The relationship of soybean mineral nutrition and Phythophthora rot is not well understood. However, Dirks et al. (1980) reported a positive relationship of disease severity with increased level of fertility. They observed a linear relationship of the number of plants killed by Phytophthora rot and the amount of N-P-K fertilizer applied. While the experimental design did not allow the separation of the influence of the individual mineral elements, based on other reports, the authors speculated that N may have played a critical role. However, in a 3-yr field study, Pacumbaba et al. (1997) found that plants fertilized with K (muriate of potash) had a greater incidence of Phytophthora rot than those fertilized with P (superphosphate), and fertilization with N, P, and K (20-20-20) resulted in the lowest disease incidence. Similarly, Schmitthenner (1999) indicated that application of large amounts of KCl prior to planting may increase damage. Xu and Morris (1998) demonstrated that application of Ca influences the developmental progress of *P. sojae* under controlled conditions and suggested that Ca fertilization under field conditions may reduce disease incidence by hampering zoospore development and release. Sugimoto et al. (2005) reported a reduction in Phytophthora rot as a result of Ca application. In their *in vitro* study, these authors applied increasing concentrations of $CaCl_2$ and $Ca(NO_3)_2$ to the agar medium and monitored the disease severity on two soybean cultivars. Disease incidence decreased in both cultivars in response to both Ca sources, but $Ca(NO_3)_2$ was more effective. The two cultivars were found to differ with regard to calcium uptake and disease reduction was related to increased tissue Ca concentrations. In addition, Sugimoto et al. (2005) observed that high concentrations of Ca reduced the release of zoospores from *P. sojae* isolates grown on agar and suggested that disease reduction could be a combination of the effect of Ca on the plant tissue and a direct effect on pathogen growth.

Sudden Death Syndrome (*Fusarium solani* (Mart.) Sacc. f. sp. *glycines*)

Sudden death syndrome (SDS) is caused by a slow growing soil-borne fungus that can cause significant yield losses (Hartman et al., 1995; Rupe and Hartman, 1999; Scherm and Yang, 1996). In fact, yield reductions in the USA due to SDS were estimated to be approximately 1.3 million tons during the three-year period of 1996-1998 (Wrather et al., 2001b).

In 1993, Rupe et al. reported on a study that examined the relationship of numerous plant nutrients and the severity of SDS in Arkansas. Results from five different multiple regression equations indicated that increased levels of exchangeable soil Na, Ca, and Mg, were associated significantly with increased disease severity in two or three of the five models. However, increased P and soluble salt levels were consistently associated with increased SDS severity across all five multiple regression equations. Based on these results, they concluded that SDS appeared to be favored by increased soil fertility. Scherm et al. (1998) conducted a field survey to investigate the relationship between SDS occurrence and various soil characteristics in Iowa. They reported that increased available K was positively correlated with increased disease severity in four out of nine commercial soybean fields and negatively correlated in one out of the nine fields. Further analyses indicated that the relationship of available K and disease severity was influenced both in magnitude and sign by the overall soil K concentration. As the overall soil K concentration increased, the magnitude of the positive correlation coefficient decreased. Thus, they concluded that nutrient management would only be of limited utility to reduce SDS in Iowa. Information to date appears to consistently indicate that favorable production environments enhance SDS and that the presence of soybean cyst nematodes results in more rapid disease progress and greater severity (McLean and Lawrence, 1993a and b; Melgar et al., 1994; Roy et al., 1989; Roy et al., 1997; Rupe et al., 1993; Rupe et al., 2000; Scherm et al., 1998).

Field investigations into the effect of elevated Cl levels did not influence SDS severity in three out of four cultivars (Rupe et al., 2000). However, in an SDS susceptible cultivar that limits Cl translocation to the shoot (Hartz 6686), elevated levels of soil Cl increased SDS severity. Because the soil K concentrations were at recommended levels, the authors attributed the observed effect to the presence of high concentrations of Cl and not K, even though the treatments were established using KCl. Interestingly, Sanogo and Yang (2001) found that the application of KCl to potted soybean plants reduced SDS severity by 36% while K_2SO_4 and KNO_3 increased disease severity compared to the control. In addition, regardless of the source, P application increased the severity of SDS in that study. Results reported by Howard et al. (1992) were consistent with those of Sanogo and Yang (2001) in that they also observed reduced SDS severity and increased SDS severity in response to KCl and K_2SO_4 application, respectively.

Other Diseases

Jeffers et al. (1982) investigated the effect of K fertilization on *Phomopsis* sp. and *Diaporthe phaseolorum* (Cke. and Ell.) Sacc. incidence and found no response except in one out of seven site years where a reduction in *Phomopsis* with increased K fertilization was observed. In contrast, Sij et al. (1985) found that increased K fertilization reduced *Phomopsis* sp. and suggested that P fertilization enhanced *Phomopsis* development. Higher levels of *Phomopsis* were observed on seed from lower as compared to upper nodes by a number of researchers including Jeffers et al. (1982) and Thomison et al (1987). Pod wall concentrations of N, P, and K were positively related and pod wall Ca concentration was negatively related to *Phomopsis* incidence on upper and lower nodes (Thomison et al., 1987). Thus, these authors suggested that reducing N and P concentrations and increasing Ca concentrations in pod walls may result in reduced *Phomopsis* seed infection. Earlier, Crittenden and Svec (1974) found that the incidence of *Diaporthe sojae* decreased as the application of K, both in the form of KCl and K_2SO_4, increased. While the most dramatic reduction of *Diaporthe sojae* infection was found at K application rates exceeding conventional application rates, even applications of less than 100 kg K ha^{-1} reduced infection.

In recent years the incidence of Asian soybean rust (*Phakopsora pachyrhizi* Sydow) has spread to soybean production regions in Africa and South America where it has become endemic. The disease was first observed in the USA in Louisiana in the fall of 2004 (Schneider et al., 2005). With the recent spread of the disease, research efforts have grown exponentially. Efforts have been focused on gaining a better understanding of the pathogen, genetic improvement of soybean genotypes, and control of the disease by chemical methods. However, studies on the relationship of mineral nutrition and Asian soybean rust have also been initiated and are ongoing at several universities in the USA (Fixen, 2007). Meanwhile, Balardin et al. (2006) reported that increased application of P and K reduced Asian soybean rust severity and disease progress in two soybean cultivars examined in a pot experiment. In addition, observational evidence also indicates that KCl application may reduce Asian soybean rust (Fixen et al., 2005).

Soil applications of calcium silicate and calcium carbonate did not reduce the incidence of Asian soybean rust in a pot experiment (Nolla et al., 2006). Similarly, calcium carbonate did not influence downey mildew (*Peronospora manshurica*) and frogeye leaf spot (*Cercospora sojina*) incidence. However, calcium silicate effectively reduced the incidence of downy mildew at 47 and 66 d but not 79 d after sowing and reduced frogeye leaf spot at all three dates after sowing (Nolla et al., 2006). In fact, frogeye leaf spot was negatively related to Si concentration in soybean leaves. In contrast to the study by Nolla et al. (2006) which indicates the benefit of silicate application resulting in increased leaf tissue Si concentration, Muchovej and Muchovej (1982) found a suppression of sclerotium-induced twin stem abnormality as a result of soil applied calcium carbonate. The application of calcium carbonate was correlated with increased tissue Ca levels, which in turn were significantly associated with reduced twin stem symptom development (Muchovej and Muchovej, 1982). In contrast, these authors found that magnesium carbonate application enhanced twin stem occurrence which they attributed to an inhibitory effect of Mg on Ca uptake.

Soybean Aphid (*Aphis glycines* Matsumura)

As opposed to the USA where the soybean aphid is an invasive species and was first discovered in 2000, it is native to Asia and has been a common pest for many years (Venette and Ragsdale, 2004; Wang et al., 1962). Soybean aphid infestations can result in a reduction in photosynthetic capacity, significant yield losses as a result of feeding damage, and can lead to losses associated with the transmission of viruses (Clark and Perry, 2002; Macedo et al., 2003; Myers et al., 2005). Yield differences between sprayed and unsprayed treatments reported by Myers et al. (2005) were about 20%. They also found significant yield differences between high K treatments and low K treatments. From a laboratory experiment using K-deficient and healthy soybean leaves, Myers et al. (2005) reported greater fecundity and survivorship of aphids feeding on K-deficient leaves. However, in their field treatments, they did not observe greater populations on K-stressed soybean plants and speculated that this may have been due to the small field plots and close proximity of the different treatments. A broad field survey including 34 production fields in Wisconsin revealed that soybean aphid population growth rates were negatively, but peak aphid densities positively, correlated with leaf tissue K, N, P, and S (Myers and Gratton, 2006). In low, medium, and high K treatments established by differential KCl applications, naturally occurring field populations had greater rates of increase and peak abundance, and aphids in clip cages had greater net reproductive rate in the low K treatment than in the medium and high K treatments. Walter and DiFonzo (2007) reported a significant increase in the asparagine proportion of the phloem with a decrease in soil K level and suggested a causal relationship with aphid population dynamics. Potassium has many functions in plants and has long been known to be involved in plant N metabolism (Marschner, 1995). Potassium deficiency often results in lower levels of starch and sucrose and increased levels of glucose and fructose as well as amino acids and amides (Evans and Sorge, 1966). For instance, K deficiency has been shown to increase asparagine concentration in soybean (Yamada et al., 2002) and is generally reflected in the accumulation of soluble N compounds in plants (Marschner, 1995). While much is to be learned about the interaction of soybean plants and aphids and the influence of the plant K status, current evidence indicates that K deficiency can not only directly cause a yield reduction but may exacerbate the losses by causing changes in plant N composition that favor aphid development and outbreak. Thus, soybean growers are well advised to carefully manage soil K fertility not only to avoid yield loss directly attributable to K function in the plant, but also to reduce the likelihood of yield reductions as a result of aphid infestation.

Other Insects

Nutrient status can influence the susceptibility of a plant to insect attack as well as its response to an attack. Nitrogen is particularly critical since N concentrations of insects and mites are generally greater than that of plants which varies greatly due to factors such as tissue type and age (Dale, 1988). For instance, reduced soybean leaf N concentration resulted in greater consumption, increased number of stadia, and increased duration of larval development of soybean looper (*Pseudoplusia includens* Walker) (Wier and Boethel, 1995).

However, N fertilization not only affects aboveground but also belowground soybean-insect interactions as demonstrated for immature stages of bean leaf beetle (*Cerotoma trifurcata* Förster) (Riedell et al., 2005). Increased P fertilization was reported to increase the density of larval velvetbean caterpillars (*Anticarsia gemmatalis*) in both years of a two-year field study in Florida (Funderburk et al., 1991). However, increased K fertility only resulted in greater velvetbean caterpillar density in one year (1987). Increased P fertility resulted in a greater density of nymphal southern green stink bugs (*Nezara viridula*) in 1986, but no effects of K and Mg fertility were reported in either of the two years. Funderburk et al. (1994) reported mixed effects of fertility level on insect predators. While high P fertility significantly increased the density of bigeyed bug nymphs (*Geocoris* spp.) in 1986 and 1987, it favored damsel bug nymphs (*Nabis* and *Hoplisotoscelis* spp.) and spiders (Araneae) only in 1986. However, Funderburk et al. (1994) concluded that the effects of the fertility treatments on velvetbean caterpillars and southern green stink bugs reported in 1991 (Funderburk et al.) were not the result of treatment effects on insect predators. In fact, positive correlations were reported between population densities of bigeyed bugs and damsel bugs with velvetbean caterpillars and southern green stink bugs in 1986 and 1987. Potassium and Mg fertility level did not affect damsel bug nymphs, bigeyed bug nymphs, or spiders (Funderburk et al., 1994).

These studies and others conducted on a wide range of plant and insect species combinations indicate the complexity of plant-herbivore interactions with regard to mineral nutrition. Busch and Phelan (1999) and Beanland et al. (2003) demonstrated that nutrient ratios of both macronutrients and micronutrients are important components of plant-insect interactions. Soybean looper and two-spotted spider mites (*Tetranychus urticae*) responded differentially to different combinations of N, S, and P proportions in the nutrient solution (Busch and Phelan, 1999). Similarly, the effects of various proportions of B, Zn, and Fe influenced the response of soybean looper, Mexican bean beetle (*Epilachna varivestis* Mulsant), and velevetbean caterpillar. While soybean plants performed poorly in the absence of B in the nutrient solution, all three insects performed best in those treatments (Beanland et al., 2003). These authors suggested that accumulation of soluble sugars and amino acids, the inhibition of lignin synthesis and cell wall reinforcement, and the modulation of small phenolic compounds in B-deficient plants could contribute to better performance of these three insects on leaves from soybean grown with B-free nutrient solution.

Soybean cyst nematode (*Heterodera glycines* Ichinohe)

Soybean cyst nematodes (SCN) remain one of the economically most important pests of soybean even though a number of effective management practices are available to producers (Niblack, 1999; Schmitt, 2004, Schmitt et al., 2004). Cultivars resistant to SCN have been and continue to be developed and are the backbone of current management strategies (Shannon et al., 2004). Rotation of soybean with non-host crops also is an effective commonly employed practice to manage SCN pressure. In light of a number of promising management strategies, it is not surprising that comparatively little effort has been expended to examine the effect of fertilizer application on SCN. However, SCN infection influences a broad range of plant nutrient dynamics including root function, root nutrient concentration, and nutrient translocation, and causes nutrient deficiency symptoms (Blevins et al., 1995; Smith et al., 2001).

Soybean cyst nematode population densities vary within and among fields (Donald et al., 1999; Avendaño et al., 2003). The infestation of SCN is affected by various factors, including soil chemical characteristics. For instance, Avendaño et al. (2004) found significant within-field correlation between spatial variation of soil pH and SCN and Ca and SCN. Similarly, Rogovska et al. (2007) reported that pH and calcium carbonate equivalent influenced SCN population densities. Pacumbaba et al. (1997) studied the influence of five application rates each of NPK, ammonium nitrate, superphosphate, and muriate of potash fertilizer applications on a number of diseases and SCN in northern Alabama over a four year period. They did not find an effect of any of the treatments on the number of SCN females. However, Rupe et al. (2000) manipulated soil Cl levels by application of KCl and observed an increase in soil SCN egg densities with increased soil Cl levels in plots planted with SCN susceptible cultivars at R2 but not at harvest. In a 3 year study in an SCN infested field, Hanson et al. (1988) found that application of KCl did not increase the yield of soybeans on a soil with a moderate K level. Similarly, they did not observe significant effects of K treatment on soybean cyst nematode counts. In contrast, on a soil with low available K, Shannon et al. (1977) reported that K fertilizer application beyond the level recommended for non-SCN-infested fields may be of benefit. Spatial distribution pattern of SCN population densities within fields make field-scale studies challenging and existing studies without spatial data difficult to interpret. The scarcity of information on the relationship of mineral nutrition and SCN in combination with the complexity of plant nutrient uptake, translocation, and metabolism in response to SCN infection warrant increased research efforts in this area.

Soybean Fertility Issues

Although there is a wealth of information about soybean fertility and excellent resources are available to both scientists and producers, not all issues have been resolved or adequately researched. Table 4 shows a list of topics we believe to require more research. These range from agronomic related production issues such as a greater understanding of the effects of irrigation water pH and composition on production to understanding plant nutrient status at the molecular level. Indications are that plant nutrition has an important influence on host-pathogen / host-pest interactions, but very little soybean specific information is available. Understanding soybean nutrition at the molecular level has the potential to allow the modification of soybean seed to target specific nutrient compositions in feed or in the many industrial uses of soybean. Additionally, there are environmental/production issues to consider. For example, band applications of fertilizer may or may not offer yield advantages, but may offer environmental benefits through reduced runoff water contamination. Lastly, deMooy et al., (1973b) concluded their detailed “Mineral Nutrition” chapter in the ASA soybean monograph 16 by discussing genetic variation and the utility of adapted cultivars. Soybean is now an even more important world-crop grown on far more hectarage and far more fertility-diverse soils than 1973. Developing cultivars that can positively respond to increased fertility management or mitigate fertility problems remains an important goal. Faster, more accurate measurements of plant nutrient status would help with screening large numbers of soybean germplasm and breeding lines.

Table 4. Soybean fertility issues in need of further research.

Production / systems level:
- Irrigation water mineral content and pH
- Crop rotation effects on soybean nutrition
    - rice
    - corn
    - wheat
- Environmentally appropriate nutrient application
- Cobalt requirements/limitations for N_2-Fixation

Plant stress responses:
- Biotic stresses:
    - disease-mineral nutrition interactions
    - insect-mineral nutrition interactions
    - nematode-mineral nutrition interactions
- Abiotic stresses:
    - drought-mineral nutrition interactions
    - water logging-mineral nutrition interactions
    - temperature-mineral nutrition interactions

Molecular / biochemical:
- Fundamental understanding of pathways and mechanisms
- Nutrient composition for targeted feed or industrial uses

Genetic resources / germplasm:
- Developing germplasm to mitigate soil fertility problems
- Developing germplasm to take advantage of better fertility management

Nutrient status technology:
- Faster, cheaper, more accurate measurements of plant nutrient status
- Site-specific nutrient management based on spatial variability of soil characteristics

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Chapter III

Property of Biodegraded Fish-Meal Wastewater as a Liquid-Fertilizer

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Introduction

The amount of fisheries waste generated in Korea is expected to increase with a steady increase in population to enjoy taste of slices of raw fish. The fisheries waste is reduced and reutilized through the fish meal production. The process, which uses fish wastes such as heads, bones or other residues, is the commonest used in the Korean industries. The first step of the fish-meal manufacturing processes is the compression and crushing of the raw material, which is then cooked with steam, and the liquid effluent is filtered off in a filter press. The liquid stream contains oils and a high content of organic suspended solids. After oil separation, the fish-meal wastewater (FMW) is generated and shipped to wastewater treatment place. FMW has been customarily disposed of by dumping into the sea, since direct discharge of FMW can cause serious environmental problems. Besides, bad smell, which is produced during fish-meal manufacturing processes, causes civil petition. Stricter regulations for this problem also come into force every year in Korea. Therefore, there is an urge to seek for an effective treatment to remove the organic load from the FMW; otherwise the fish meal factories will be forced to shut down.

Biological treatment technologies of fish-processing wastewater have been studied to improve effluent quality (Battistoni and Fava, 1995; Park et al., 2001). The common feature of the wastewaters from fish processing is their diluted protein content, which after concentration by a suitable method would enable the recovery and reuse of this valuable raw material, either by direct recycling to the process or subsequent use in animal feed, human food, seasoning, etc. (Afonso and Borquez, 2002). It has been also reported that the organic wastes contain compounds, which are capable of promoting plant growth (Day and Katterman, 1992), and seafood processing wastewaters do not contain known toxic or carcinogenic materials unlike other types of municipal and industrial effluents (Afonso and Borquez, 2002). Although these studies imply that FMW could be a valuable resource for agriculture, potential utilization of this fish wastes has been limited because of its bad smell (Martin, 1999). There is an increasing need to find ecologically acceptable alternatives to overcome this problem.

Aerobic biodegradation has been widely used in treatment of wastewaters, and recently references to the use of meso- and thermophilic microorganisms have become increasingly frequent (Cibis et al., 2006). During the biodegradation, the organic matter is biodegraded mainly through exothermic aerobic reactions, producing carbon dioxide, water, mineral salts, and a stable and humified organic material (Ferrer et al., 2001). There have been few reports that presented the reutilization of biodegraded waste products as liquid-fertilizer: a waste product of alcoholic fermentation of sugar beet (Agaur and Kadioglu, 1992), diluted manure streams after biological treatment (Kalyuzhnyi et al., 1999), and biodegraded fish-meal wastewater in our previous studies (Kim et al, 2007; Kim and Lee, 2008). Therefore, aerobic biodegradation is considered to be the most suitable alternative to treat FMW and realize a market for such a waste as a fertilizer.

The growth of plants and their quality are mainly a function of the quantity of fertilizer and water. So it is very important to improve the utilization of water resources and fertilizer nutrients. The influence of organic matter on soil biological and physical fertility is well known. Organic matter affects crop growth and yield either directly by supplying nutrients or indirectly by modifying soil physical properties such as stability of aggregates and porosity that can improve the root environment and stimulate plant growth (Darwish et al., 1995). Incorporation of organic matter has been shown to improve soil properties such as aggregation, water-holding capacity, hydraulic conductivity, bulk density, the degree of compaction, fertility and resistance to water and wind erosion (Carter and Stewart, 1996; Franzluebbers, 2002; Zebarth et al., 1999). Combined use of organic and inorganic sources of nutrients is essential to maintain soil health and to augment the efficiency of nutrients (Lian, 1994). Three primary nutrients in fertilizers are nitrogen, phosphate, and potassium. According to Perrenoud’s report (1990), most authors agree that N generally increases crop susceptibility to pests and diseases, and P and K tend to improve plant health. It has been reported that tomato is a heavy feeder of NPK (Hebbar et al., 2004) and total nitrogen content is high in leaves in plants having a high occurrence of bitter fruits (Kano et al., 2001). Phosphorus is one of the most essential macronutrients (N, P, K, Ca, Mg, S) required for the growth of plants, and the deficiency of phosphorus will restrict plant growth in soil (Son et al., 2006). However, the excessive fertilization with chemically synthesized phosphate fertilizers has caused severe accumulation of insoluble phosphate compounds in farming soil (Omar, 1998), which gradually deteriorates the quality as well as the pH of soil. Different fertilization treatments of a long-term field experiment can cause soil macronutrients and their available concentrations to change, which in turn affects soil micronutrient (Cu, Fe, Mn, Zn) levels. Application of appropriate rates of N, P and K fertilizers has been reported to be able to increase soil Cu, Zn and Mn availabilities and the concentrations of Cu, Zn, Fe and Mn in wheat (Li, et al., 2007). It has been also reported that higher rates of fertilizers suppress microbial respiration (Thirukkumaran and Parkinson, 2000) and dehydrogenase activity (Simek et al., 1999). Recently, greater emphasis has been placed on the proper handling and application of agricultural fertilizers in order to increase crop yield, reduce costs and minimize environmental pollution (Allaire and Parent, 2004; Tomaszewska and Jarosiewicz, 2006).

Hydroponics is a plant culture technique, which enables plant growth in a nutrient solution with the mechanical support of inert substrata (Nhut et al., 2006). Hydroponic culture systems provide a convenient means of studying plant uptake of nutrients free of confounding and uncontrollable changes in soil nutrient supply to the roots. Thus, it is fit for test of fertilizing ability of liquid fertilizers. The technique was developed from experiments carried out to determine what substances make plants grow and plant composition (Howard, 1993). Water culture was one of the earliest methods of hydroponics used both in laboratory experiments and in commercial crop production. Nowadays, hydroponics is considered as a promising technique not only for plant physiology experiments but also for commercial production (Nhut et al., 2004; Resh, 1993). The technique has been also adapted to many situations, from field and indoor greenhouse culture to highly specialized culture in atomic submarines to grow fresh vegetables for the crew (Nhut et al., 2004). Hydroponics provides numerous advantages: no need for soil sterilization, high yields, good quality, precise and complete control of nutrition and diseases, shorter length of cultivation time, safety for the environment and special utility in non-arable regions. Application of this culture technique can be considered as an alternative approach for large-scale production of some desired and valuable crops.

Prevention of slowing down deteriorative processes is required after a liquid fertilizer was produced by aerobic biodegradation in order to maintain its quality during the period of circulation in market. Generally, the lower the pH, the less the chance that microbes will grow and cause spoilage. It has been known that organic acids can lower the pH and have a bacteriostatic effect (Zhuang et al., 1996), although a number of other methods have also reported for microbial control (Agarwal et al., 1986; Curran et al., 1990; Stratham and Bremner, 1989). A means of a more long-term preservation of the liquid fertilizer is required for a higher additional value.

In this study, a large-scale biodegradation was successfully carried out for three days in a 1-ton reactor using the original FMW, and properties of the biodegraded FMW, such as phytotoxicity, amino-acid composition and its change during long-term preservation, concentrations of major and noxious components, and fertilizing ability on hydroponic culture plants were determined to examine the suitability of the biodegraded FMW as a fertilizer.

Experimental

Microorganisms and Media

The potential aerobically-degrading bacteria used in this study were isolated from commercial good-quality humus and from compost and leachate collected at three different sites of composting plants. In order to select potential microorganisms, all isolates were spread on four different agar plates: 1% skim milk agar for detection of proteolytic microorganisms; 3.215% spirit blue agar for detection of lipolytic microorganisms; and starch hydrolysis agar (5 $g \cdot l^{-1}$ of beef extract, 20 $g \cdot l^{-1}$ of soluble starch, 10 $g \cdot l^{-1}$ of tryptose, 5 $g \cdot l^{-1}$ of NaCl, and 15 $g \cdot l^{-1}$ of agar, pH 7.4) and cellulose agar (10 $g \cdot l^{-1}$ of cellulose powder, 1 $g \cdot l^{-1}$ of yeast extract, 0.1 $g \cdot l^{-1}$ of NaCl, 2.5 $g \cdot l^{-1}$ of $(NH_4)_2SO_4$, 0.25 $g \cdot l^{-1}$ of K_2HPO_4, 0.125 $g \cdot l^{-1}$ of $MgSO_4 \cdot 7H_2O$, 0.0025 $g \cdot l^{-1}$ of $FeSO_4 \cdot 7H_2O$, 0.025 $g \cdot l^{-1}$ of $MnSO_4 \cdot 4H_2O$, and 15 $g \cdot l^{-1}$ of agar, pH 7.2) for detection of carbohydrate-degrading microorganisms, respectively. To investigate the effect of salt concentration on cellular growth of the isolates, each pure cell was also spread on the four agar (skim milk agar, spirit blue agar, starch hydrolysis agar, and cellulose agar) plates containing various concentrations of 1, 2 and 3.5% NaCl additionally. All agar plates were incubated at 45℃ until change of color or a clear zone around each colony appeared.

Screening of potential bacterial antagonists against the other isolated microorganisms was carried out by the use of perpendicular streak technique as described by Alippi and Reynaldi (2006). After the test, seven microorganisms were finally screened, and they were *Bacillus subtilis*, *Bacillus licheniformis*, *Brevibacillus agri*, *Bacillus coagulans*, *Bacillus circulans*, *Bacillus anthracis* and *Bacillus fusiformis* (Kim et al., 2007). Each pure culture was maintained on 1.5% Nutrient agar plate at 4℃ until used, and transferred to a fresh agar plate every month. The potential degrading ability of each pure culture was also checked every month.

Preliminary Experiment

Aerobic biodegradation was preliminary carried out in a 100-ml syringe that served as the reaction vessel (Cho et al., 2006). Under supply of sterile oxygen, 0.2 g (wet weight basis) of mixed isolates (5% inoculums) were suspended in the syringe with 40 ml of the original FMW (pH 6.5±0.2) obtained from a fish-meal factory. The pH of the original FMW was not adjusted before autoclaving because it was always measured to be 7.0±0.3. The original FMW was sterilized at 121℃ for 15 min. For faster biodegradation, the inoculated cells were previously acclimated for two days in the original FMW under an aerobic condition. The syringes prepared in this way were incubated in a shaking incubator at 45℃ and 180 rpm. The gas produced by the mixed microorganisms during incubation was analyzed by gas chromatography (GC). At the same time liquid broth was taken from the syringe to measure the concentrations of chemical oxygen demand- dichromate (COD_{Cr}) and total nitrogen (TN).

Liquid-Fertilization of FMW

In our previous study (Kim et al., 2007), liquid-fertilization of FMW was carried out first in a 5-l continuous stirred reactor with the original FMW at two different dilution ratios (8-

and 32-fold), based on the preliminary experiment. The mixed isolates (20g wet cell) were suspended in the bioreactor filled with 4 l of sterile diluted FMW, and the reactor was operated at 45℃ and 200 rpm. For faster biodegradation, the inoculated cells were acclimated previously as mentioned above. During aerobic biodegradation, the changes of the concentration of dissolved oxygen (DO), pH and oxidation-reduction potential (ORP) were observed by real-time measurement, and those of other reaction parameters (COD_{Cr} and TN) were analyzed after sampling.

To scale up the liquid-fertilization process of a lab-scale production, a large-scale biodegradation was carried out in a 1-ton reactor. The characteristics of the original FMW are given in Table 1. The pH of FMW was not adjusted, and 30-l liquid broth of seven microorganisms (on the same amount of each pure culture) grown in exponential phase of growth were seeded into the reactor filled with 600 l of original FMW. The bioreactor was operated at 42±4℃, and air was supplied from two blowers (6.4 $m^3 \cdot min^{-1}$ of capacity) into the reactor through ceramic disk-typed diffusers. The aeration rate was 1,280 $l \cdot min^{-1}$. Ten-fold diluted ‘Antifoam 204’ was used when severe foams occurred during biodegradation. Samples from the bioreactor were collected periodically. The concentrations of DO, COD_{Cr} and TN were measured with ORP and pH.

Table 1. Characteristics of the original FMW

Constituents	Concentration ($mg \cdot l^{-1}$)
COD_{Cr}	115,000±13,000
TN	15,400±1,300
BOD_5	68,900±7,600
NH_4^+-N	2,800±600
NO_3^--N	0
NO_2^--N	0

Seed Germination Test

According to the method of Wong et al. (2001), seed germination tests for 50-, 100-, 250-, 500-, and 1,000-fold diluted final broths, which were taken from the biodegradation of original FMW in a 1-ton reactor, were carried out against control in order to evaluate the phytotoxicity of the biodegraded FMW. Two commercial fertilizers, C-1 (A-company, Incheon, Korea) and C-2 (N-company, Kimhae, Korea), frequently used in Korea were also tested for the comparison of their phytotoxicity. Five milliliters of each sample were pipetted into a sterile petri dish lined with Whatman #1 filter paper. Ten cress (*Lepidium sativum*) seeds were evenly placed in each dish. The plates were incubated at 25 ºC in the dark at 75% of humidity. Distilled water was used as a control. Seed germination and root length in each plate were measured at 72 h. The percentages of relative seed germination (RSG), relative root growth (RRG) and germination index (GI) after expose to the sample were calculated as the following formula (Zucconi et al., 1981; Hoekstra et al., 2002):

$$\text{RSG (\%)} = \frac{\text{Number of seeds germinated in the biodegraded FMW}}{\text{Number of seeds germinated in control}} \times 100$$

$$\text{RRG (\%)} = \frac{\text{Mean root length in the biodegraded FMW}}{\text{Mean root length in control}} \times 100$$

$$\text{GI (\%)} = \frac{\text{RSG} \times \text{RRG}}{100}$$

Hydroponic culture

To test the fertilizing ability of the biodegraded FMW produced in a 1-ton reactor, a hydroponic culture system was applied to cultivate red bean and barley in a mini-hydroponic culture pot ($5\times12\times8$ cm^3) against control. Tests were carried out on the biodegraded FMW at various dilutions (50, 100, 250, 500 and 1,000-fold). The tests were also carried out on 1,000-fold diluted commercial-fertilizers in order to compare with the fertilizing ability of the biodegraded FMW. The hydroponic culture pot was composed of a glass vessel and a plastic screen inside. In each pot, ten seeds of red bean or twenty-five seeds of barley were put on top of the plastic screen, respectively, and approximately 300-ml solution of the biodegraded FMW at various dilution ratios was filled underneath the plastic screen. The seeds were soaked the entire time, and roots grew through the pore of the plastic screen after seed germination. After set-up of the hydroponic culture system, each pot was initially covered with aluminum foil to keep seeds in dark before seed germination. After seed germination, the pot placed by the window all day long to provide the necessary sunlight for plant's growth. Day and night temperatures of air were maintained at 22±2℃ and 18±2℃ by natural ventilation and heating. Water temperature was 15±3℃, and the relative humidity in the room was 60% on the average**.** The fertilizer solution was refreshed every four days. The growth of plants was observed periodically, and height, thickness of stem, number of leaf, and length of leaf of each plant were measured.

Preservation of Liquid Fertilizer

Various concentrations (0.5, 1 and 3%) of lactic acids were used to preserve for the biodegradated FMW. The preservation was carried out for twelve months in a 1 l plastic bottle, which was stored at room temperature. During the preservation, samples were periodically taken under a clean bench for the odor evaluation by panel. The panel was composed of twenty persons. The change of level of amino acids was also analyzed during the period of the preservation.

Analyses

The concentrations of cations (NH_4^+) and anions (NO_2^- and NO_3^-) were estimated by IC (Metrohm 792 Basic IC). The columns used in these analyses were Metrosep C2-150 and Metrosep Supp 5-150 for cation and anion, respectively. The concentrations of COD_{Cr} and TN concentrations were analyzed by the Water-quality Analyzer. The five days biological oxygen demand (BOD_5) was analyzed by the OxiDirect BOD-System. The composition of amino acids, and concentrations of major and noxious components in biodegraded FMW and commercial liquid-fertilizers were analyzed at Science Lab Center Co., Ltd (Daejeon, Korea) by our request.

For determination of nitrogen and carbon dioxide gases, 20 μl samples (injection volume) were taken for GC/TCD (Perkin Elmer Instruments) analysis. The columns used were a 'molecular sieve 13X' and 'carboxen 1,000' for nitrogen and carbon dioxide, respectively. In analyses of both gases, the following conditions were equally applied: the carrier gas was helium at a flow rate of 30 $ml \cdot min^{-1}$ and the injector and the detector temperatures were 100 and 200℃, respectively. However, the oven temperature for nitrogen gas was 40℃, and that for carbon dioxide gas was 40℃ for 3 min initially then increased to 170℃ with a rate of 30$℃ \cdot min^{-1}$.

Results and Discussion

Metabolic Characteristics of Seven Microorganisms

In our previous study (Kim and Lee, 2008), metabolic characteristics of seven microorganisms used in the biodegradation of FMW were found. Among the seven microorganisms, *Bacillus fusiformis* had the highest values of O_2 consumption rate and N_2 production rate under an aerobic condition, which resulted in increase of pH during biodegradation. *Bacillus licheniformis* showed the similar characteristic with high CO_2 production rate. However, *Brevibacillus agri*, *Bacillus coagulans* and *Bacillus circulans* had relatively lower O_2 consumption rate with lower N_2 and CO_2 production rates. Between diverse microbial populations, interaction always occurs and both of them may benefit from the interactions. Coexistence in the mixed culture occurs only if between species competition is weaker than within species. Recently, a new model with introduction of mutualism between competitive species has been proposed (Zhang, 2003). Thus, mutualism among seven microorganisms could promote coexistence and enhance the carrying capacity of the system, since they did not show any antagonism.

There was no effect on cellular growth of each microorganism at the concentrations of 1 and 2% of NaCl. However, the effect was distinct at 3.5%. In general, the salt concentration of the original FMW varies, since the concentration of salt in a raw material (fish wastes) used for the production of fish meal varies. The salt concentration of the original FMW used in this study was measured to be less than 1% (0.6±0.1%). Thus, it is concluded that the low salt concentration of FMW could not affect the growth of the seven microorganisms used in the biodegradation.

Preliminary Experiment for Biodegradation of FMW

From our preliminary experiments (Kim et al., 2007) carried out in a 100-ml syringe, it was found that the concentrations of COD_{Cr} and TN in original FMW were much reduced in the end by the seven microorganisms under a condition of O_2 supplement, compared with those under a condition of no O_2 supplement. When O_2 was supplemented continuously, the production of CO_2 gas was increased with increase of N_2 production. However, the production of CO_2 gas was very small without supplement of O_2. This result indicates that the greater mineralization of the organic matter took place under an aerobic condition. It is known that oxygen consumption is a general index of microbial metabolism (Tomati et al., 1996). Therefore, the seven microorganisms were found to be fit for active mineralization of FMW under an aerobic condition.

The effect of dilution ratio of FMW on biodegradation was also examined in our previous study (Kim et al., 2007), since cellular metabolism is dependent on substrate concentration (Maria et al., 2000). This result showed that the oxygen consumption rate by the seven microorganisms in 100-ml syringe vessel tended to increase when more diluted FMW was used as substrate, i.e., faster biodegradation took place with more diluted FMW, which resulted in faster removal rates of COD_{Cr} and TN. The maximum rates of gas productions of CO_2 and N_2 during the biodegradation were the highest with 8-fold diluted FMW, and the microbial population also tended to increase with more diluted FMW.

Liquid-Fertilization of FMW

To industrialize the biodegraded FMW as liquid-fertilizer, data obtained in laboratory equipment should be transferred to industrial production. For this reason, it is necessary to investigate both the biological and technological aspects of the system in the large scale.

Biodegradation of FMW in a 5-l reactor

In our previous study (Kim et al., 2007), we examined the characteristics of aerobic biodegradation of FMW in a 5-l reactor using 8-fold diluted FMW. The removal percentages of COD_{Cr} and TN were more prominent with slight decrease of COD_{Cr}/TN ratio, compared with the results of the syringe experiment using the same diluted FMW. This is because oxygen is able to be supplied more sufficiently into a reactor than a syringe vessel. It has been known that the COD/N ratio may influence biomass activity, and therefore on the metabolic pathways of organic matter utilization (Ruiz et al., 2006). Based on this information, the cell activity and metabolic pathways of the seven microorganisms may be maintained steadily during the biodegradation. The change of pH was not considerable and the weight of sludge decreased approximately half, which means that some fractions of suspended solids were biodegraded. Even when more diluted (32-fold) FMW was biodegraded, almost the same trend was seen in reduction of COD_{Cr} or TN, and the pH was also not changed considerably. However, it was noticeable that a strong unpleasant smell (mainly a fishy smell) remarkably disappeared in the end.

Biodegradation of FMW in a 1-ton reactor

Problems of scale-up in a bioreactor are associated with the behavior of liquid in the bioreactor and the metabolic reactions of the organisms. Biological properties, especially various constants involved in kinetic equations are dependent on scale-up, although the metabolic patterns remain unchanged. The typical differences of bioreactions have been known between large-scale and lab-scale reactors: i) the biomass yield is reduced at large-scale; ii) more metabolic by-products are produced at large-scale; and iii) limiting substrate gradients are present at large-scale as measured at different heights in the bioreactor (Bylund et al., 1998; Xu et al., 1999). Therefore, transport limitation is considered as one of the major factors responsible for phenomena observed at large-scale. In this study, a large-scale FMW biodegradation were attempted in a 1-ton bioreactor, and the result is shown in Fig. 1. After 5 h, DO level in the reactor started to decrease by the active biodegradation of seven microorganisms. The DO level could be maintained over 2 $mg \cdot l^{-1}$ during two days biodegradation, but it decreased thereafter. The final DO level was 1.25 $mg \cdot l^{-1}$. In aerobic processes, oxygen is a key substrate and because of its low solubility in aqueous solutions a continuous transfer of oxygen from the gas phase to the liquid phase is decisive for maintaining the oxidative metabolism of the cells. Generally, it has known that DO level in a bioreactor should be maintained over 1 $mg \cdot l^{-1}$ for aerobic fermentation (Tohyama et al., 2000). Therefore, the supply of oxygen into the 1-ton reactor could meet the demand of oxygen by the microorganisms during the biodegradation.

The pH was 6.99 at the beginning and decreased down to 6.15 after 20 h. Then the pH was recovered slowly and its final value was 6.86. However, in our previous study (Kim et al., 2008), pH had a tendency to increase during the biodegradation because of insufficient supply of oxygen. These results imply that microorganisms did different metabolism, which was dependent on the availability of dissolved oxygen. The value of ORP started at 18.2 mV. The value of ORP decreased rapidly after 45 h and the final value reached to 0.6 mV. The decrease in the ORP value resulted from the decrease in DO level. In this study, the values of ORP maintained positive during biodegradation, but they did not in our previous study (Kim et al., 2007). A complete aerobic biodegradation can take place when the value of ORP maintained in a positive range during the biodegradation. However, unpleasant odor can be easily produced under incomplete aerobic biodegradation (Zhang et al., 2004). From all the results, maintenance of DO level found to be very important and ORP could be a key parameter to operate biodegradation of FMW in a large-scale. ORP was reported to be used as a controlling parameter for regulation of sulfide oxidation in anaerobic treatment of high-sulfate wastewater (Khanal and Huang, 2003), and on-line monitoring of ORP has been proved to be a practical and useful technique for process control of wastewater treatment systems (Guo et al., 2007; Yu et al., 1997). As a result, process optimization is required in a large-scale operation, especially aeration rate in this case. After 52 h of biodegradation, the concentrations of COD_{Cr} and TN in original FMW reduced to 25,100 and 4,790 $mg \cdot l^{-1}$, respectively.

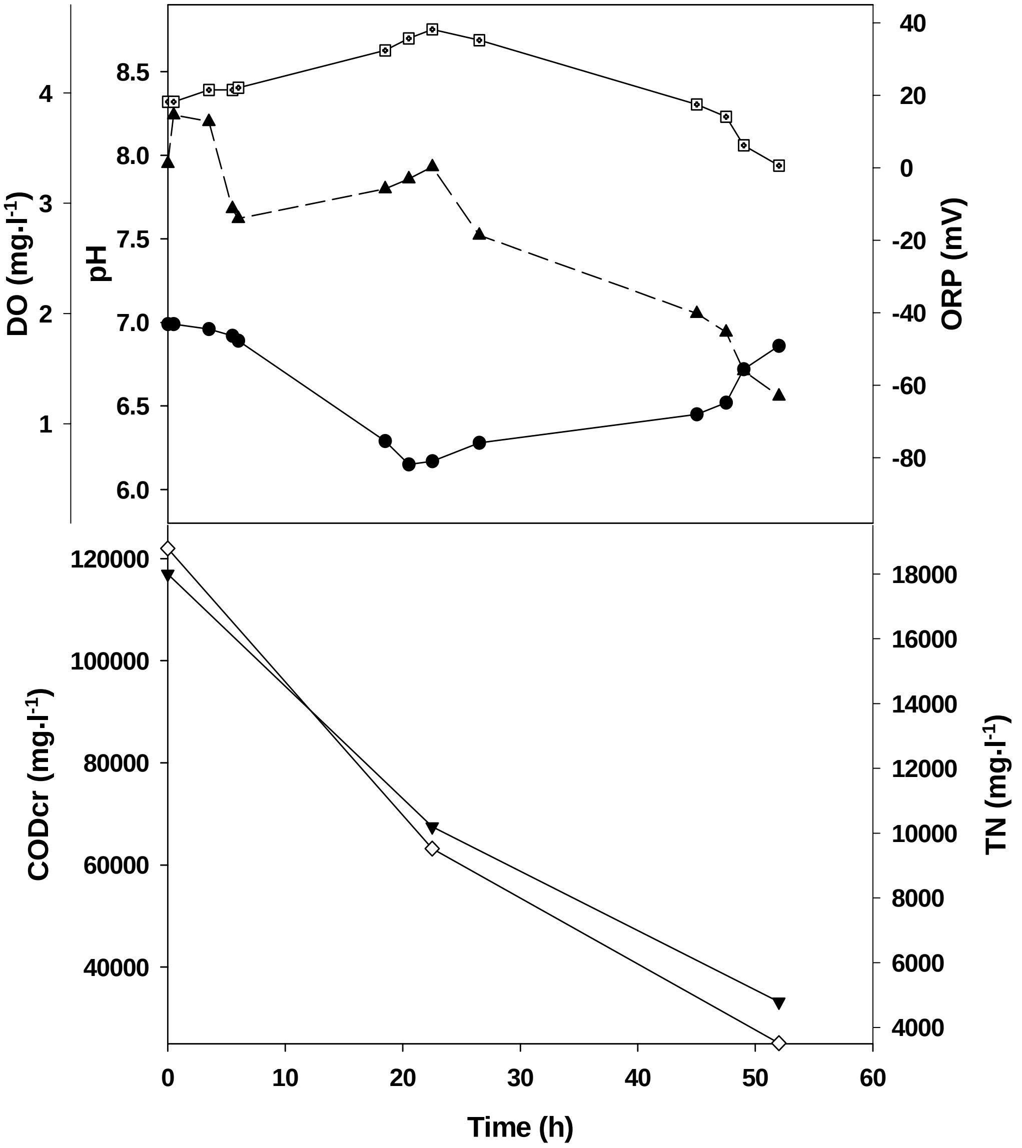


Figure 1. Changes of ORP(▣), DO(▲), pH(●), CODcr(◇) and TN(▼) during the biodegradation in a 1-ton reactor.

Properties of Biodegraded FMW as A Fertilizer

Since FMW contains various compounds potentially useful for diverse plants, an attractive application is its use as a fertilizer; however, this could have limitations due to some toxic characteristics of the waste. For this reason, it is necessary to prove the non-toxic properties and fertilizing ability of the final biodegraded FMW.

Phytotoxicity of biodegraded FMW

Organic matters hold great promise due to their local availability as a source of multiple nutrients and ability to improve soil characteristics. Sufficient aeration promotes the conversion of the organic matters into nonobjectionable, stable end products such as CO_2, SO_4^{2-}, NO_3^-, etc. However, an incomplete aeration may result in accumulation of organic acid, thus giving trouble to plant growth if the fertilizer is incorporated into the soil (Jakobsen, 1995). In our study (Kim et al., 2007), a test for seed germination was accomplished to examine the phytotoxicity of the biodegraded FMW produced in a lab scale. All cress seeds used in the experiment were germinated in one day, and thus, this method was found to be not sensitive. The same result was reported by Wang and Kentri (1990). They reported that seed germination was regarded as a less sensitive method than root elongation when used as a bioassay for the evaluation of phytotoxicity. Instead, the germination index (GI), which combines the measure of relative seed germination (RSG) and relative root growth (RRG) of cress seed (*Lepidium sativum*), has been reported to be the most sensitive parameter used to evaluate the toxicity (Zucconi et al., 1981). When phytotoxicity of the biodegraded FMW was assayed based on GI, its average value was only 8.0% with low root elongation, and the GI values had a tendency to increase with increase of dilution ratio of the biodegraded FMW. The reduction in values of GI indicates that some characteristics existed had an adverse effect on root growth. This may be attributed to the release of high concentrations of ammonia and low molecular weight organic acids (Fang and Wong, 1999; Wong, 1985), since cress used in this study is known to be sensitive to the toxic effect of these compounds (Fuentes et al., 2004). The phytotoxicity caused by organic compounds can be remedied either by increasing the period of aerobic decomposition (Wong et al., 2001) or by reducing the concentration of substrate (Maria et al., 2000).

In this study, the phytotoxicity of the biodegraded FMW produced in a 1-ton reactor was examined at various dilution ratios and compared twith those of two commercial fertilizers (Fig. 2 and Fig. 3). As shown in Fig. 3, the values of GI tended to increase with increase of dilution ratio of the biodegraded FMW. The GI values of the biodegraded FMW at 50- and 100-fold dilution were less than 20% with low root elongation (shown in Fig. 2), and the GI value was close to 50% at 250-fold dilution. A GI of 50% has been used as an indication of phytotoxin-free compost (Zucconi et al., 1985). According to this GI criterion, the biodegraded FMW required more than 250-fold dilution to reach stabilization of the organic matter to maintain the long-term fertility in soil. At 1,000-fold dilution, the GI value was found to be close to 90%. This GI value was compared with those of two commercial fertilizers at the same dilution, since 1,000-fold diluted liquid fertilizer was used in horticulture for general purpose. The GI value of the biodegradative FMW was much better than C-1 and comparable to that of C-2. This result implies that the biodegradation of FMW in a 1-ton reactor was successfully carried out, and thus the development of a liquid-fertilizer from FMW was feasible.

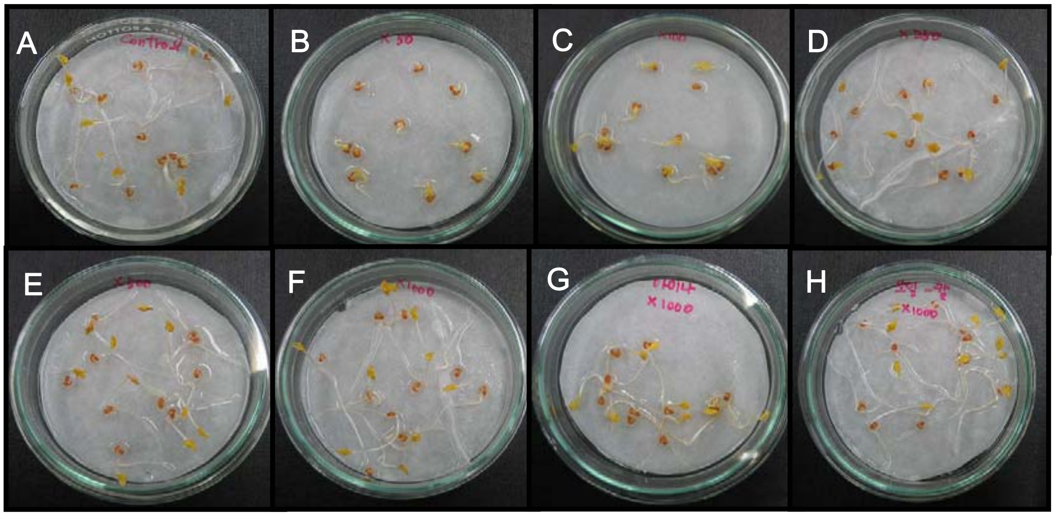


Figure 2. Results of seed germination test of the biodegraded FMW on various dilution ratios. (A) Control (water); (B) 50-fold; (C) 100-fold; (D) 250-fold; (E) 500-fold; (F) 1,000-fold; (G) 1,000-fold diluted C-1; and (H) 1,000-fold diluted C-2.

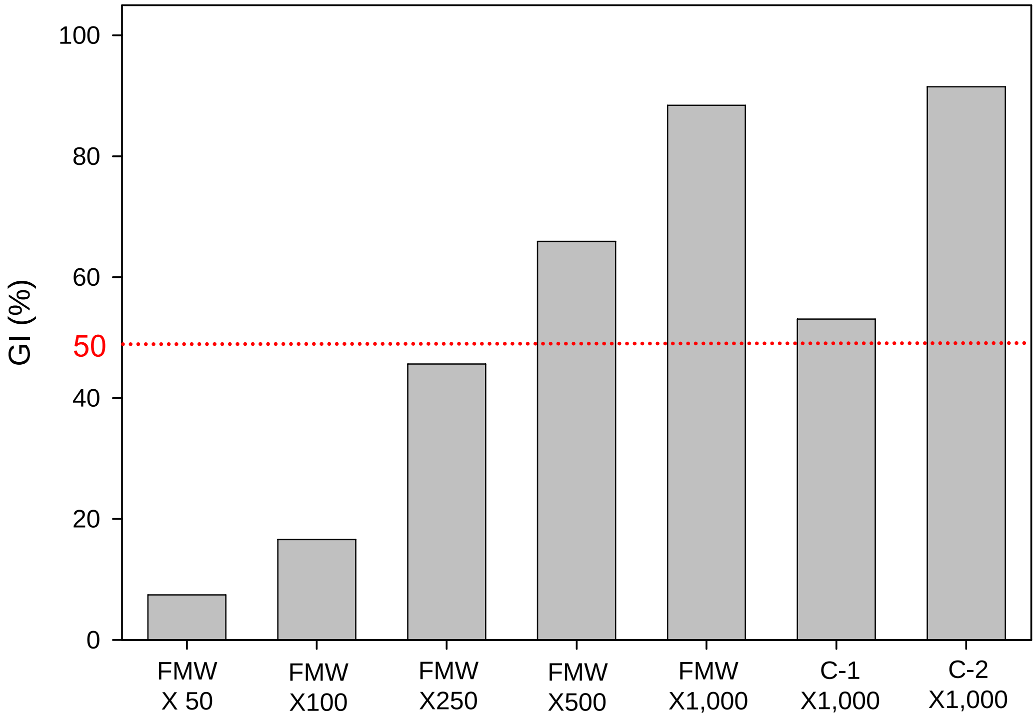


Figure 3. Percentages of germination index (GI) at various dilution ratios of the biodegraded FMW produced in a 1-ton reactor. The results are compared to those of 1,000-fold diluted commercial fertilizers, C-1 and C-2.

Composition of amino acids of biodegraded FMW

Amino acids are an essential part of the active fraction of organic matter in a fertilizer. The growth of plants depends ultimately upon the availability of a suitable balance of amino acids, and their composition might also be used as a means of assessing biodegradation. The success of the scale-up process for the production of liquid fertilizer from FMW can be verified by determining the composition of amino acids. From this point of view, it is necessary to analyze the amino-acid composition of the biodegraded FMW produced in a 1-ton bioreactor. In our previous study (Kim et al., 2007), it was found that the amino-acid content of the biodegraded FMW produced in a lab scale was almost twice that of non-biodegraded FMW, and higher than that of the biodegraded FMW under the condition of insufficient oxygen supply. This implies that the biodegradation of FMW and its environmental condition are very important to increase the content of amino acids. The higher content of amino acids is probably due to the higher degree of mineralization of FMW, which indicates release of more nutrients available for plants. It was also found that the levels of amino acids in the biodegraded FMW was comparable to those in commercial fertilizers.

In this study, the composition of amino acids of the biodegraded FMW produced in a 1-ton reactor was analyzed and compared with those in two commercial fertilizers (Table 2). The level of total amino acids was 5.60 g·100g sample^{-1}, which was a little bit better than that produced in our previous study (Kim and Lee, 2008). However, the level of total amino acids in the biodegraded FMW was lower, compared with those in two commercial fertilizers. This implies that mineralization of FMW in a 1-ton reactor was not as great as that in a lab-scale reactor (Bylund et al., 1998; Xu et al., 1999). Especially, the levels of aspartic acid, alanine and lysine were low, whereas those of proline and glycine were relatively high. The difference may be due to the composition of the original FMW, since it was found to be dependent on the nature of the raw material processed in the factory (Kim et al., 2007). Moreover, the composition of sulfur-containing amino acids, cysteine and methionine, were relatively high in the biodegraded FMW. It has been reported that the sulfur-containing amino acid, methionine is a nutritionally important essential amino acid and is the precursor of several metabolites that regulate plant growth (Amir et al., 2002).

Concentrations of major and noxious components in biodegraded FMW

It is very important to improve the utilization of fertilizer nutrients, since the growth of plants and their quality are mainly a function of the quantity of fertilizer. Organic matter affects crop growth and yield directly by supplying nutrients (Darwish et al., 1995). Combined use of organic and inorganic sources of nutrients is essential to augment the efficiency of nutrients (Lian, 1994). For this reason, concentrations of three primary nutrients (N, P, and K) in the biodegraded FMW were anaylized together with noxious components and compared with those in commercial fertilizers. The results were tabulated in Table 3. The concentrations of N, P and K in the biodegraded FMW were 1.49, 0.28 and 0.41%, respectively, and were much lower than those in commercial fertilizers. However, the concentrations of noxious components in the biodegraded FMW were much lower than those in commercial fertilizers. The noxious components, Pb, As, Cd, Cu and Ni were not detected, and the concentrations of Cr and Zn were much lower than the standard concentrations.

Table 2. Comparison of amino-acids composition of the biodegraded FMW with those of commercial fertilizers[a]

Amino acid	Source of liquid fertilizer		
	Biodegraded FMW[b]	Commercial C-1	Commercial C-2
Aspartic acid	0.49	0.90	0.58
Threonine	0.18	0.23	0.21
Serine	0.21	0.21	0.23
Glutamic acid	0.78	2.96	0.89
Proline	0.50	0.09	0.61
Glycine	1.06	0.31	1.25
Alanine	0.60	1.00	0.98
Valine	0.15	0.39	0.24
Isoleucine	0.14	0.28	0.13
Leucine	0.24	0.42	0.26
Tyrosine	0.07	0.17	0.05
Phenylalanine	0.19	0.22	0.18
Histidine	0.20	0.28	0.25
Lysine	0.29	1.54	0.53
Arginine	0.31	0.23	0.35
Cystine	0.04	n.d.[c]	0.04
Metionine	0.13	n.d.	0.01
Tryptophan	0.02	n.d.	0.02
Total	5.60	9.23	6.81

[a] composition of amino acids was based on dry weight (g•100g sample^{-1}).
[b] produced in a 1-ton reactor.
[c] n.d. means 'not detected'.

Table 3. Comparison of concentrations of major and noxious components present in the biodegraded FMW with those present in commercial fertilizers

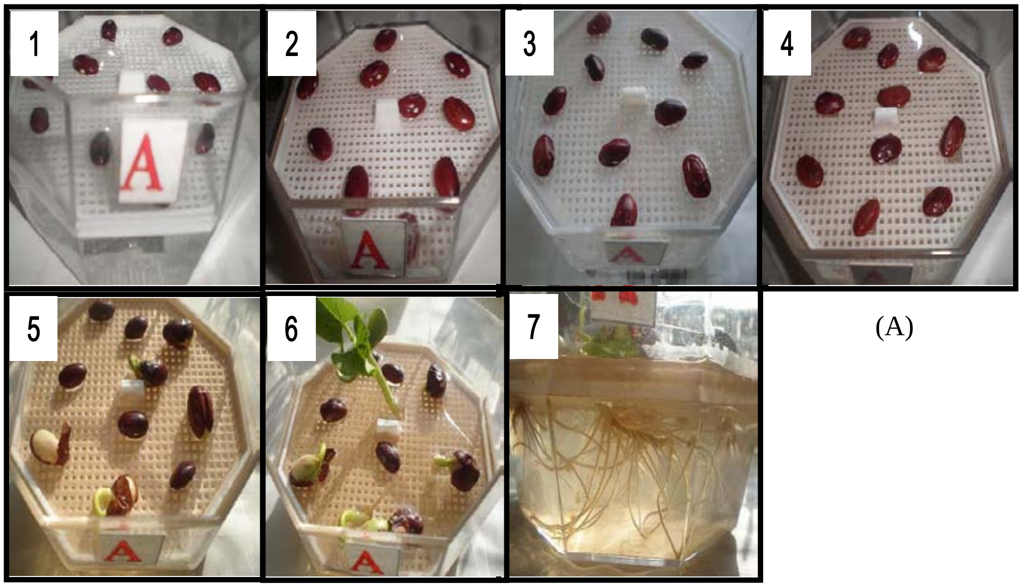
Measurement		Source of liquid fertilizer		
		Biodegraded FMW[a]	Commercial C-1	Commercial C-2
N, P, K (%)	N	1.49	4.83	3.80
	P_2O_5	0.28	2.86	2.83
	K_2O	0.41	2.10	3.04
Noxious Compounds (mg·kg^{-1})	Pb	n.d.[b]	0.63	0.31
	As	n.d.	0.88	0.36
	Cd	n.d.	0.08	0.03
	Hg	0.01	n.d.	n.d.
	Cr	0.20	3.52	3.44
	Cu	n.d.	3.24	2.24
	Ni	n.d.	1.54	2.32
	Zn	1.61	4.39	3.51

[a] produced in a 1-ton reactor.
[b] n.d. means 'not detected'.

Hydroponic culture on biodegraded FMW

The fertilizing ability of the biodegraded FMW at various dilution ratios was tested in a hydroponic culture system, which was applied to cultivate red bean and barley. The tests were also carried out on 1,000-fold diluted commercial-fertilizers simultaneously. Results of hydroponic culture of red bean on diluted biodegraded-FMW and commercial fertilizers were tabulated in Table 4. As seen in Table 4, elongation of root was slow after seed germination in all diluted FMW. However, roots elongated soon after development of root. The fastest growth was achieved at 100-fold dilution, which growth was comparable to those of 1,000-fold diluted commercial-fertilizers. In Fig. 4, the change of red bean's growth from the seed at 100-fold dilution is shown against control. At the same dilution of 1,000-fold, growth of red bean cultivated on the biodegraded FMW was not as good as that cultivated on commercial fertilizers. A decreasing order of growth in the culture of red bean was C-1 > C-2 > biodegraded FMW. According to Perrenoud's report (1990), N, P and K are primary nutrients and considered to improve plant health. The deficiency of phosphorus restricts plant growth in soil (Son et al., 2006), although the excessive fertilization with chemically synthesized phosphate fertilizers has caused severe accumulation of insoluble phosphate compounds (Omar, 1998). Therefore, relatively lower growth of red bean on the biodegraded FMW may be due to the shortage of NPK components, as represented in Table 3.

In culture of barley, the best growth was achieved at 500-fold dilution in which the growth was seen evenly in root and stem (Fig. 5). This growth of barley was comparable to that cultivated on 1,000-fold diluted commercial-fertilizers. As seen in Table 5, the growth in root and stem at 50-fold dilution was especially lower than that of control. However, effect of dilution on barley was not as sensitive as that on red bean.



(A)

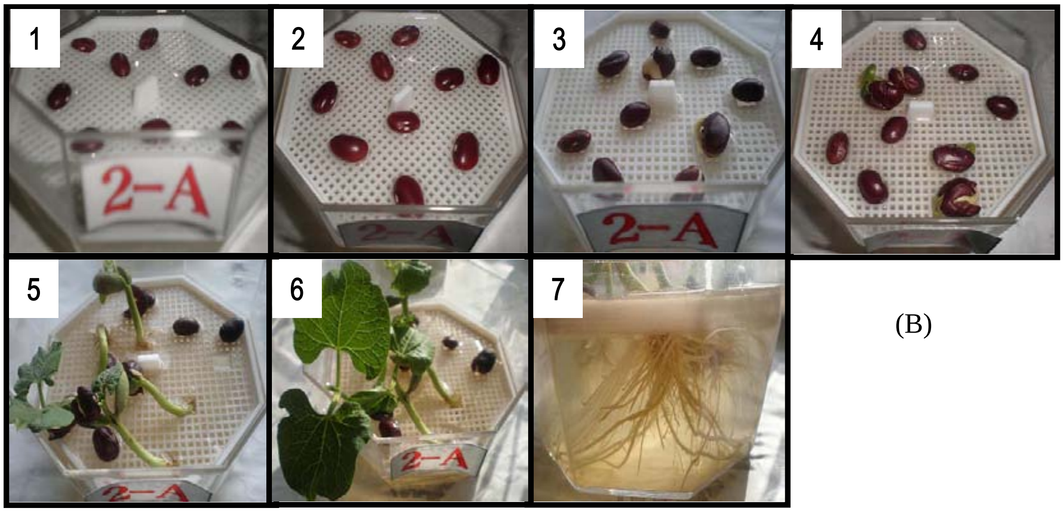
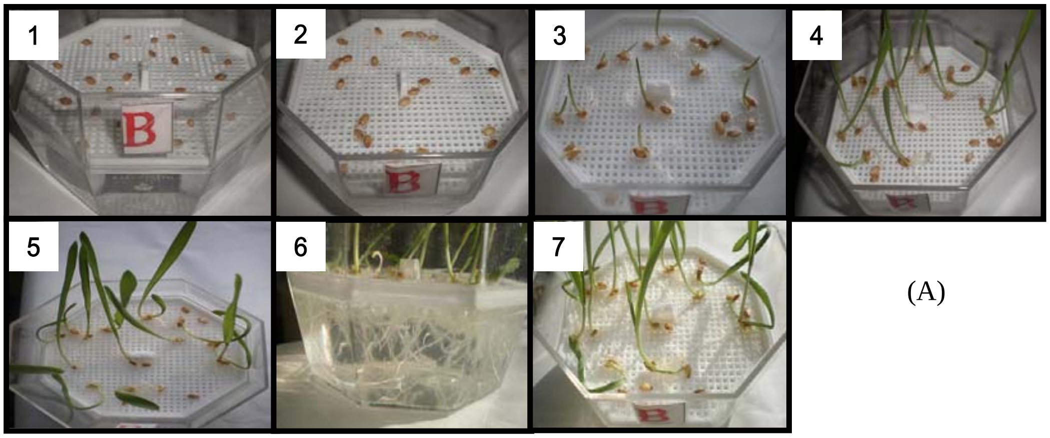


Figure 4. Results of hydroponic culture of red bean in control (A) and 100-fold diluted FMW (B). Each figure shows the growth of red bean from the seed along the cultivation time. (1) day 1; (2) day 2; (3) day 5; (4) day 8; (5) day 12; (6) day 14 and (7) roots on day 14.

Table 4. Results of hydroponic culture of red bean on diluted biodegraded-FMW and commercial fertilizers

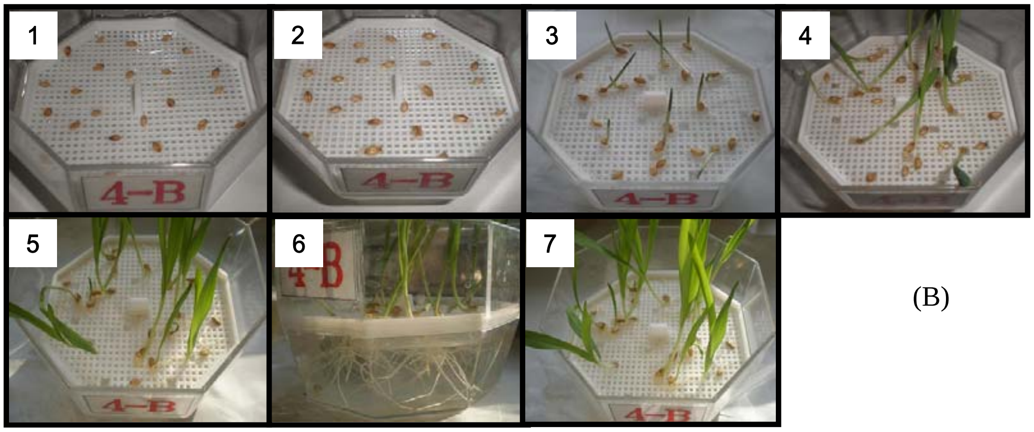
Measurement	Control (Water)					Dilution of biodegraded FMW														
						50-fold					100-fold					250-fold				
	Time (day)					Time (day)					Time (day)					Time (day)				
	2	5	8	12	14	2	5	8	12	14	2	5	8	12	14	2	5	8	12	14
Height (cm)	-	-	1.5	3.0	6.0	-	-	-	1.0	2.0	-	2.0	4.0	6.0	9.0	-	-	1.0	3.0	4.5
Thickness of stem (cm)	-	-	0.2	0.3	0.5	-	-	-	0.2	0.2	-	0.2	0.3	0.4	0.5	-	-	0.2	0.3	0.3
Number of leaf	-	-	-	-	2	-	-	-	1	1	-	-	-	2	2	-	-	-	1	2
Length of leaf (cm)	-	-	-	-	3.0	-	-	-	1.0	2.0	-	-	-	1.5	4.0	-	-	-	1.0	1.5

Measurement	Dilution of biodegraded FMW										Commercial fertilizer									
	500-fold					1,000-fold					1,000-fold, C-1					1,000-fold, C-2				
	Time (day)					Time (day)					Time (day)					Time (day)				
	2	5	8	12	14	2	5	8	12	14	2	5	8	12	14	2	5	8	12	14
Height (cm)	-	-	1.0	3.0	4.0	-	1.0	2.0	4.0	5.5	1.0	4.0	4.5	5.0	8.0	-	2.0	3.0	4.0	6.0
Thickness of stem (cm)	-	-	0.2	0.3	0.4	-	0.2	0.2	0.3	0.4	0.2	0.3	0.3	0.4	0.5	-	0.2	0.3	0.4	0.5
Number of leaf	-	-	-	1	2	-	-	-	1	2	-	-	-	2	2	-	-	-	2	2
Length of leaf (cm)	-	-	-	1.0	1.5	-	-	-	1.0	2.0	-	-	-	1.5	4.5	-	-	-	1.0	3.0



(A)

Figure 5 A. Results of hydroponic culture of barley in control (A) Each figure shows the growth of barley from the seed along the cultivation time. (1) day 1; (2) day 2; (3) day 5; (4) day 8; (5) day 12; (6) roots on day 12 and (7) day 14.



(B)

Figure 5. Results of hydroponic culture of barley in 500-fold diluted FMW (B). Each figure shows the growth of barley from the seed along the cultivation time. (1) day 1; (2) day 2; (3) day 5; (4) day 8; (5) day 12; (6) roots on day 12 and (7) day 14.

Table 5. Results of hydroponic culture of barley on diluted biodegraded-FMW and commercial fertilizers

Measurement	Control (Water)					Dilution of biodegraded FMW														
						50-fold					100-fold					250-fold				
	Time (day)					Time (day)					Time (day)					Time (day)				
	2	5	8	12	14	2	5	8	12	14	2	5	8	12	14	2	5	8	12	14
Height (cm)	-	1.7	5.8	8.3	8.5	-	1.2	2.6	4.4	5.2	-	2.1	4.1	6.5	7.5	-	2.4	4.3	6.7	7.6
Thickness of stem (cm)	-	0.1	0.2	0.2	0.2	-	0.1	0.2	0.2	0.2	-	0.1	0.2	0.2	0.2	-	0.1	0.2	0.2	0.2
Number of leaf	-	1	2	2	2	-	1	2	2	2	-	1	2	2	2	-	1	2	2	2
Length of leaf (cm)	-	1.2	4.6	6.5	6.8	-	0.7	1.3	3.1	3.5	-	1.6	3.0	5.0	5.5	-	1.9	3.0	5.0	5.6

Table 5. (Continued)

Measurement	Dilution of biodegraded FMW										Commercial fertilizer									
	500-fold					1,000-fold					1,000-fold, C-1					1,000-fold, C-2				
	Time (day)					Time (day)					Time (day)					Time (day)				
	2	5	8	12	14	2	5	8	12	14	2	5	8	12	14	2	5	8	12	14
Height (cm)	-	2.9	5.5	7.8	8.1	-	1.5	3.9	5.6	6.3	-	2.8	5.6	7.1	7.7	-	1.8	4.8	8.6	9.0
Thickness of stem (cm)	-	0.1	0.2	0.2	0.2	-	0.1	0.2	0.2	0.2	-	0.1	0.2	0.2	0.2	-	0.1	0.2	0.2	0.2
Number of leaf	-	1	2	2	2	-	1	2	2	2	-	1	2	2	2	-	1	2	2	2
Length of leaf (cm)	-	2.4	4.2	5.7	6.1	-	0.9	2.7	3.8	4.2	-	2	4.3	5.3	5.7	-	1.3	3.8	6.6	7.0

Preservation of biodegraded FMW

After a liquid fertilizer is produced, it is required to prevent the deteriorative process in order to maintain its quality during the period of circulation in market. In this study, lactate was used as a means of a more long-term preservation of the biodegraded FMW.

Effect of lactate on preservation

The effect of lactate addition on the quality of the biodegraded FMW was investigated for twelve months, and the results are tabulated in Table 6. The biodegraded FMW were stored at the various concentrations of lactate as a reagent for preservation. At the beginning of the experiment, the biodegraded FMW emitted relatively light ammonia smell. In cases of control and addition of 0.5% lactate, unacceptable odors were produced within one and half months by putrefaction. The addition of 1% lactate could preserve the biodegraded FMW for six months with emission of the soy sauce-like smell, but deteriorative process was slowly proceeded hereafter. After ten months, ammonia smell was emitted. Addition of 1% lactate caused the change of pH from 7.8 to 5.7, with increase of ORP from - 10 to 73.2 mV, which in turn had a higher bacteriostatic effect. When 3% lactate was added to the biodegraded FMW, the soy sauce-like smell was emitted and retained for twelve months without any unpleasant odor. It has been reported that sweet-smell soy sauce had eighteen different free amino acids (Jingtian et al., 1988). As a result, the addition of 3% lactate prevented the growth of spoilage microorganisms with keeping high content of amino acids effectively for one year.

Change of amino-acid composition

The change of amino-acids composition was investigated during one-year preservation by addition of 1% lactate, and the results were tabulated in Table 7. Until six months of the preservation, the levels of several amino acids slightly increased as storage time elapsed. During first six months, the level of total amino acids increased from 5.60 to 5.81 g·100g sample^{-1}, and the pH of the liquid broth decreased further to 5.3 due to the production of amino acids.

Table 6. Effect of lactate addition on the quality of the biodegraded FMW during the preservation at room temperature[a]

Addition of lactate (%)	Storage time (day)														
	0	10	20	30	45	60	75	90	120	150	180	210	240	300	360
0 (control)	L	A	A	O											
0.5	L	L	A	A	O										
1	L	S	S	S	S	S	S	S	S	S	S	L	L	A	A
3	L	S	S	S	S	S	S	S	S	S	S	S	S	S	S

[a] symbols represent different smell: ‘A’ means ammonia smell; ‘O’ means odor by putrefaction; ‘L’ means relatively light ammonia smell; and ‘S’ means soy sauce-like smell.

This implies that useful microorganisms maintained their minimum activity under the addition of 1% lactate, which resulted in emission of soy sauce-like smell. However, this effect of 1% lactate could not continue hereafter. The final level of total amino acids was reduced to 2.46 g·100g sample^{-1} with ammonia emission. This is probably due to the consumption of lactate and amino acids by putrefactive microorganisms present in the liquid broth of FMW (Adour et al., 2002). As a result, the addition of 3% lactated is required for a long-term preservation of the biodegraded FMW.

Table 7. Change in amino-acids composition during preservation by addition of 1% lactate[a]

Amino acid	Storage time (day)			
	0	30	180	360
Aspartic acid	0.49	0.49	0.51	0.32
Threonine	0.18	0.18	0.21	0.08
Serine	0.21	0.21	0.21	0.10
Glutamic acid	0.78	0.79	0.82	0.26
Proline	0.50	0.51	0.52	0.18
Glycine	1.06	1.07	1.08	0.42
Alanine	0.60	0.61	0.62	0.18
Valine	0.15	0.15	0.16	0.06
Amino acid	Storage time (day)			
	0		0	
Isoleucine	0.14	0.15	0.17	0.04
Leucine	0.24	0.25	0.26	0.06
Tyrosine	0.07	0.08	0.09	0.02
Phenylalanine	0.19	0.20	0.21	0.06

Table 7. (Continued)

Histidine	0.20	0.21	0.24	0.38
Lysine	0.29	0.30	0.32	0.14
Arginine	0.31	0.31	0.30	0.10
Cystine	0.04	0.04	0.04	0.03
Metionine	0.13	0.13	0.13	0.02
Tryptophan	0.02	0.02	0.02	0.01
Total	5.60	5.70	5.81	2.46

[a]composition of amino acids was based on dry weight (g•100g sample^{-1}).

Conclusion

Seven thermophilic microorganisms, which had proteolytic, lipolytic and carbohydrate-degrading functions, were used in order to reutilize the wastewater generated during the process of fish-meal production (FMW). Their growth was not influenced by the salt concentration contained in FMW, and mutualism among seven microorganisms could promote coexistence and enhance aerobic biodegradation. A lab-scale aerobic biodegradation of FMW were successfully achieved, and its data were transferred to a large-scale reactor. A large-scale FMW biodegradation were attempted in a 1-ton bioreactor. During the biodegradation of FMW, the level of DO could be maintained over 1.25 mg·l^{-1}. The level of DO in the liquid broth was found to be decisive influence on the quality of final fermented broth, and ORP to be a key operation parameter in biodegradation of FMW in a large-scale. A complete aerobic biodegradation can take place without unpleasant odor when the value of ORP maintained in a positive range.

Properties of the biodegraded FMW were determined to examine the suitability of the biodegraded FMW as a fertilizer. The result of the phytotoxicity test showed that the biodegraded FMW required more than 250-fold dilution to reach stabilization of the organic matter to maintain the long-term fertility in soil. At 1,000-fold dilution, the GI value was found to be close to 90%, which was comparable to those of commercial fertilizers. The level of total amino acids in the biodegraded FMW was 5.60 g·100g sample^{-1}, which was lower than those in two commercial fertilizers. Especially, the levels of aspartic acid, alanine and lysine were low, whereas those of proline and glycine were relatively high. Moreover, the composition of sulfur-containing amino acids, cysteine and methionine, were relatively high in the biodegraded FMW. The concentrations of N, P and K in the biodegraded FMW were 1.49, 0.28 and 0.41%, respectively, which were lower than those in commercial fertilizers. However, the concentrations of noxious components in the biodegraded FMW were much lower than the standard concentrations. The fertilizing ability of the biodegraded FMW at various dilution ratios was tested in a hydroponic culture system. The fastest growth in culture of red bean was achieved at 100-fold dilution, which growth was comparable to those of 1,000-fold diluted commercial-fertilizers. In the hydroponic culture of barley, the best growth was achieved at 500-fold dilution in which the growth was seen evenly in root and stem, which was comparable to that cultivated on 1,000-fold diluted commercial-fertilizers. Effect of dilution on barley was not as sensitive as that on red bean.

During the period of circulation in market, the biodegraded FMW is required to be maintained its quality as a liquid fertilizer. The addition of 3% lactate prevented the growth of spoilage microorganisms with keeping high content of amino acids effectively for one year, whereas the addition of lower concentrations of lactic acids could not preserve properly and resulted in putrefaction in the end.

Acknowledgments

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Chapter IV

Fertilizer Application on Grassland – History, Effects and Scientific Value of Long-Term Experimentation

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Abstract

Organic as well as mineral fertilizers were used for centuries to improve quantity and quality of forage produced on permanent grassland. In many regions, application of organic fertilizers on farm land resulted in creation or in enlargement of oligotrophic plant communities highly valuated by nature conservation today. Industrial production of synthetic fertilizers started in the middle of the 19th century and since that time, long-term fertilizer experiments were established. Ten grassland experiments at least 40 years old are still running in Austria, Czech Republic, Germany, Great Britain, Poland and Slovakia.

The present paper demonstrates that short and long-term effects of fertilizer application on plant species composition differ substantially. In contrast, short-term experiments cannot to be used to predict long-term effects. On oligotrophic soils, highly productive species supported by short-term N fertilizer application can completely disappear under long-term N application whilst other nutrients such as P become limiting. Under P limiting conditions, species characteristic for low productive grasslands (sedges, short grasses and some orchids) can survive even under long-term N application. It is more likely that enhanced P soil content causes species loss than N enrichment.

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Residual effect of fertilizer application differs substantially among individual types of grassland and nutrients applied. Decades long after-effects of Ca and P application were revealed in alpine grasslands under extreme soil and weather conditions decelerating mineralization of organic matter. In extreme cases, resilience of the plant community after long-term fertilizer application can take more than several decades. Changes in plant species composition may even be irreversible. At lower altitudes with less extreme soil and climatic conditions, residual effect of fertilizer application is generally substantially shorter.

From the comparison of long-term *vs* short-term nutritional effects, it was concluded that long-term fertilizer experiments are irreplaceable as many existing models and predictions can be validated only by means of long-term manipulation of plant communities and their continuous observation and documentation. In conclusion, the authors give examples of how to apply forward-looking grassland research on existing long-term experiments and explain the extraordinary value that is provided by plant-soil-environment equilibrium.

History of Fertilizers Use on Grassland

The application of organic and mineral fertilizers on grasslands has been performed since hundreds of years. The most prominent example of organic fertilizer application, which has been applied in many regions for centuries, is paddock manuring (Hejcman *et al.* 2002). The principle of paddock manuring consists of the transport of nutrients by livestock on pasture. Livestock that grazes on pasture during day time is kept in small mobile fenced enclosures during the night. Most of the nutrients taken up elsewhere on pastures are excreted as feces and urine during the night within this enclosure. After a certain time, about one week in most cases, the enclosure is moved forward to areas to be fertilized. In the past, those fertilized areas were most frequently used for hay making in subsequent years to benefit from the fertilizer effect on biomass production and forage quality. To restrict oligotrophic vegetation dominated by *Nardus stricta*, a tussock grass of low forage quality and biomass production, sheep paddock manuring has been utilized in Carpathian Mountains in Romania, Ukraine, Slovakia and in the Czech Republic (Fig. 1).

Transportation and application of nutrients by humans has been documented in many regions in Europe since centuries. For example, in the Giant Mts. (Krkonoše, Riesengebirge) located in the borderland between the Czech Republic and Poland, hay was transported from sub-alpine grasslands down the valleys across a distance of 10 – 15 km during the 17th to 20th century (Fig. 2). This hay was used as feed to cows and to goats kept in cow houses in the valleys. The organic fertilizer nutrients originating from that hay were applied to grassland or arable land in close vicinity of the farm houses. As a consequence, biomass production of grasslands at lower altitudes was promoted, whereas nutrient depletion occurred on grasslands in the sub-alpine vegetation zone (Hejcman *et al.* 2006).

Figure 1. Paddock manuring has been used in the Carpathian Mts. for centuries. Sheep are kept over night in small mobile enclosure that is moved across the grassland plot in intervals of about one week. (Slovakia 2006, photo Michal Hejcman).

Figure 2. Transport of hay from sub-alpine grasslands in the Giant Mts. at the end of 19th century. Men carried up to 80 kg and women up to 50 kg of hay across the distance of 10 – 15 km with super-elevation of almost 1000 m (the Czech Republic, photo archive KRNAP).

The same principle of fertilizer application on the one side and nutrient depletion on the other was described by Bakker (1989) in The Netherlands. In the Iron Age (800 – 0 BC), sandy soils of Drenthian region were so poor in nutrients that after a time of fallow and pasture soil fertility was insufficient to grow crops whereas loamy soils elsewhere in Europe maintained more fertile.

It is well documented that replenishment of nutrients has been practiced by applying organic material to arable fields, such as woodland litter, sods and waste from the settlement and cattle dung when the stubble was grazed. It is important from the vegetation science point of view that the maintenance of arable soil fertility was thus promoting the enlargement of oligotrophic heathland and grassland in less fertile agricultural regions.

In Europe, slurry application on grassland looks back to a long-term tradition. Slurry, a liquid organic fertilizer composed of urine and livestock feces, was produced in mountaineous regions where bedding straw or other organic material was rare. In the Alps, slurry production and application systems have already been used in the middle age. In the Giant Mts., slurry has been applied on grassland since the 16th century. The system of fertilizer application was introduced by German colonists from the Alps. Slurry pit was placed in front of farm house in sloping terrain and slurry was scraped away from the barn manually or washed up by running water. The grassland located below the pit was called Grass Garden and consisted of a system of small ditches connected to the slurry pit through a central channel. In spring time, running water from a water course was used to dilute slurry and so uniformly applied on Grass Garden as an organic fertilizer. Because of high biomass production and forage quality in the Grass Garden and its close vicinity to farm houses, this area was exclusively used for hay production.

Figure 3. Although sub-alpine vegetation zone of the Giant Mts. is no longer utilized for agricultural production, remains of farm houses with slurry pits are still well conserved. Slurry pit on the photograph was used until 1938. (Czech Republic 2004, photo Michal Hejcman).

Apart from organic fertilizer, the use of mineral and chemical-synthetic fertilizers has long-term tradition as well. Romans, for example, used lime, limestone or marl. Probably the oldest well known and largely used organo-mineral fertilizer was wood ash. The content of organic compounds in this fertilizer is dependent on intensity and temperature of combustion process. If combustion is intensive enough, wood ash contains almost entirely alkaline mineral fertilizer rich in K, Ca, Mg, P, Si and many trace elements such as Zn, Co, Fe (Campbell 1990). The content of individual nutrients and ratios among them are dependent on original chemical composition of wood and are thus specific of species combusted. Further, the volume of ash obtained from an equivalent amount of wood is dependent on combustion temperature. In an experiment conducted by Etiégni and Campbell (1991), ash yield decreased by approximately 45% when combustion temperature increased from 538 to 1093°C. The metal content tended to increase with temperature, although K, Na and Zn decreased.

Wood ash has been frequently mixed with slurry or manure before application on grassland or arable land in the 19th century. Such treatment increased ash fertilizer value because of N enrichment (Semelová *et al.* 2008). In many tropical regions, small scale slush and burning agriculture still takes advantage of high fertilizer value of wood ash (Menzies & Gillman 2003). In industrialized countries, wood ash is a fertilizer with a certain future perspective, *e.g.* when produced by combustion of renewable organic material. In addition to fertilizer application, wood ash has also been used as a binding agent, a glazing base for ceramics, an additive to cement production and an alkaline material for the neutralization of wastes. In former times, wood ash was highly valued and was repurchased and sold in many regions of Central Europe in 18th and 19th century.

It is common knowledge, that mineral fertilizers were used long before the principles of plant mineral nutrition were discovered by Sprengel in 1826 and 1828 and popularized by Liebig in 1840 and 1855 (van der Ploeg *et al.*, 1999). According to the Humus Theory, the addition of mineral compounds such as salts of P, Ca, K and other elements increased availability of organic compounds in the soil. When taken up by plants, these elements increased biomass production of agricultural crops. The rejection of the Humus Theory finally resulted in industrial production of mineral and chemical-synthetic fertilizers across the world. The first superphosphate was manufactured by Lawes in his factory in Deptford in England in 1842 (Leigh 2004). In the Austrian Monarchy for example, superphosphate has been industrially produced since 1856 in Ústí nad Labem (currently in the Czech Republic, Vaněk *et al.* 2007).

Long-Term Grassland Fertilizer Experiments

There are some reasons that take grassland fertilizer application and nutrient status on a special position in practical agriculture as well as in agricultural research. As described earlier, grassland suffered throughout centuries from nutrient depletion through removal of organic material (dung and forage) and subsequent transfer to agricultural land or high productive lowland grassland. That is why historians declare “grassland as the mother of arable land”. In consequence, floristic composition and forage quality changed significantly in less-favored agricultural areas, often in favor of currently endangered species. Additionally, grassland forage is by nature only convertible through ruminants and, to a lower but not significant amount, by horses. In other words, accumulated nutrients in grass crops appear in organic fertilizers, hence providing the dominant nutrient source on dairy and beef farms. Albeit the availability of considerable amounts of recycled nutrients, agronomists introduced increasingly mineral and chemical-synthetic fertilizer with intensification of grassland during the last century. However, the effects of either nutrient source or a combination of both have not been known until that time, and so experimentalists set up fertilizer experiments that focused on a wide range of crop and soil parameters that were supposed to be influenced by nutrient application. Further, type and frequency of grassland utilization directly interferes with both the amount and date of nutrient supply during the growing season. In factorial field experiments, fertilizer application rate and type were therefore often combined with type and frequency of grassland defoliation through grazing and/or cutting.

The authors hypothesize that lessons learned from currently running long-term experiments can significantly contribute to improve our knowledge on fertilizer nutrient effects on grassland. Although problems of under nourishment of grassland have been solved in central Europe, de-intensification of agricultural land in view of nutrient preservation, eutrophication prevention and nature protection requires expert knowledge on fertilizer effects. Further, the key to successful grassland management lies in understanding the impact of defoliation and fertilizer application on inter-specific plant competition. However, although beef and milk production benefits from improved sward ameliorated through long-term nutrient addition, inter-species competition is still not well understood. The following description of selected long-term grassland experiments and scientific outcome provided by the scientists involved in experimentation shall demonstrate how these experiments can contribute to research at the interface of agriculture, soil science, botany, and landscape ecology. The intention of the presentation is to demonstrate value and potential contribution of long-term experiments to current and future scientific research.

In this chapter, experiments are summarized that are running for at least 40 years. Some of them were described in more detail, especially those which are not generally known to scientific community. Summarizing long-term experiments is a very difficult mission as results from many of them have never or only in local languages been published. Recently, many long-term experiments were closed worldwide as their potential for grassland research was not recognized and acquisition costs for maintaining experimental work increased substantially.

In 2006, the Grass Garden (GG) near Meadow Chalet (Semelová *et al.* 2008) composed of a piece of land fertilized with organic fertilizers, and an unfertilized control was identified in the sub-alpine vegetation zone of the Giant Mts. (Czech Republic). The highly contrasting plant species composition between fertilized and control plots was first described in 1786 (Haenke *et al.* 1791) and repeatedly during the 19th century. The oldest written record concerning GG was made in 1778, but GG was probably established together with Meadow Chalet in the second half of the 16th century. The positive effect of organic fertilizers (farmyard manure FYM, slurry, and woody ash) on biomass production and its forage quality was well known in the Giant Mts. already in the 18th century. In 1748, for example, grassland management instructions probably inspired by GG were laid down by count Spork, a progressive experimenter in agriculture and were released and applied to practice. According to these instructions, an intensively fertilized meadow, nicknamed "Grass Garden", should be established in the neighborhood of each mountain chalet.

GG near Meadow Chalet has been permanently marked on maps and has been delimited by boundary stones in the field. Therefore, the border between plots with different fertilizer regimes has been stable for at least the last 250 years and was accurate to a few decimeters (Fig. 4). Further, GG was permanently utilized by owners of the Meadow Chalet, who regularly fertilized the grassland year after year with mixtures of slurry and wood ash. Fertilized and unfertilized control plots were cut or occasionally grazed by livestock. Different fertilizer regimes were applied at least for 200 years until the management was terminated in 1944. Since that time, fertilized plots and surrounding grasslands have been set aside. Given these specific features, the GG was denoted probably as the oldest grassland fertilizer experiment in existence (Semelová *et al.* 2008).

With respect to species composition, *Deschampsia cespitosa* and *Avenella flexuosa* were dominant grasses in fertilized plot and *Nardus stricta* in unfertilized control plots during the last 220 years. This indicates high stability of differences in plant species composition created by contrasting long-term fertilizer regime under extreme climatic and soil conditions of the sub-alpine vegetation zone.

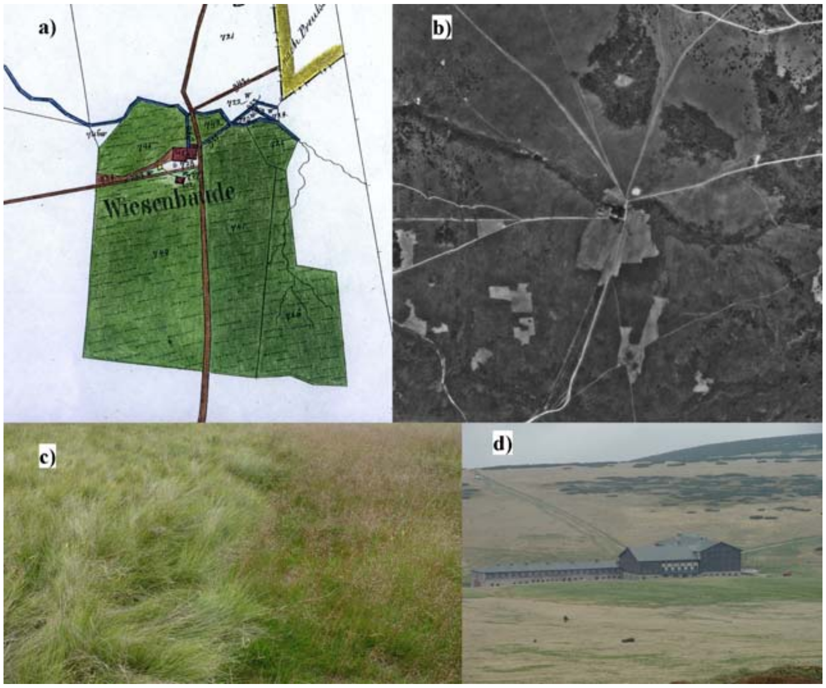

Figure 4. Cadastral map of Grass Garden (GG) near Meadow Chalet created in 1840–1841 (a), arial photograph of GG taken by the Czechoslovak army in summer 1936 - freshly cut plots are marked by a light color, most of the GG area was freshly cut (b), border between control and fertilized plot was still well visible and accurate to a few decimeters in summer 2006 (c), Meadow Chalet together with still well-visible GG (dark green color in front of the building) in spring 2004 (d) (Czech Republic, photo (c) and (d) Michal Hejcman).

The Park Grass Experiment (PGE, Fig. 5) set up by Lawes and Gilbert in Rothamsted (England, UK) in 1856 was recognized by Silvertown *et al.* (2006) as the oldest still running fertilizer experiment on permanent grassland in the world. According to author's knowledge, PGE is the best documented and published long-term grassland fertilizer experiment worldwide. More than 170 scientific papers and contributions have been published from this unique experiment until 2006. The experiment was established on homogeneous grassland on 2.8 ha area. Treatments imposed in 1856 included unfertilized control and various combinations of FYM with mineral P, K, Mg, Na, with N applied as either sodium nitrate or ammonium salts. The difference in soil acidity with the various nutrient inputs had a major effect on species composition measured in 1964. To extend the range of pH values, most plots were divided into four subplots with the aim to control surface soil pH values of 5, 6 and 7.

Figure 5. Aerial photograph of Park Grass Experiment taken in May 2005. (photo Rothamsted Research ©)

Still running Palace Leas hay meadow experiment in Cockle Park in North England was established on an experimental farm of the University in Newcastle in 1896 (Shiel 1986, Shiel & Batten 1988). The treatments used were based on what was considered forward-thinking best practice at the end of the 19th century. Nitrogen fertilizers were ammonium sulfate and sodium nitrate. Phosphorus fertilizer used since 1896 up to 1976 has been basic slag (syn. Thomas phosphate). In 1976, the only change of treatments occurred when basic slag was replaced by triple superphosphate supplying the same amount of phosphorus (100 kg N, 66 kg P_2O_5, 100 kg K_2O). In 2007, 14 treatment combinations of FYM, N, P and K fertilizers in total were available in this experiment creating a wide range of grassland ecosystem productivity, plant species composition and soil chemical properties.

Two prominent long-term grassland fertilizer experiments exist in Germany. The Rengen Grassland Experiment (Fig. 6) was established by Prof. Ernst Klapp on low productive grassland dominated by *Nardus stricta* and *Calluna vulgaris* in 1941 in the Eifel Mountains close to the border with Belgium (Schellberg *et al.* 1999, Hejcman *et al.* 2007a). In 1941, the soil of the study site was cultivated using a grubber in order to create a seed bed and was then reseeded with a mixture of productive grasses and herbs. Since then, fertilizer was applied in a single annual dressing every year except for nitrogen (two dressings). Six treatments combinations of Ca, N, P and K fertilizer were applied annually: an unfertilized control, Ca, CaN, CaNP, CaNP-KCl, and CaNP-K_2SO_4. Adjacent to that first block including 5 replicates each, a second ungrubbed block consisting of the same experimental treatments exists.

Figure 6. Arial photograph of the Rengen Grassland Experiment taken in late June 2005. This experiment is probably the oldest still running grassland fertilizer experiment with proper experimental design and publications in Germany. Fertilizer treatments were arranged in five complete randomized blocks (photo Michal Hejcman).

The "Weiherwiese" experiment was established on an alluvial meadow near the village Steinach (Bavaria, South East Germany) in 1933. Since that time, five treatments are still running under a two cut system. The last modification of the experiment was performed in 1971 so that 22 fertilizer treatments have been available since 1971, *i.e.* a combination of mineral N, P and K fertilizers with liquid manure application. Long-term fertilizer application created grassland plots considerably varying in aboveground biomass production ranging from 4 to 11 t of DM ha^{-1} (Diepolder *et al.* 2005).

In the 1960s, a series of long-term fertilizer experiments aiming to investigate the rational use of N fertilizers were established by Prof. Velich in the Czech Republic (Velich 1986). This series covered a wide gradient of grassland productivity from oligotrophic vegetation dominated by tuft grass *Nardus stricta* to alluvial highly productive meadows with dominant rhizomatous grass *Alopecurus pratensis*. The change in political regime in the 1990s resulted in low interest in fertilizer application research in post-communistic countries of Central Europe. As a consequence, the majority of long-term fertilizer experiments were closed not only in the Czech Republic, but also in Poland, Slovakia and Romania. The last still running long-term grassland fertilizer experiment in the Czech Republic exists near the village Černíkovice (35 km south of Prague, Fig. 7) and was established on a highly productive alluvial meadow in 1966 (Honsová *et al.* 2007). The following treatments arranged in four complete randomized blocks were applied under two and three cut management, unfertilized control, PK, $N_{50}PK$, $N_{100}PK$, $N_{150}PK$ and $N_{200}PK$ treatments. Ammonium nitrate (50 – 200 kg N ha^{-1}), superphosphate (40 kg P ha^{-1}) and potassium chloride (100 kg K ha^{-1}) have been applied from the start of the experiment onwards. Fertilizer application was terminated in half of each experimental plot in 1992 and since that time, residual effect of fertilizer application on grassland functioning have been investigated.

Figure 7. Original aim of the Černíkovice experiment was to investigate efficiency of nitrogen fertilizer application on biomass production, forage quality and nitrogen leaching. (Czech Republic, May 2005, photo Michal Hejcman).

Four long-term grassland fertilizer experiments were established in various regions in Slovakia on semi-natural grassland at altitudes ranging from 130 to 850 m a. s. l.. Two proportions of N:P:K applications were studied: 1:0.3:0.8 and 1:0.15:0.4. Results have shown that as an average across multiple years, cuts and sites, the increase in N rates from 50 to 200 kg ha^{-1} with both proportions of nutrients applications promoted a linear increase in dry matter production (Michalec *et al.* 2007). The last still running experiment from this series was established in 1961 in Veľká Lúka close to Zvolen town. In Austria, similar series of still running long-term grassland fertilizer experiments used for investigation of rational use of N fertilizers was established in 1960s (Trnka *et al.* 2006, Watzka *et al.* 2006). In Poland, the most prominent experiment is the Krynica (Szarny Potok) experiment established on low productive mountain grassland dominated by *Festuca rubra* and *Nardus stricta* in 1968. The experiment consists of seven treatment combinations of mineral N, P and K fertilizers in five replicates. All treatments were cut twice per year. Thirty five years of varied fertilizer application resulted in formation of completely different plant communities (Galka *et al.* 2005).

Effect of Fertilizer Application on Plant Species Composition of Grasslands: Short-Term versus Long-Term Experimental Results

When determining which nutrient primarily limits plant growth, fertilizer application experiments should be short, avoiding that indirect effects of a non-limiting nutrient emerge (Güsewell *et al.* 2002). On the other hand, the time dependence of fertilizer effects on plant species composition of grassland becomes visible only in long-term experiments where changes in species composition are monitored over a number of years. Generally, fertilizer application affects plant species composition by two ways, (1) directly by affecting physiological processes in individual plant species and (2) indirectly by change in competition for resources such us limiting nutrient, water and light among individual species. Over a long term, direct and indirect effects together generate a nutrient driven shift in plant species composition (Tilman, 1982). A direct toxic effect of high rates of applied N or P is known in stress tolerant species well adapted to nutrient poor conditions. *Nardus stricta* for example, survives much lower rates of applied P than *Avenella flexuosa* or tall growing forage grasses (Hejcman *et al.* 2007b). High levels of applied N are restricting spread and growth of legumes. Velich (1986) concluded that retreat of legumes in plots with high doses of N can be caused by different ways, (1) indirectly by competition with tall grasses, (2) directly by a high sensitivity of legumes to increased nitrate concentrations in the soil affecting inhibition of nitrogenase activity and feedback on glutamate pathway. Further, direct toxic effect of ammonium ions applied in fertilizer on several grassland moss species was reported by Solga & Frahm (2006).

Short and long-term effects of fertilizer application on plant species composition can differ substantially. Short-term N application can support tall grasses with high forage quality, but long-term N application on oligotrophic soils can result in predominance of unproductive species of miserable forage quality. This becomes obvious from two long-term grassland fertilizer experiments running for decades. In Rengen Grassland Experiment, sedges (*Carex panicea*, *Carex pilulifera*) and short grass *Briza media* are still dominant species in treatments with application of Ca and N after 64 years of fertilizer application (Hejcman *et al.* 2007a). A unique feature of Rengen Grassland Experiment, however, is the presence of orchid species in CaN treatment. Obviously, *Dactylorhiza maculata* and *Platanthera bifolia* were able to tolerate long-term application of ammonium nitrate. This finding denies frequently presented conclusion of observational studies about high toxicity of N fertilizers for orchid species. Many orchids are able to tolerate N application if this is not accompanied by an increase in biomass production under low soil P content. A relevant reason for a retreat of orchids from grasslands in the last decades is probably the application of N together with P, but not of N alone. Tall growing forage species were completely missing in CaN treatment in the Rengen Grassland Experiment although initial plant species composition was equal in all treatments. Tall growing grasses (*Alopecurus pratensis, Arrhenatherum elatius* and *Trisetum flavescens*) prevailed in treatments with CaNP or CaNPK application.

Similar results were obtained in Krinica experiment in Poland. Species characteristic for plant communities of oligotrophic acid soils (*Nardus stricta, Vaccinium myrtillus*) were found on plots fertilized with 90 kg N ha^{-1} year^{-1} but not in treatments with NPK application after 35 years of fertilizer application (Galka *et al.* 2005). In NPK treatments, tall grass (*Holcus mollis*) and dicotyledonous species characteristic for nutrients rich plant communities (*Alchemilla* sp. and *Chaerophyllum aromaticum*) were dominant. In the Park Grass Experiment, species typical of mountainous grasslands disappeared from plots receiving N, P, and K fertilizers. The addition of P reduced species richness, the application of K along with P reduced species richness further, but the biggest negative effects were found when N and P were applied together (Crawley *et al.* 2005).

Results from these experiments stress the importance of long-term studies in fertilizer research. Short term N fertilizer application can result in support of tall growing species and biomass production within several years following N treatment. But, in a long-term perspective N application can cause complete elimination of tall species from fertilized plots. Generally, N fertilizer application increases uptake of other nutrients which become limiting for highly productive species after several or many years of N application depending on initial soil conditions. Decades long N fertilizer application thus paradoxically supports species with high P use efficiency characteristic for low productive grasslands. According to Janssens *et al.* (1998), the highest number of species is found below the optimum P content of the soil for plant nutrition. Species rich grasslands highly valuated by nature conservation thus persist only in localities with limited P supply. Agronomists have learned, that long-term N fertilizer application can be used to increase P extraction from previously P fertilized grassland and so deplete soil P, induce P deficiency and restore species rich vegetation.

In many areas of Central or Western Europe, expansion of *Molinia caerulea* was revealed in semi-natural or naturally low productive grasslands in the last decades. *M. caerulea* is a grass that is well known to respond positively to N addition even when foliar N/P ratio is above 30, indicating strong P limitation to majority of other grassland species (Kirkham 2001). Massive expansion of this species was therefore connected with human activities which substantially increased atmospheric N deposition and so shifted natural ecosystems from N to P limited. Wassen *et al.* (2005) investigated species richness in herbaceous terrestrial ecosystems, sampled along a transect through temperate Eurasia representing a gradient of declining levels of N deposition from 50 kg ha^{-1} $year^{-1}$ in western Europe to natural background value of less than 5 kg ha^{-1} $year^{-1}$ in Siberia. It was demonstrated that many more endangered plant species persist under P than N limited conditions. Enhanced soil P content is more likely to be the cause of species loss than N enrichment. The conclusion that high P concentration in the soil is not consistent with high species richness was recently supported by many grassland studies performed on landscape level (see Klimek *et al.* 2007, Marini *et al.* 2007, Wellstein *et al.* 2007).

Application of P and K supports growth of legumes and thus increases the capacity for N fixation. In the Park Grass Experiment, proportions of grass, legumes and other species distinctly differed between treatments. Grasses consistently dominated (<90%) N fertilized plots, whereas legumes were abundant (> 30%) on plots receiving P and K but not N. Intermediate ratio of the three functional groups appeared on unfertilized plots (Silvertown *et al.* 2006). Although PK fertilizer application generally increases the portion of legumes in the sward, it increases inter-annual variability of plant species composition as well. After 40 years of experimentation in a long-term trial published by Honsová *et al.* (2007), a previously found positive effect of PK application on legumes (*i.e.* 10.1% and 4.2% in PK and control treatment, respectively) has not been found. The conclusion is, that legume’s persistence is limited in grassland as many species posses relatively short turn-over period. In a P and K unlimited ecosystem, nitrogen fixing legumes increase N availability and thus support growth of grasses and *vice versa* after N depletion.

It was often thought that N enrichment is detrimental to vascular plant species richness, but results from several long-term experiments supported that this is not necessarily so if N application is not accompanied by application of other growth limiting nutrient like P. Together with soil and climatic conditions, long-term fertilizer (NPK) application is a leading factor of plant species composition in semi-natural grasslands.

Residual Effect of Fertilizer application on Grassland Functioning

Although many grassland fertilizer experiments have been performed worldwide, information about residual effects of fertilize application on grassland ecosystem functioning is still rare. The reason is that probably many researchers terminated activities with cessation of fertilizer application in their experiments or looked for residual effects for several years only. Recently, some papers were published about decade’s long residual effect of fertilizer application in mountainous low productive grasslands on acid soils. In *Nardus stricta* dominated grassland, for example, residual effect of short term fertilizer application, P and Ca application especially, was detected even 40 years after the final nutrient addition. Hejcman *et al.* (2007b) recorded restricted cover of *Nardus stricta* on the benefit of other grasses with more rapid leaf growth (*Avenella flexuosa* and *Anthoxanthum alpinum*) almost 40 years after the last application in the Giant Mts. (Fig. 8).

Figure 8. The long-term experiment established and fertilized by Dr. Helena Štursová from 1964 to 1967 was rediscovered according to treatments with P application in 2004. *Nardus stricta* was still reduced in P treatments and so color of the sward was dark green hence highly contrasting with the almost yellow neighborhood (Czech Republic, photo Michal Hejcman).

In treatments that received quick lime (CaO), soil pH and Ca concentration was still increased and white concretions of $CaCO_3$ were revealed in several cases during soil sampling in 2004 (Fig. 9).

Figure 9. In acid low productive alpine grassland, lime can affect grassland functioning for many years after final application. White concretions represent calcium carbonate revealed in humus soil horizon 37 years after final application of quick lime. (Czech Republic, August 2004, photo Michal Hejcman).

Similar results were obtained from the experiment established by Dr. Werner Lüdy on alpine pasture near Interlaken in the Swiss Alps in the 1930s. In Ca treatments especially, species typical of low productive *Nardus* grassland were still reduced even after 40 years. The content of N and P in the leaves of selected species was still higher in plots once having received N or P (Hegg *et al.* 1992). The effect of Ca application on plant species composition, soil Ca content and soil pH was still detectable even after almost 70 years. The results of this long-term study indicate that the resilience of mountaineous ecosystems (the ability to re-establish original species composition after a disturbance or stress period; Lepš *et al.* 1982) may be particularly low in response to disturbance that substantially change soil pH or other key determinants of belowground processes (Spiegelberger *et al.* 2006).

A very prominent example of residual effect is Grass Garden in the Giant Mts. Increased Ca, Mg and P concentration in the soil and plant biomass was recorded there 62 years after the last fertilizer application (Semelová *et al.* 2008, Fig. 4). *Nardus stricta* was still the dominant species in unfertilized control and almost missing in previously fertilized plots. On the other hand, *Deschampsia cespitosa* together with *Avenella flexuosa* were dominant species in previously fertilized plots and almost missing in the control. In contrast to both studies discussed above, in which perturbation by fertilizer application was induced only over a short term, long-term fertilizer application in the case of GG had a long-term “stable after-effect” on differences in plant species composition. Results from the above studies and GG indicate that in the case of alpine grassland, resilience of a plant community after short-term perturbation by fertilizer application can be achieved after many years. However, a change of plant community after long-term perturbation can take more than several decades or may even be irreversible.

At lower altitudes with less extreme soil and climatic conditions, residual effect of fertilizer application is generally substantially shorter. In extreme, significant effect of fertilizer application on plant species composition can disappear within several years after termination of nutrient application. For example, although NPK fertilizer treatments created plant communities with different dominant species and the effect of treatment was highly significant in the last of seven years of nutrients application, differences among treatments were not significant two years after the cessation of nutrient application and cutting management (Pavlů *et al.* 2007). *Alopecurus pratensis*, a dominant grass in NPK treatments, as well as *Trifolium repens*, a dominant legume in PK treatments, almost disappeared and were replaced by *Festuca rubra* in all treatments. The reason for such a short residual effect in unmanaged swards was probably shallow soil profile and sandy soils developed on granite with very low sorption capacity. Nutrients, nitrogen especially, were probably quickly leached from the soil profile.

Although mineral nitrogen is considered highly mobile in the soil profile especially on sandy soils, Heyel & Day (2006) recorded enhanced biomass production of dune grassland ecosystem and increased N concentration in plant tissues nine years following N fertilizer application. Applied nitrogen was probably quickly incorporated into organic matter. Long-term retention of applied N in the ecosystem was primarily facilitated by increased biomass, predominately in roots and increased pools of plant litter. Willems & Nieuwstadt (1996) investigated long-term after-effects of fertilizer application on above-ground phytomass and species diversity in calcareous grassland in The Netherlands. Treatments previously receiving

9 years of fertilizer application (treatment $N_{170}P_{50}K_{50}$ and $N_{110}P_{350}K_{110}$, numbers indicate annually applied nutrients in kg ha^{-1}) initially highly fluctuated in productivity after cessation of N, P and K application. Biomass production comparable to control was reached in the sixth and eighth year after the last fertilizer application for the $N_{170}P_{50}K_{50}$ and $N_{110}P_{350}K_{110}$ treatments, respectively. In $N_{170}P_{50}K_{50}$ treatment, species number doubled and approached the control after 5 – 6 years thereafter remaining constant for the following year. In $N_{110}P_{350}K_{110}$ treatment, however, species number increased more slowly and reached the level of control only 14 years after the last fertilizer application. This was probably because of the grass/forbs ratio that had decreased to a level of 1 at slower rates than in $N_{170}P_{50}K_{50}$ treatment. In treatment with high amount of P, *Festuca rubra* gained dominance during an 8 year's period after the last fertilizer application although this grass is generally considered as an indicator of low nutrient levels. A further species richness in this experiment was driven by biomass production because of negative correlation between aboveground biomass production and number of species.

In Estonia, Wooded Meadow fertilizer experiment at Laelatu had been running on calcareous species rich grassland from 1961 to 1981 (Sammul *et al.* 2003). Four treatments were applied in a complete randomized block's design, control, $P_{26}K_{50}$, $N_{35}P_{26}K_{50}$ and $N_{100}P_{26}K_{50}$. Annual P and K dressings were 26 and 50 kg ha^{-1}, respectively. $N_{35}P_{26}K_{50}$ and $N_{100}P_{26}K_{50}$ treatments received additionally 35 and 100 kg N ha^{-1} annually. According to Kull & Zobel (1991), the productivity of some plots was still significantly higher and species density still lower as compared to the control even 8 years after termination of fertilizer application. Concentration of P in humus horizon was still increased in treatments receiving P, thus indicating long-term residual effect of P application. Niinemets & Kull (2005) published results obtained 8 – 17 years after termination of fertilizer application (Fig. 10).

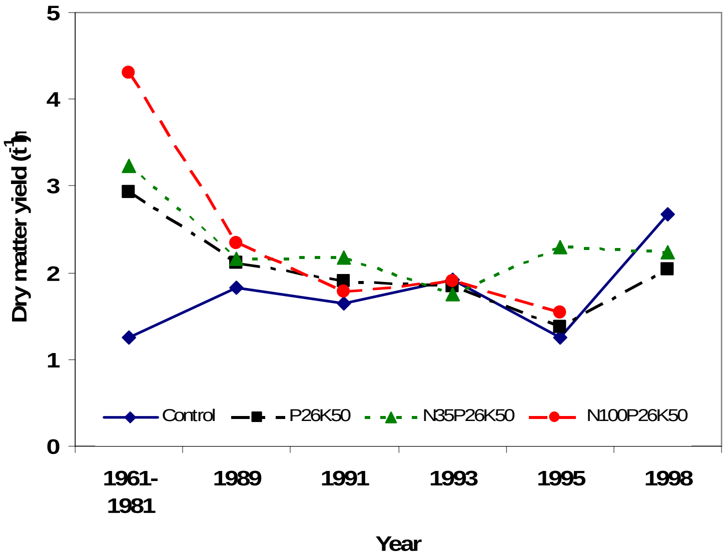

Figure 10. Aboveground biomass production in the Wooded Meadow fertilizer experiment at Laelatu in Estonia (based on data published by Niinemets & Kull 2005). Biomass production in the interval of years 1961 – 1981 is a mean of yields over the period of fertilizer application. Data collected in the interval 1989 – 1998 is a residual effect after termination of fertilizer application in 1981. Treatment abbreviations represent nutrients applied and numbers indicate annual application rates in kg ha^{-1}.

In this experiment, the residual effect of fertilizer application on biomass production was visible only in selected seasons. For example, the effect of $N_{35}P_{26}K_{50}$ treatment on biomass production was significantly different from the control in 1995. High variability in biomass production and varying results obtained throughout individual seasons stresses the importance of long-term studies in grassland research, mainly because conclusions based on the data obtained from one season can be misleading. There was a strong correlation between above-ground biomass productivity and soil P availability in all investigated years after termination of fertilizer application.

Mountford *et al.* (1993) investigated the effect of five nitrogen input treatments (0, 25, 50, 100 and 200 kg N ha^{-1}) applied for five years on species diversity of wetland site in England. P and K removed in biomass were replaced with the cut made in early July each year. Fertilizer nutrients promoted grasses, particularly *Lolium perenne* and *Holcus lanatus* which came to dominate the sward at the expense of most other species. In treatments receiving high levels of N, the sward became taller and species diversity declined. All *Carex*, *Juncus* and moss species became less common in all N fertilized treatments, producing apparently a more mesotrophic plant community. Short living species and wetland forbs also declined with increased N application probably because of shading. Legumes declined under high levels of N, but remained common in plots receiving 25 kg of N per ha. Further, Mountford *et al.* (1996) evaluated residual effect of N fertilizers throughout nearly four years after final application. Most species continued to be more abundant in the control than in treatments to which fertilizer was applied for five years. Reversion time required for regeneration of original plant species composition was estimated on 3, 5, 7 and 9 years for 25, 50, 100 and 200 kg N ha^{-1}, respectively. A 23 years of intensive NPK fertilization resulted in a drastic reduction of species diversity in sub-montane grassland in the Czech Republic (Královec & Prach 1997). The number of species per 4 x 2 m plot increased substantially from 5.6 in the last year of fertilizer application (320 kg N ha^{-1} applied annually) to 20.6 after six years without fertilizers under continuous cutting.

To join protection of endangered plant species in grasslands with requirements of farmers to produce adequate forage quantity and quality, sensitivity of target plant species to fertilizer input must be known. Silvertown *et al.* (1994) investigated effects of a range of replicated fertilizer treatments applied for six years and long-term after-effects of fertilizer application on the flowering population of green-winged orchid *Orchis morio*. Throughout six years, equivalent amounts of N, P and Mg and 80% of K of what was removed with the annual hay crop has been applied as inorganic fertilizers. As a result, a significant decrease in flowering spike numbers was observed, although the rates of N applied were relatively low (22 to 88 kg N ha^{-1}). Two applications of P at a rate of 40 kg ha^{-1} during the six year’s period greatly reduced flowering spike numbers, probably for a much longer period than 20 years. Except when 40 kg P ha^{-1} were applied, that decrease was closely related to an increase in biomass production following fertilizer application, suggesting that the remaining vegetation outcompeted *Orchis morio*. The effect of P on *Orchis morio* was out of all proportion to its effect on hay yield, suggesting that it may have been toxic to the orchid. Similar result was obtained by Dijk & Olff (1994) for *Dactylorhiza majalis*, a common orchid in wet European grasslands. The application of 250 kg N ha^{-1} (as NH_4NO_3) and/or 80 kg P ha^{-1} (as NaH_2PO_4)

per year was sufficient to decrease the frequency, total biomass and flowering of *Dactylorhiza majalis* in *Juncus acutiformis* meadow in the Netherland.

A decrease in the above ground biomass production after cessation of fertilizer application is generally considered as an important prerequisite for the development of high species richness in semi-natural grassland. The level of biomass production which must be achieved is evidently specific for individual grassland plant communities. Olff & Bakker (1991) recorded a major increase in species richness when the dry matter biomass production of semi-natural grasslands on sandy and peaty soils decreased below 4 t ha^{-1}. In this long-term study, a strong decrease in standing crop from 8 to 3 t ha^{-1} was observed on peaty soils whilst the initial biomass production of 3 t ha^{-1} did not decrease on sandy soils during 14 years after final fertilizer application. In a similar experiment by Oomes (1990), two moderately productive grasslands on humic sandy and heavy clay soils were monitored for 11 and 14 years after the fertilizer application was stopped. Monitoring was performed under a two cut regime. On sandy soil, the annual dry matter yield decreased from 10.2 to 6.5 t ha^{-1} in four years and to 4.1 t ha^{-1} after nine years without nutrient input. During the first three years, the dry matter yield on clay soil decreased from 10.2 to 5.0 t ha^{-1} and after 6 years, the production paradoxically increased again. In the lower productive grassland on sandy soil the number of species that invaded the sward was higher compared to clay soils.

It is clear that the rate of decline in productivity induced by termination of fertilizer application together with cutting and removal of biomass varies according to soil type and climatic region. Time necessary to induce nutrient deficiency in former intensively utilized grassland depends on yield-limiting nutrient content. In the case of K and N, yield limiting deficiency can be achieved within 2 – 10 years after cessation of fertilizer application. In the case of P, deficiency took about decades and can be hardly achieved in real time on low productive grasslands in harsh climatic conditions with low nutrient turnover. Such big differences among nutrients can be ascribed to different behavior and cycling of individual elements in the ecosystem.

In grassland, the cycling of N is a complex process and majority of N is fixed in soil organic matter (Whitehead 1995). Plants absorb only mineral N (NH^{4+} and NO_3^-). The soil pool of mineral N can be reduced by N leaching and plant uptake connected with subsequent biomass removal. In addition to export of N from the system, the pool of mineral N is a function of mineralizability of the soil organic matter, N fixation performed by legumes especially and air N deposition. Reducing the soil mineral N pool is therefore a long- term process directly linked with the pool of soil organic matter. In extreme soil and climatic conditions of alpine grasslands, increased concentration of N in biomass of selected plant species was recorded even 37, 40 and 62 years after final N fertilizer application (Hegg *et al.* 1992, Hejcman *et al.* 2007b, Semelová *et al.* 2008). The applied N was probably quickly incorporated into fungus protein preventing its leaching because of slow mineralization of organic matter under cold conditions.

K cycling and availability differ substantially between grassland soils. K is deficient especially on sandy and peaty soils with little clay content and/or limited sorption capacity. The cation exchange sites of a soil may readily absorb K ions from the soil solution and retain them in a form that is immobile and yet still available for plant uptake. If applied in high quantities, K shows higher leaching on sandy than on clay soils (Kayser & Isselstein

2005). If available in sufficient quantities in the soil, uptake of K is "luxury" as the plants generally take more K than they need for their nutrition. K deficiency can hardly be achieved under grazing management as about 90% of ingested K is excreted and returns to the pasture particularly with urine. However, under cutting management, K deficiency may arise as K is removed in higher quantities in plant biomass as compared to N. Residual after-effects of K fertilization are thus relatively short in contrast to other nutrients like P or Ca.

In low productive ecosystems, residual effect of P fertilization can take decades. In areas fertilized for a many years, substantial soil pool of plant unavailable P frequently establishes, especially on acid or calcareous soils. Most such P is bound in soil organic matter or in inorganic complexes, thus leaching of P from the soil profile is prevented. Depletion of total P pool by plant extraction is very slow even under continual removal of harvested biomass. In low productive grasslands, amount of P taken up by cutting is only several kilograms per ha annually (see Silvertown *et al.* 1994, Schellberg *et al.* 1999) and decrease of P concentration in soil solution is equilibrated by P release from plant unavailable forms.

Mechanism of P uptake by plants probably explains their preference for acid or alkaline soils. According to Tyler (1994), the inability of plants to solubilize phosphate compounds in limestone soils is a key factor in the calcifuge behaviour of plants. In this experiment, growth rate of calcifuge plant species (*Carex pilulifera*, *Avenella flexuosa*, *Holcus mollis, Luzula pilosa*, *Nardus stricta* and *Veronica officinalis*) which have been transplanted into limestone soil (pH 8) increased two to three times with the addition of $CaHPO_4$ as compared to untreated control where plants maintained symptoms of high P deficiency.

Conclusion

Some long-term experiments on grassland are rarely documented and can even not be discovered due to missing documentation and publication. Others, however, continuously provide scientific output that considerably contributes to agricultural science and teaching. The future of many long-term experiments in Europe is still open. As long as traditional grassland research is exclusively following up the initial research objectives that once have been set up decades ago, continuation cannot be verified in many cases. Seeking for research objectives that can compete with modern agricultural research is a prerequisite of maintaining management, sampling, plant and soil analyses as well as data storage, evaluation and publication. Of course, long-term experiments are very valuable due to long data series and uniform treatment application. Over and above, the long-term near equilibrium of soil-plant-nutrient interaction in many experiments provides extraordinary scientific value to recently announced future research priorities (Lemaire *et al.*, 2005) such as climate change impact on vegetation and global carbon sink and source.

Objectives that can be applied to those extraordinary field experiments are manifold. For example, as grassland by far exceeds the spatial extension of arable land worldwide, it is one of the most important sinks for carbon. Hence, the dynamics of carbon sequestration, accumulation and release can best be studied under *ceteris-paribus* conditions in long-term grassland experiments. Unfortunately, many long-term experiments cannot provide historical plant and soil material (an advantage that the PGE can offer) so that comparison across past ecological gradients cannot be considered. To the author’s knowledge, a common data base containing grassland experiments is still missing, but would be very useful to integrate experimental findings into a network leading to a higher level of knowledge. The Global Change and Terrestrial Ecosystems Soil Organic Matter Network (SOMNET, www.rothamsted.ac.uk/aen/somnet) provides information on long-term experiments, but by far most of them are placed on arable land. An initiative to consolidate experimental work on long-term grassland experiments is still missing.

As another example of research application, field plots under uniform long-term treatments provide soil and sward conditions that allow testing of the effect of applied experimental factors under differing environments such as soil microbial activity and respiration, nutrient turnover, transpiration as well as water and nutrient use efficiency. Many short-term grassland experiments fail to discover treatment effects due to adaptation of floristic composition and sward conditions on previously introduced treatments. Not so in long-term experiments, where near equilibrium can be considered. Further, there is no other opportunity to investigate satisfactorily the influence of climate on grassland productivity under ambient outdoor conditions than by studying long-term data series.

Further, long-term experiments on grassland provide the required facilities to study the impact of nutrient application on the environment and its consequences for animal and human nutrition. For example, it is often not considered that grassland forage is part of the food chain from beef and milk to human nutrition although food and feed safety is a high ranking issue not only in the delevoped countries. In this respect it is important that many fertilizers, P fertilizers especially, contain risk elements (As, Cd, Cr, Pb *etc.*) that are consumed by ruminants with grassland forage. Models predicting long-term behavior of such elements in the grassland ecosystems and their transfer into food chain can be validated only by means of long-term research.

Today, scientific potential of long-term grassland experiments is still underestimated as many of them have either already been terminated or will soon be given up without adequate publication of the results. The authors hope that this contribution will increase interest of scientists in long-term research hence paying attention to existing long-term grassland experiments. They provide an excellent basis for research and restore irreplaceable scientific information.

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Chapter V

Precision Fertilizer Management on Grassland

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Abstract

The recycling and utilization of animal excreta with slurry is an integral element of dairy farming on grassland. Slurry is the most important source of fertilizer nutrients on animal farms, its application, however, is still not satisfactorily solved. The major concern is that part of the applied nutrients are inefficiently utilized for plant growth due to a misbalance of local growth conditions within a grassland field and nutrients on offer. The authors therefore hypothesize that precision fertilizer management can help to improve nutrient use efficiency by reducing loss through site-specific management.

There is evidence that improper use of nutrients on agricultural fields potentially increases the amount of nutrients released to the environment. The need to improve nutrient handling by applying up-to-date agricultural technology is therefore described. Experimentalists and farmers are faced with the problem of how to determine within-field heterogeneity to which they can respond by using recently developed precision application techniques. Such heterogeneity pertains to soil as well as to crop characteristics. Hence, it is discussed if mapping procedures and sensors can be utilized to detect heterogeneity.

A slurry application technique is described and a layout of system components is presented based on current research. The impact of such technique can yet not be anticipated due to lack of experimentation. Therefore, a simulation model has been

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applied to estimate the effect to reduced losses through precision fertilizer application on nutrient budget and losses within a typical dairy farm on grassland in temperate climates.

Introduction

Precision agriculture (PA) technology has rapidly developed during the past decades especially on arable crops with the aim to facilitate economically feasible and sustainable handling of means of production and resources through site-specific management. Manifold applications of precision agriculture exist (*e.g.* precision livestock agriculture) and so some authors dispute that PA could more generally be explained as the use of information technology in all of agriculture (Plant, 2001). However, site-specific management is dominating within PA applications. It is widely accepted in research as well as in farm practice as a prospering discipline that enables to respond adequately to within-field heterogeneity with many kinds of field work, *e.g.* sowing, herbicide spraying, mechanical weeding, and fertilizer application. On grassland, PA has not yet developed like on arable land although in view of sustainable utilization of fertilizer it would bring about some advantages (Goulding *et al.*, 2007). The objective of the present paper is to introduce the state of the art of precision application of organic fertilizer on grassland and on forage crops based on currently published knowledge and own experimentation of the authors. The main focus is on the discussion of detecting field heterogeneity and the development of a technique that allows responding to that heterogeneity aiming to support adaptation of nutrient supply to local nutrient demand. Further, the authors put precision fertilizer application into context with the overall nutrient management on grassland farms and give an example of impact of that technology on nutrient budget.

The Need to Render Nutrient Management Precisely

Earlier scientific work emphasises that agricultural practise contributes significantly to the global nutrient cycle, eutrophication and N release to soil and atmosphere (Jarvis, 1993). Although the nutrient input with fertilizer and feed into agricultural farms in kilogram per hectare and year has increased crucially during the past decades, the nutrient use efficiency still remains low on a global scale (Bussink and Oenema, 1998). At the same time, the total annual amount of nutrients leached and volatilized is considerably high on intensively managed farms. N use efficiencies have been calculated in different ways, but the overall conclusion is that imported as well as internally produced N is converted into product N only to a little amount (Børsting *et al.*, 2003; Groot *et al.*, 2003; Schröder, 2005). Several studies indicate that a linear relationship exists between the N input on a grassland farm and the calculated surplus (Bleken *et al.*, 2005). Moreover, even if the overall farm nutrient budget is in balance, N turnover from plant biomass to beef and milk and, in parallel, to excreta can be very high and so can be the mobile N fraction that is exposed to leaching and volatilization.

Grassland forage is preferably utilized by ruminants bringing about two implications on nutrient cycle, surplus and handling, and all of it is relevant in precision agriculture. Firstly, ingested N is excreted for the most part and converted into mobile N fractions in faeces and urine. Additionally, recycling of ingested surplus N through blood, lever and saliva is an immanent characteristic of the rumino-hepathic cycle, leading to considerable N excretion rates mainly with urine when N is available in excess. In a two years study with suckler cow herds on permanent grassland, Schellberg *et al.* (2006) calculated that most of N ingested was excreted as urine, the most labile N fraction on farm. This fraction contributed annually between 20.3 and 27.5 % to the total N in circulation.

Secondly, due to the recycling of excreted N, slurry and farmyard manure are the major fertilizer types on grassland that require special attention in terms of storage, specification of nutrient content, as well as rate and technique of application. On the other hand, chemical-synthetic fertilizers play only a minor role or can even be neglected when the total farm budget is sustained by N_2 fixation through legumes and N deposition.

To underpin the importance of internal recycling of nutrients, an outline of the N flow on dairy farms is given in figure 1 for two situations, (i) in grassland regions where arable cropping is not suitable for climatic, topographic, and soil structural reasons and where grassland forage is the only resource for production compared to (ii) regions where arable cropping is practicable and advantageous. The existence of arable crops on a dairy farm is decisive in terms of the unloading of the farm N budget because nutrients in slurry can be converted into marketable products and so be exported in appropriate form. For example, with a cereal crop DM yield of 8 t ha^{-1} and a grain N content of 2.5 %, the total N export would be 200 kg ha^{-2}. If not marketed but internally converted to milk, the same harvest would substitute the equivalent amount of N in alternatively imported feed concentrates, provided that the energy concentration and related intake and milk production per unit of either feed would be the same. That way, internally converted self-produced feed concentrates can substantially donate to closed nutrient cycling within the dairy farm and to the N discharging of the environment.

On modern dairy farms, slurry is the main source of fertilizer nutrients containing significant amounts of primary and essential nutritive elements. From a total of 107 manure samples, Van Kessel and Reeves (2000) determined 4.5, 1.8, 0.9 and 2.9 kg m^{-3} of total N, NH_4^+, P and K in slurry, respectively. The excellent fertilizer value of slurry on grassland has been confirmed in earlier agricultural studies (*e.g.* Schröder, 2004). On the other hand, in many experiments nutrients in slurry have been identified as a potential source of losses to the environment, especially if excess amounts are applied beyond the use capacity of the soil or if improperly applied (Newton *et al.*, 2003, Thome *et al.*, 1993). But, even if applied properly, part of the N in slurry circulating across the dairy farm is inevitably lost to the environment, often referred to as unavoidable losses.

Slurry nutrients that are under control either in the cow house or in the container can be more efficiently handled by means of precision agriculture techniques as compared to current conventional management. In Figure 1, four tracks have been identified that allow control. The advantage of site-specific application is firstly to be seen in the release of a dosage of slurry N that matches the local requirements as precisely as possible. In contrast, the unawareness of site-specific requirements of N, P and K may lead to either local nutrient

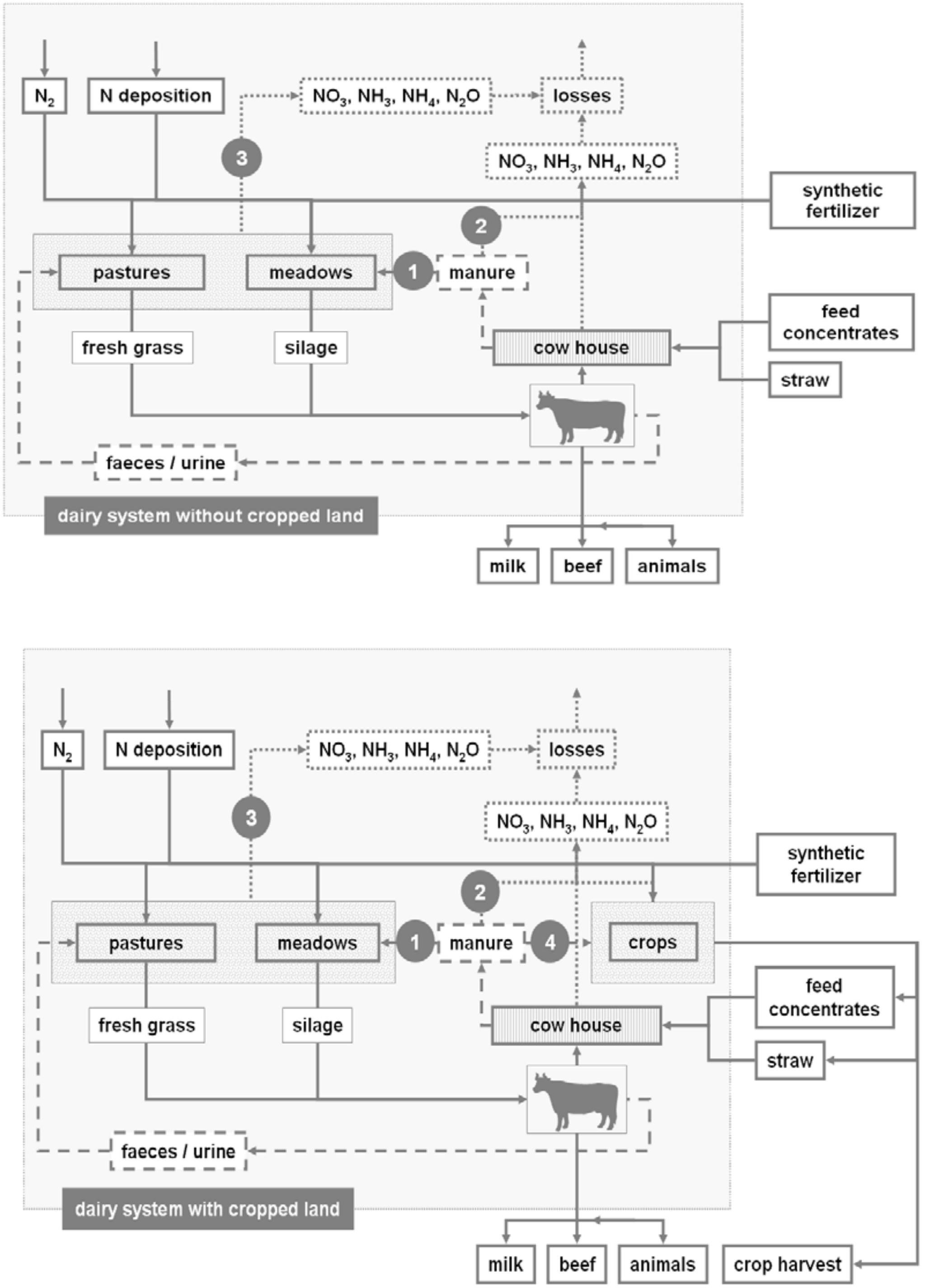

Figure 1. Generalized scheme of the nitrogen (N) flow in a dairy system without (upper) and with (lower) cropland. Dashed lines: flow of excretal N, dotted lines: path of N losses. Number 1 – 4 indicate paths of N that can be influenced by near-ground site-specific slurry application on either grassland or cropped land.

deficiency or surplus. Secondly, a side-effect of near-ground precision application techniques is that volatilization of NH_3 would be considerably reduced. Thirdly, if local surplus N is minimized by precise application procedure, N losses throughout the growing season that might appear due to soil N saturation will be reduced. It should be mentioned here that not all N flows can be encountered with precision agriculture techniques, although recent work has demonstrated that even faeces and urine spot distribution with cattle grazing can be controlled. Additionally, the correct calculation of aliquots of slurry applied to individual field plots by means of commercial PC software would already improve precision of nutrient management. Here it becomes obvious, that precision agriculture and conventional techniques should be linked and should not be separated from each other.

One approach to handle recycled N on farms in an environmentally friendly way is to calculate precisely the required N substitution after harvest on a per plot basis. With respect to uneven distribution of nutrients within farms, there is a need to monitor soil nutrient content from time to time. However, such approach does currently not consider heterogeneity within fields. The authors therefore hypothesize that site-specific application of nutrients can potentially better adapt to the local demand and so minimize losses. The question of precise management of nutrients on farms is not a new issue, but with site-specific application technology developing rapidly, the spatial dimension is increasingly recognised. Following best practise for nutrient management as recommended by Goulding *et al.* (2007) ("apply fertilizers evenly, and well away from watercourses"), site-specific slurry application is seen as a significant contribution to environmentally friendly and efficient nutrient use on grassland.

The Problem of Within-Field Heterogeneity

Per definition, site-specific management aims to control zones within fields that require variable adjustment of kind of treatment. Such management zones can be defined as portions of a field that expresses a homogeneous combination of yield limiting factors for which a single rate of a specific crop input or management intervention is appropriate (Doerge, 1998). For experimental reasons it might appear advantageous that field heterogeneity exists on which precision agriculture application under development can be appropriately tested. For site-specific management in practice, however, heterogeneity is difficult to handle as causes and effects on the spatial distribution of dry matter yield have not yet been sufficiently explored. In this context, within field heterogeneity on grassland mainly means the spatial variation in DM yield and forage quality on the one hand and in soil properties including nutrient status on the other. The demonstration of within-field variability of yield relies on the spatial resolution of yield data and subsequent geo-statistical data processing. Jordan *et al.* (2003) compared different sampling combinations on a silage field in Ireland that led to similar yield maps, except for the widest (50 m · 50 m rectangular) sampling pattern.

In contrast to arable land, grassland provides several growths and hence allows several fertilizer applications during the year, so temporal variability of yield is as well of great interest. There are indications that the spatial distribution among cuts of the same year can vary significantly (Jordan *et al.*, 2003). Bailey *et al.* (2001) concluded from variogram analyses on N yield in cut grassland that the pattern of variability of N yield remained quite constant with time. It can therefore be suggested, that N yield distribution maps can be considered as a calculatory basis for N application maps. However, the quality of information that can be derived from historical yield potential on sites within the field has not yet been sufficiently proven. The authors discuss spatial heterogeneity with respect to the mineralization-immobilisation-turnover (MIT) cycle (Whitehead, 1995) and, although causes of MIT variability still remain unclear, they propose to respond to it aiming to maximise N efficiency.

The spatial variation of soil nutrients differs with the chemical element under observation. Some have been identified as being quite conservative whereas others exhibit considerable change. Hence, as important as the spatial is the spatio-temporal distribution of soil and crop properties determining local yield as well as nutrient extraction and fertilizer application. Shi *et al.* (2002) observed on a grassland field in Ireland that nugget/sill ratios of soil variables in semi-variograms did not change significantly between two years with three samplings each. They also found that spatial distribution of soil extractable K exhibited not only the highest within-field variability but was also highly unstable over time, whereas pH, P, Mg and S were found temporally stable. Spatial distribution of soil fertility data were statistically and geostatistically analyzed also by Geypens *et al.* (1999) in 4 adjacent grassland fields and on 1 arable field in Belgium. Similarly to the previously mentioned study, K exhibited as well high coefficients of variation.

Only few experiments have focussed on spatial and temporal heterogeneity on grassland and so the present findings need to be confirmed across a wider range of environments and nutritional status. But, experiments already indicate that the strategy of soil sampling, if any is necessary, needs to be adapted to the expected coefficient of variation. The ranking of spatial variability of soil chemical properties can be summarized from current knowledge approximately as follows: pH < N < P < C < Mg < K, whereas the spatio-temporal follows: pH < N = C < Mg = S < P < K (Shi *et al.*, 2002).

The Response to Within-Field Heterogeneity – Site-Specific Fertilizer Application

Site-specific fertilizer application technique requires geographical position of features linked with information on amount of fertilizer to be applied within these features. In principle, three approaches to obtain the required information are conceivable. An application map for nutrients on grassland can be calculated on previously determined spatial yield distribution with the intention to simply substitute extracted nutrients, but with the restriction that the quality of prediction of historical yield data might be limited. Small forage harvesters carrying an integrated weighing system have been developed for grassland research that can rapidly cut samples and automatically separate aliquots for laboratory analyses. From such plant samples, the amount of nutrients to be substituted with fertilizers has then to be calculated either from additional nutrient analyses or through estimates of critical nutrient concentration (Lemaire and Gastal, 1997). Alternatively to yield measurements, a diagnosis of plant nutrient status based on small samples has been developed for highly productive perennial ryegrass (Beaufils, 1973; Walworth and Sumner, 1986; Bailey *et al.*, 1997a, Bailey *et al.*, 1997b; Bailey *et al.*, 2000). This technique has successfully been tested on grassland silage fields to predict the spatial distribution of nutrient sufficiency status, thus avoiding laborious and time consuming clipping of voluminous sward samples. The third approach is the one that uses N sensors (www.yara.de) aiming to obtain reflectance properties above canopies that allow the detection of local N deficiencies. However, this technique has only been tested successfully in cereals but not yet calibrated on species rich and diverse grassland. So, there is a need to verify that this technique is applicable on grassland where manifold interactions exist of spectral properties with floristic composition, standing biomass, sward density, height, and plant morphology.

At present there is no rapid technique available or sufficiently tested that allows to measure and map spatial yield distribution within fields without manual or mechanical clipping, except one development that is provided by a New Zealand enterprise, the rapid pasture meter (www.c-dax.com, www.farmworkspfs.co.nz). This instrument reads the pasture height by sensors that are mounted in a metal frame pulled through the pasture. However, the rapid pasture meter does not allow measurements in taller grass stands, *e.g.* in silage fields. Further, calibration is required to convert sensor data adequately into dry matter data depending on species composition and density of the sward. In conclusion, there is an important gap in technological development that hinders the site-specific application of fertilizers due to missing “ground truth information”.

Provided that the spatial and temporal heterogeneity of soil nutrient status and potential nutrient extraction could be either mapped or detected on-the-go, the problem remains how to calculate local N demand without knowing future N extraction of the momentary growing grass crop. Even with grassland growth models, the simulation of yield performance and N extraction would implicate intolerable uncertainty of prediction, as growing conditions cannot be anticipated over a long term. The response of yield and N extraction to fertilizer application and its variation throughout multiple years can be proven by long-term field trials (Hejcman and Schellberg, this issue). On grassland, such annual yield variation can mainly be explained by the response to changes in actual precipitation that is difficult or even impossible to predict.

Unless the above discussed uncertainties, the development of site-specific fertilizer application techniques has been considered attractive by research groups on species rich and frequently utilized grassland. With chemical-synthetic fertilizer, application technique will be the same on grassland as on arable land. However, with slurry as the main nutrient source on grassland farms, new application techniques need to be developed. Requirements of site-specific slurry application on grassland are listed in table 1. Albeit current difficulties to determine local nutrient demand, technical realisation of site-specific application is possible. For rapid determination of organic N and NH_4^+ content in slurry, the accuracy of traditional analyses have been compared in quick tests (Van Kessel and Reeves, 2000), where Quantofix-N-Volumeter (Klasse and Werner, 1987) performed best. Recently, a near-infrared reflectance spectroscopy (NIRS) sensor has been developed that allows rapid and comfortable measurements of N content during filling of the slurry tank (Dolud, 2005). This technique can potentially be applied also on the running tank trailer after calibration.

Table 1. Requirements of site-specific slurry application technique and a ranking of its technical standard and precision

	technical standard[1]	precision[2]
	detection of nutrient demand	
DM yield or nutrient yield map[3]	4	2
online detection of crop nutritional status[3]	5	n.a.
	slurry application	
determination of nutrient content in slurry	2	2
permanent homogenisation of slurry	1	n.a.
spreading technique	2	2
rapid slurry flow rate control	2	2
measurement of flow rate	2	4
	navigation and mapping	
measurement of travelling speed	1	1
high-resolution GNSS monitoring	1	1
as-applied-map	2	n.a.

[1] ranking from *easily achievable* = 1 to *laborious, time consuming or technically not yet available = 6*

[2] ranking from *sufficient* = 1 to *insufficient* = 6

[3] alternatively required

n.a. = information not available

With the development of trailing hose systems and injectors, precision of slurry distribution has been optimized. Transverse distribution has been tested on a test bench, indicating that most spreaders available in Germany and Austria showed good distribution even on slopes (Sauter, 2004). The measurement of actual flow rate is as well possible with good precision through electronic flow meters installed between tank and spreader. Navigation and mapping during application is not a challenge too, but the comparison of as-applied maps with application maps is still missing.

Based on the above discussed technical and methodological requirements, a flow diagram demonstrating the course of action in site-specific slurry application has been laid out in figure 2. The procedure follows current research activities at Bonn University (Germany). The technical realization of slurry flow control is based on three main components, (i) a control software that reads the amount of slurry to be applied in the digital map depending on the GNSS position in the field, (ii) a control valve linked to the micro-controller that steers the outlet of the slurry container and (iii) the flow meter that is checking the actual flow in a feedback loop. The design of an existing technique is given in figure 3. Preliminary testing of the system demonstrated that the development of the electronic control was most challenging.

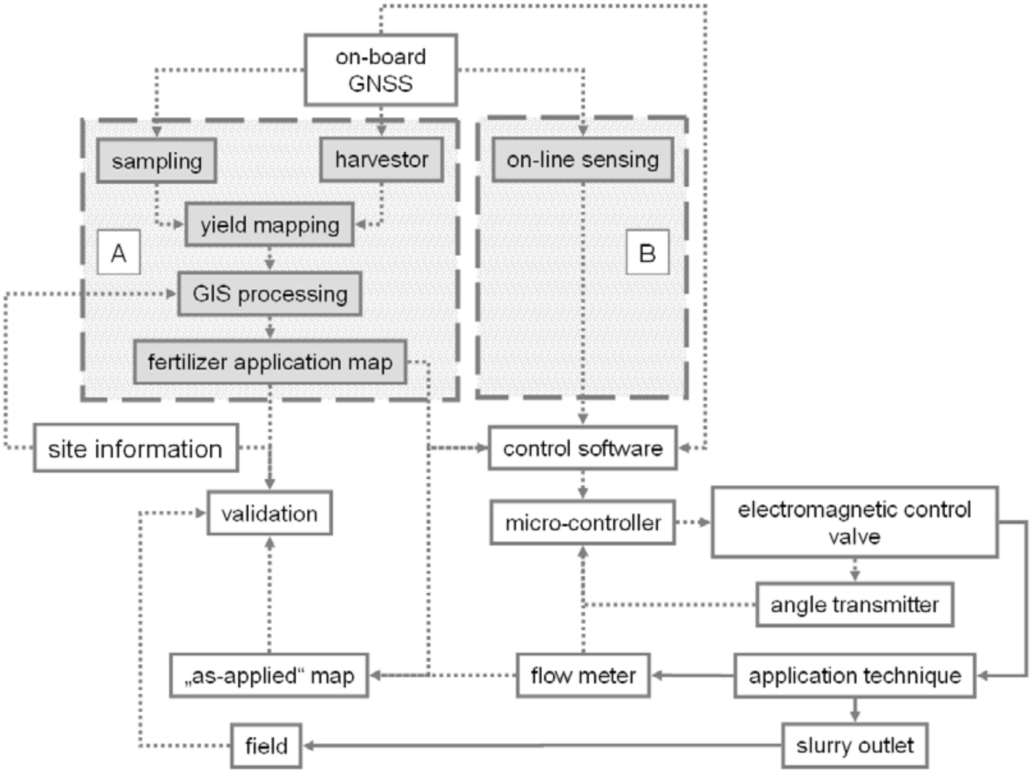

Figure 2. Scheme of a site-specific slurry application system based either on (A) “hard” data acquisition on crop status and yield through sampling or harvest mapping and (B) “soft” data acquisition of present crop status by means of close range or remotely sensed crop detection technology. Dotted lines indicate data flow, solid lines indicate flow of slurry.

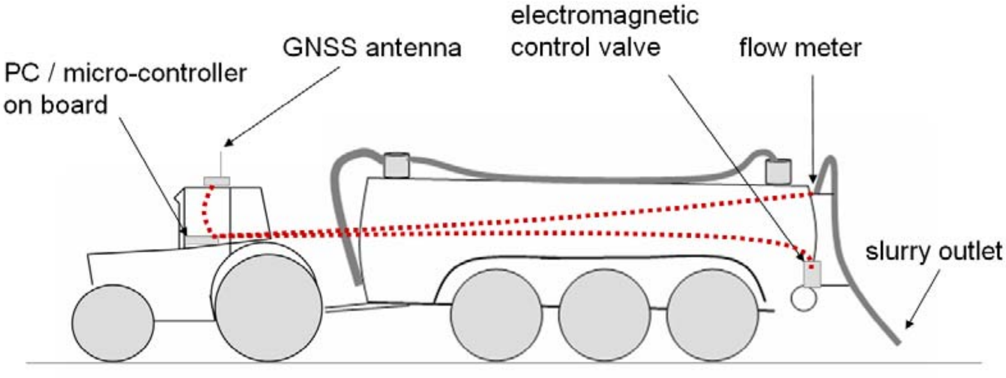

Figure 3. Design of a site-specific slurry application technique based on *a priori* calculation of an application map on grassland.

Impact of Precision Slurry Application on Farm Nutrient Cycling and Management

The inequality of slurry application in grassland or on forage fields in relation to local nutrient uptake is a major source of nutrient losses. Hence, fine-tuning of slurry application would reduce N losses but narrow the overall nutrient margin of the farm. Accordingly, farmers would have to reduce the import of additional fertilizer in case that the N export via losses would be reduced.

Simulation settings and procedure

To prove these hypotheses, the GRASFARM simulation model (Schellberg and Rademacher, 2003) was applied to simulate the impact of fine-tuning N application and reduction of N losses on the annual farm gate balance (N budget). N loss was meant as deriving from surplus N at locations within the field where N is applied according to the field average of N required but above local demand. The presupposition is that with growing field heterogeneity the probability increases that site-specific slurry application reduces N losses. In other words, if N is equally distributed although local potential for N uptake and yield varies considerably, much surplus N is leached. On the other hand, if N applied is locally lower than demand, yield would decrease but N losses would remain unaffected. The simulation study focussed only on nitrogen as one of the most prominent and yield limiting chemical elements in agricultural production.

Table 2. Parameter settings of simulation runs with the GRASFAM model. See text for details

parameter	value	unit
number of cows	100	
live weight of cow	650	kg
begin day of lactation	30	
end day of lactation	335	
N content milk	0.56	%
daily milk yield	25 - 35	kg cow^{-1} day^{-1}
energy concentration grassland forage	6.3	MJ NEL kg^{-1}
protein concentration grassland forage	180	g kg^{-1}
grassland dry matter yield	0.5 – 1.5	t ha^{-1}
silage dry matter on offer	14	kg cow^{-1} day^{-1}
pasture dry matter on offer	20	kg cow^{-1} day^{-1}
N_{min} loss total	variable	
N loss during slurry application	0.2	
N loss with urine on pasture	0.8	
N loss with faeces on pasture	0.1	
N deposition	20	kg ha^{-1} yr^{-1}
N_2 fixation rate per 1 % DM contribution of white clover	4	kg ha^{-1} yr^{-1}

Given the farm parameters in table 2, Monte Carlo simulations were conducted based on a normal distribution of N_{min} loss at an average of 20 % (parameter value 0.2) and a standard deviation of 0.05. Further, two scenarios were compared, a conventional slurry application against a reduced N loss strategy calculated without surplus N within sub-fields and with negligible losses. It is generally known that the N return to the field with slurry and the content of mineralized soil N is strongly dependant on stocking rate, yield potential and (feed intake dependent) milk yield. Hence, these parameters were varied in the simulation runs. It has to be mentioned here that a change in annual dry matter yield in the simulation model brings about a change in cow number per hectare. If the daily amount of forage on offer per cow is a constant value, increasing DM yield per unit area provides an increasing number of feed rations per year, hence increasing the maximum possible stocking rate. In the current simulation, the stocking rate on the farm increased by a factor of 0.16 per ton of available annual dry matter yield on one hectare.

Simulation results – Effects of minimizing N_{min} loss through site-specific fertilizer application on N budget

Lowering N_{min} losses increases the N farm gate balance (figure 4), confirming that the N margin on the virtual farm decreases. Increasing average daily milk yield generally performed higher N budgets, but the amount of N that can be saved by site-specific application through a reduction in N_{min} loss was calculated as being independent from milk yield, as indicated by parallelism of the curves in figure 4. At 20 kilogram milk per cow and day, the N budget (without chemical-synthetic fertilizer) was -30.8 and - 4.7 kg N ha^{-1} yr^{-1} without and with N loss reduction technology, respectively. With site-specific application of N, the budget was balanced already at about 25 kg milk, whereas this was the case with 20 % losses under conventional N application only at 28 kg.

Monte Carlo simulation – Variability of N budget

The question behind a subsequent Monte Carlo simulation was in how far the risk of failing the optimum N budget is influenced by reducing in N_{min} loss through site-specific application of N in organic fertilizer. The simulation study included two levels of milk yield (25 *vs* 35) and three levels of grassland productivity (5, 10, 15 t ha^{-1}) as indicated in table 3. A variation in N_{min} loss (0.2 $^{+}/_{-}$ 0.05 SD) as affected by site-specific slurry application significantly influences not only the average but also the variation of N budget. Results indicate that the effect of grassland area and related stocking rate on the grassland farm is strong in this respect. Albeit the effect of grassland productivity, mean N budget is also changed by milk yield, but the variation (*i.e.* standard deviation in table 2) remains much lower with 6.50 and 7.07 at 25 and 35 kg milk, respectively. Further, SD of N budget is high under extensive (5 t ha^{-1}) as compared to intensive production (15 t ha^{-1}), in other words, the risk that the N budget misses the optimum remains higher.

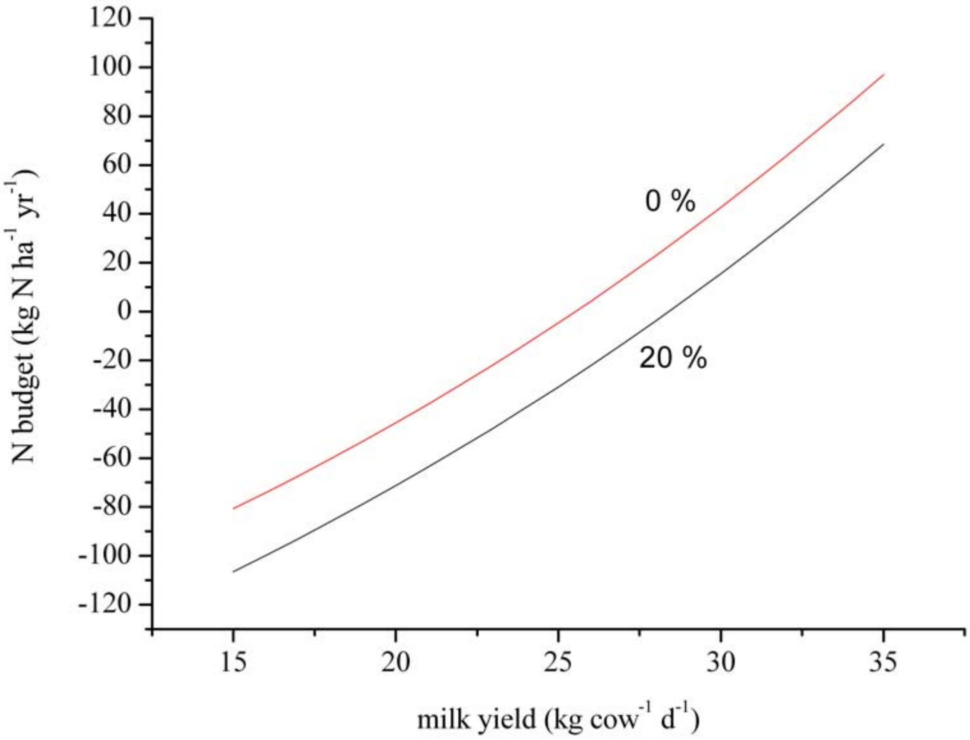

Figure 4. Change in annual N budget (B_N) on a grassland dairy farm without chemical-synthetic N fertilizers as a function of annual milk yield (Y_m) with (20%) and without (0 %) losses of mineralized soil N, demonstrating a conventional and a site-specific application technique of slurry, respectively. Result of GRASFARM simulation runs with parameter settings given in table 2 under *ceteris paribus* conditions. See text for further explanation.

Statistics:

$B_N = A + B\cdot M_Y + C\cdot M_Y^{\,2}$					
N_{min} loss (%)	A	B	C	SD	n
0	-145.7	2.43	0.128	0.150	21
20	-174.8	2.80	0.118	0.185	21

Table 3. Monte Carlo statistics of simulation runs (n = 999 permutations) on the effect of mineral N losses on N farm gate balance for given daily milk production and grassland yield.

daily milk production (kg d^{-1} cow^{-1})	25		25	35
DM yield t ha^{-1}	5	15	10	
mean N budget (kg N ha^{-1} yr^{-1})	- 65.0	4.1	-30.5	68.9
standard deviation	9.28	3.7	6.50	7.07
standard error	0.29	0.11	0.21	0.22
range	61.2	24.6	42.8	46.6

Conclusions

Precision fertilizer application must be seen as part of an integrated nutrient management system which includes a sequence of measures to improve use efficiency and to minimize losses. Figure 5 displays the interrelation of parameters that should be considered to calculate within farm nutrient distribution and per-field application on grassland. If precision agriculture is defined as generally applying new information technology on farms, the management of slurry may not solely pay attention to site-specific management. In fact, the entire procedure of slurry production, storage and field application illustrated in figure 5 would benefit from control by new technology. Present agricultural PC software allows the control over the animal stock, its feed intake, milk production and hence also its excretion of nutrients that appear in slurry (ref. 1 in figure 5). Further, it has been discussed in the present study that determining slurry nutrient content by NIRS technique will soon be applicable (ref. 2). The administration of agricultural field on farms by geographic information system (GIS) can simply be implemented and so can the planning of grazed and cut areas, the area and frequency of grassland under slurry fertilization as well as the underlying soil maps, topography and protected unfertilized areas within the watershed (ref. 3, 4). Some data evaluation software provide GIS linked data base within which nutrient balance calculation is possible including the estimation of N_2 fixation by legumes and local atmospheric deposition (ref. 5). A typical application of precision agriculture is that of sensing the canopy for characteristics that permit conclusions on its nutrient status (ref. 6) as discussed previously. Finally, an active site-specific control of nutrient application with slurry would contribute mainly to spatial precision of the described procedure (ref. 7).

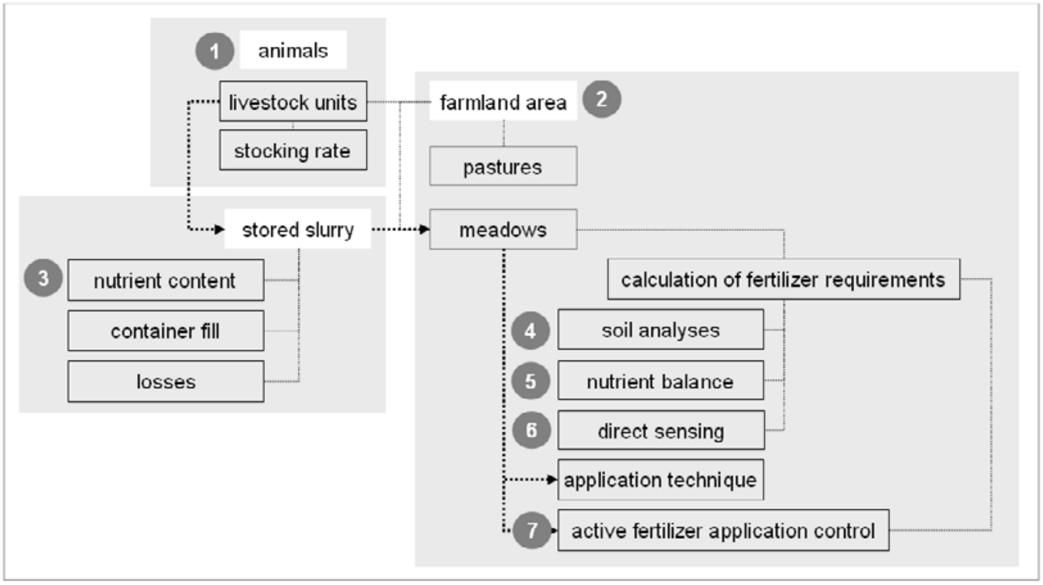

Figure 5. Interrelation of farm parameters relevant for calculation of nutrients applied with slurry on a grassland farm. Indices indicate working points of precision agriculture. See text for further explanation.

The significance of site-specific application technique in relation to other instruments and measures as presented here are difficult to assess. The key information is still missing to what extent site-specific application techniques contribute to efficient and environmentally friendly utilization of resources and means of production, although its potential to do so is without controversy. Due to lack of experimentation, the use efficiency of fertilizer nutrients in slurry when applied according to local demand can yet not be determined. Long-term field experimentation would be required to determine the nutrient input and output at numerous locations within a heterogeneous field. It is clear that the overall amount of nutrients applied would be exactly the same when either calculated as the sum of all sub-fields or as an average per entire field area. However, advisors and farmers would be enabled to more precisely determine actual nutrient demand using precision agriculture technology. It is also important in view of environmental compatibility of production that when applying nutrients site-specifically, local nutrient surplus on low productive sub-fields can be avoided and so losses be reduced. On the other hand, together with direct sensing of canopy nutrient status it will be most ambitious, expensive and only be practical and economically feasible on big farms or in cooperation with agricultural contractors.

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Chapter VI

Organic Fertilization as Resource for a Sustainable Agriculture

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Abstract

The application of conservative agricultural practices, as shallow tillage, crop rotations and organic amendments, could usefully sustain plant performances and increase soil fertility, thus playing an important role in the sustainable agriculture. Therefore, the aim of this research was to study the possibility to reduce the agronomic inputs, investigating the effects of different soil tillage, crop rotations and nitrogen (N) fertilization strategies on a rain-fed durum wheat crop. To accomplish this goal, a two-year field trial (2002 and 2004) was carried out in a typical Mediterranean environment (Apulia Region, Southern Italy), determining wheat yields and quality, N uptake, N utilization, plant N status and the most important soil properties.

In a split split-plot experimental design with three replications, two tillage depths, conventional (40-45 cm depth) and shallow (10-15 cm) were laid out in the main plots. Sub-plots were three two-year crop rotations, industrial tomato-durum wheat (TW), sugar beet-durum wheat (SBW) and sunflower-durum wheat (SW). Sub sub-plots (40 m^2 each) were the following N fertilization strategies which supplied durum wheat 100 kg N ha^{-1}: 1. mineral (Nmin); 2. organic (Ncomp), with Municipal Solid Waste (MSW) compost; 3. mixed (Nmix), with MSW compost and mineral N; 4. organic-mineral (Nslow), with a slow N release fertilizer. These treatments were compared with an unfertilized control (Contr).

The results showed no significant difference between the trial years in wheat yields and, between the soil tillage depths, poor variation in wheat grain production, protein content, grain and straw N uptake, thus showing the possibility to reduce the arable layer without negatively affect the durum wheat performances.

With reference to the crop rotations, grain yields significantly decreased in the following sequence: SBW, TW and SW, with 5.05, 4.74 and 4.52 t ha^{-1}, respectively, while grain protein contents sequence was: SW, SBW and TW (12.0, 11.2 and 10.6 %).

Among the N treatments, the most interesting responses were almost always obtained with Nmix, that showed not only the significantly highest grain yields (5.29 t ha^{-1}), but also protein content similar to that of Nmin treatment. The Ncomp achieved, in comparison with Nmin and Nmix treatments, lower grain yields, protein contents and total N uptake.

On the whole, these findings highlighted that only the partial substitution of the mineral fertilization with the organic one is a sustainable technique, to obtain good wheat performances and reduce the environmental risks due to the high levels of mineral fertilizers application. Furthermore, another useful advice for the farmers could be the determination of pre sowing mineral N, the application of MSW compost before wheat sowing and the continue monitoring of plants N status during cropping cycles.

Key Words: Winter Wheat; Mineral and Organic Fertilization; Yield; N Uptake and Efficiency; Plant and Soil N Indicators; Soil Properties

Introduction

The development of a sustainable agricultural system, but at the same time profitable, depends on both the enhancing of the nitrogen (N) utilization efficiency and the improvement of agricultural practices. Mahler et al. (1994) suggest that the maximization of N utilization efficiency is an increasingly important objective in the most cropping systems, because of economic and environmental pressures. The reduced soil tillage practices and the utilization of crop rotations are indispensable for improving resources sustainability, yet their long-term effects have to be still assessed (Lòpez-Bellido et al., 1997). In fact, the environment of many agricultural systems under Mediterranean conditions is highly deteriorated because of soil erosion and decreased fertility, as a consequence of high mineralization rate, leaching and runoff of fertilizers that pollute either the surface water, or the ground one.

Rasmussen and Collins (1991) indicate that conventional tillage with moldboard plow increases organic matter loss from soils, while no or minimum tillage systems increase the microbial activity. In addition, the physical processes that disrupt the soil structure, such as cultivation, may influence soil N mineralization and nitrification, due to their effects on soil porosity and aeration and because the physical disruption may bring microbial populations into contact with fresh, previously unavailable, soil substrate. Conversely, a deep tillage may be used to obtain a good seedbed, to kill weeds, to undo the damage caused by previous traffic over the land or to increase the permeability of the surface or subsoil layers, which will allow better aeration and drainage in the soil, improving root penetration and influencing water retention properties (Silgram and Shepherd, 1999).

Crop rotations usually increase soil organic matter and they have a marked influence on the N use efficiency and on the utilization of N sources. In fact, the rotation prompts changes in various N sources, affecting their availability for plants and, as a consequence, the N efficiency is greater when crop rotation is adopted, as found by different authors (Badaruddin and Meyer, 1994; Yamoah et al., 1998). Furthermore, its positive effects over monocultures are well documented (Mcewen et al., 1989; Christen et al., 1992) and, in dry areas, the grain yields are higher when cereals follow a legume as this saves N and breaks the disease cycle of grains. The effect of the preceding legume on the graminaceous crop yield is frequently quantified in terms of the amount of N required to obtain the same performance of the highest N fertilizer application (Peterson and Varvel, 1989). Conversely, there is a need for a major research effort to study the problem of crop rotations of wheat with the industrial crops in a semiarid climate, such as the Mediterranean region. In any case, sustainable agriculture has revitalized the interest in crop rotations and their effect on yield and N utilization efficiency to promoting profitable and efficient agriculture.

The amount of N fertilizer required under semiarid climates is largely affected by the seasonal rainfall and then the time, amount and source of N supply should be identified according to environmental conditions. As a consequence, to find the best time and amount of N fertilizer in different environments, the plant and soil N status as indicators of yielding performance should be developed. Jarvis et al. (1996) suggest that it is not possible to use just one N indicator to predict how much N will be released from the various organic N sources and therefore, when this fertilization occurred, a single, unique indicator could be an illusion. In addition, it is well know that the application of organic matter amendments to soil increases soil fertility (Sikola and Yakovchenko, 1996; Rees and Castle, 2002) and different authors (Eghball and Power, 1999a; Montemurro et al., 2005) found that phosphorus (P) and N supplied with the application of compost or manure resulted in similar grain yield. Therefore, the utilization compost to manage nutrient inputs for crop production presents certain challenges. Surface compost application may not be efficient as its incorporation because of additional N loss or nutrient stratification. Eghball (2000) reports that N mineralization was similar in no till and conventional tillage even though the compost was surface applied in no till. Eghball and Power (1999b) find similar corn yields in no till and conventional tillage in three of four trial years and, considering that compost application rates were similar in both tillage, they concluded that surface application of compost did not result in significant N loss. In this framework, several research has shown interesting results obtained with the application of Municipal Solid Waste (MSW) on different species (Eriksen et al., 1999; Montemurro et al., 2005; Montemurro et al., 2006a; Montemurro and Maiorana, 2007). However, there is still a lack of knowledge about the effects of different N fertilization strategies on wheat performance, N utilization and N status, especially when sustainable management (crop rotations and soil tillage) was adopted.

In the light of these considerations, the objectives of the present research were: i) to determine how soil tillage, crop rotations and MSW compost application affected yield, yield quality, N uptake and N utilization efficiency in durum wheat cropped under Mediterranean conditions; ii) to investigate the soil concentrations of organic matter, nutrients and potential toxic elements; iii) to study the possible use of N indicators for saving N supply in wheat production.

Material and Methods

The Site of Study and the Experimental Weather Conditions

The field experiments were conducted in Foggia (41° 27’ lat. N, 15° 36’ long. E, 90 m above sea level), in a typical flat area of Southern Italy, the Apulian “Tavoliere”, on the Experimental Farm of the Research Unit for Cropping Systems in Dry Environment. The main crops in the area are durum wheat, sugar beet, sorghum, maize, sunflower, industrial tomato, pulses or vegetables, while the cultural practices usually applied consist in deep soil tillage, plentiful use of mineral fertilizers, continuous cropping of cereals.

The soil is a Vertisol of alluvial origin, classified by Soil Taxonomy-USDA as Fine, Mesic, Typic Chromoxerert. Before the begin of the research, ten soil samples (0-50 cm layer) were taken from the whole experimental field, air dried, ground to pass a 2-mm sieve and then analyzed as follows for determining the most important soil parameters: total N = 1.22 g kg^{-1} (Kjeldahl digestion and distillation method); organic matter = 20.7 g kg^{-1} (Springer and Klee method); pH = 8.13 (1 : 2.5 soil water suspension, McLean, 1982); sand = 29.2 %, clay = 37.5 % and silt = 33.3 % (hydrometer method).

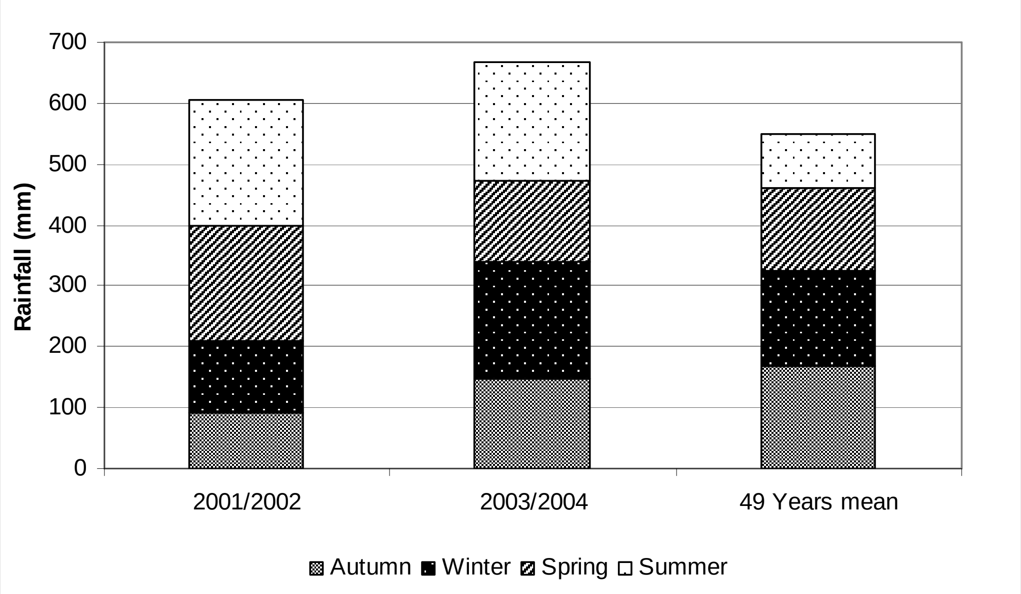

Figure 1. Annual and seasonal rainfall during the field experiment period and the long-term average (49 years).

The climate is “accentuated thermomediterranean”, as classified by UNESCO-FAO, with winter temperatures which can fall below 0 °C, summer temperatures which can rise above 40 °C and rains unevenly distributed during the year, being concentrated mainly in the winter months. Particularly, the weather during the trial period was characterized by total annual rainfall of 604.7 and 666.2 mm, respectively for 2002 and 2004, vs. 550.8 mm of the long-term averages 1952-2000 (figure 1). However, the difference between the experimental years

(2002 and 2004) and the long-term period was mainly due to the higher amount of rainfall recorded in the summer months. The average annual temperatures were 15.10 °C in 2002 and 14.86 °C in 2004, both lower than 15.43 °C of the 1952-2000 period. Furthermore, the weather variability was higher during the wheat cropping cycles (figure 2) than in the 2002 and 2004 trial years. In particular, the rainfall was 339.0, 557.1 and 491.4 mm for the September 2001 - June 2002, September 2003 - June 2004 and September - June of the long-term period (1952-2000), respectively. A high variability was also recorded for the mean annual temperature (13.18, 12.68 and 13.53 °C, respectively).

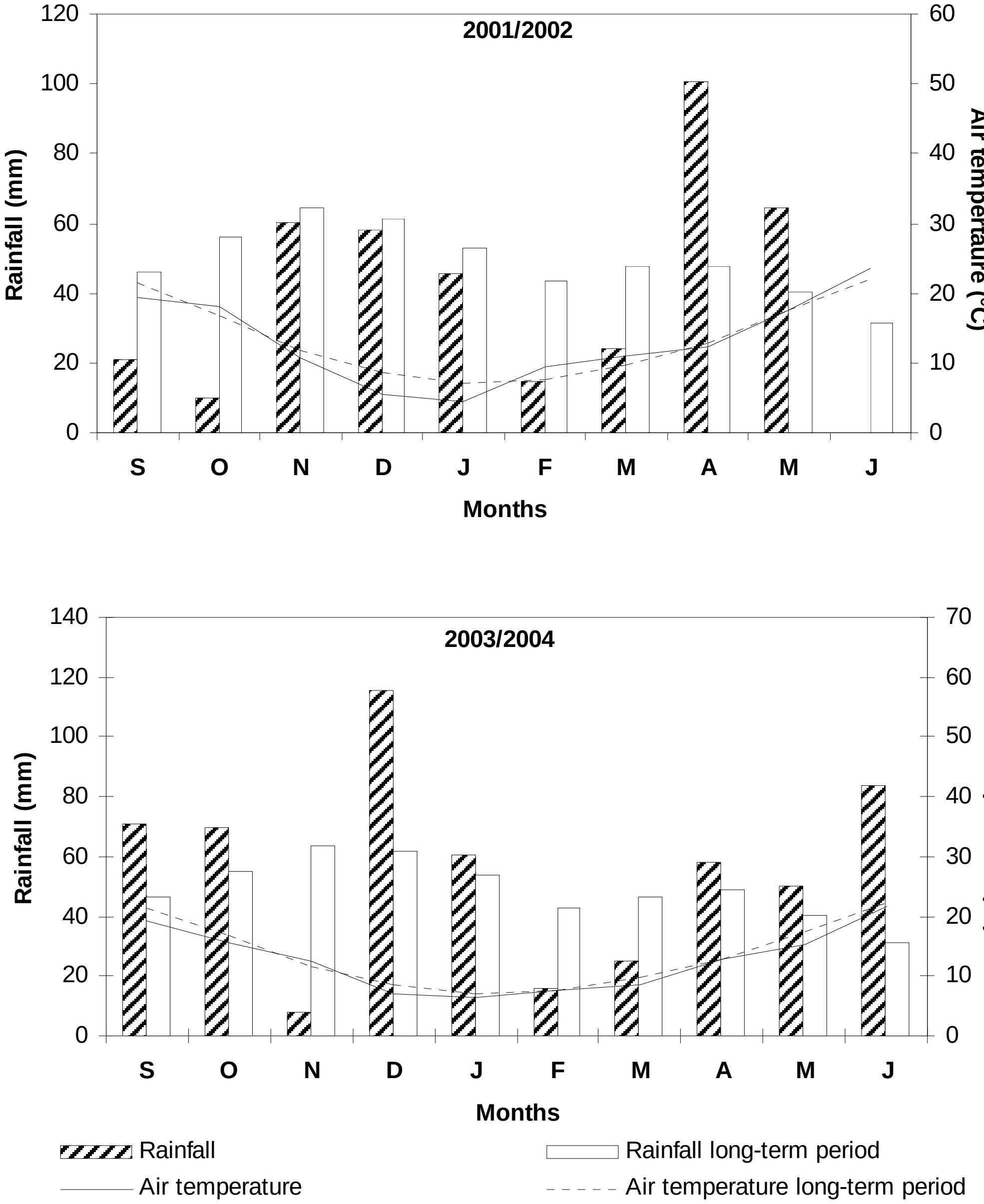

Figure 2. Monthly average air temperature and rainfall during the wheat cropping cycles (2001-2002 and 2003-2004) in comparison with the long-term period (1952-2000).

Experimental Treatments and MSW Characteristics

Durum wheat (*Triticum durum* Desf., cv. Simeto) was sown in the farming years 2001-2002 (indicated as 2002) on November 30, and 2003-2004 (2004) on November 17. In a randomized complete block experimental design with a split-split plot arrangement and three replications, the main plots were the following tillage systems: conventional tillage (indicated as CT) and shallow tillage (ST). The CT treatment included moldboard plowing (40-45 cm deep), disk harrowing and vibrating tine cultivator to prepare a proper seedbed. The ST treatment consisted of two disk harrowing and vibrating tine cultivator (10-15 cm deep) before plant sowing. Sub-plots were three two-year rotations: industrial tomato-durum wheat (TW); sugar beet-durum wheat (SBW); sunflower-durum wheat (SW).

Sub sub-plots of 40 m^2 each (8 by 5 m) were the following different strategies of N fertilization, that supplied durum wheat 100 kg N ha^{-1}: mineral (Nmin); organic, with MSW compost application (Ncomp); mixed (Nmix), with 50 kg N ha^{-1} of organic N (MSW compost) and 50 kg N ha^{-1} of mineral N; organic-mineral slow N release fertilizer (Nslow). These fertilizing treatments were all compared with an unfertilized control (Contr). For Nmin, mineral N was applied in two equal amounts at the wheat sowing (as ammonium sulfate) and at 75 (as ammonium nitrate) days after sowing (DAS) in 2002. In 2004, the second share of mineral N was distributed at 111 DAS. The MSW compost was uniformly broadcasted for Ncomp (100 kg ha^{-1} of organic N) in one solution about one month before sowing. In Nmix, the MSW compost was spread about one month before sowing (50 kg ha^{-1} of organic N), while the mineral N fertilizer (50 kg ha^{-1} as ammonium nitrate) was applied during plants growth at the same date of Nmin treatment (75 and 111 DAS for 2002 and 2004, respectively). In Nslow treatment, the organic-mineral slow N release fertilizer was all broadcasted at the sowing. Its composition was: total N = 290 g kg^{-1}; organic N = 50 g kg^{-1}; mineral N (as urea) = 240 g kg^{-1}; total organic carbon = 180 g kg^{-1}; organic matter = 310 g kg^{-1}.

The P fertilizer (50 kg P_2O_5 ha^{-1}) was applied at the time of main soil plowing, whereas no K fertilizer was broadcasted in both years, since Southern Italy soils often contain good amounts of this element.

The same compost was used in both years and was industrially obtained through the aerobic transformation of Municipal Solid Waste by selective collection. The composting process was based on mechanical separation of selected organic materials aimed to recuperate the organic fraction suitable for composting. This fraction was submitted to be ground by a hammer mill followed by a sieving process. Finally, the material was composted in an open space and both the homogenization and oxygenation processes were ensured by means of a continuous monitoring of humidity and temperature and by turning over the material. The main chemical characteristics of MSW were as follows (expressed on dry matter obtained at 40 °C): total N = 1.47 mg kg^{-1}; Cu = 330 mg kg^{-1}; Zn = 751 mg kg^{-1}; Pb = 670 mg kg^{-1}; Ni = 217 mg kg^{-1}; total organic carbon = 13.75 g kg^{-1}; extracted total organic = 7.67 g kg^{-1}; humified organic carbon = 2.51 g kg^{-1}; C/N = 9.55. Total, extracted and humified organic carbon were determined according to the Springer and Klee method, modified by Sequi et al. (1986), whereas heavy metals content was determined by atomic absorption spectrometry, according to Page et al. (1982) methodologies. Despite the use of the organic fraction of

MSW, the compost presented heavy metal amounts higher than the current limit values allowed by the Italian legislation (Legislative decree No 217, 2006). In spite of that, this compost was used in order to investigate, in much unfavorable conditions, the heavy metals accumulation in the soil.

Plant and Soil Measurements at the Harvesting and during Wheat Cropping Cycles

In both trial years, during the wheat plant growth, at the main phenological phases (tillering, internodes elongation, booting, anthesis and milky maturity stage), plant samples were harvested to determine the following plant indicators: the Leaf Area Index (LAI), the leaves' green index (SPAD readings, Minolta 502), the nitrate content of the stems base (Nitrachek, MERCK) and the total N content of the different parts of plant (Fison CHN elemental analyzer mod. EA 1108). The SPAD readings were measured at the mid-length on fully expanded leaves from 10 plants and the nitrate content was measured at the stems base of the same plants, whereas the LAI and the total N content were determined on one linear meter of plants. Furthermore, the pre sowing soil mineral N ($N\text{-}NO_3$ + $N\text{-}NH_4$ exchangeable, recorded at 0-40 cm soil depth) was recorded before sowing of both each experimental year and elementary plot, as soil N indicator.

At the anthesis stage and at the harvesting time of every trial year, one linear meter of plants was randomly harvested from each elementary plot, divided into leaves, stems and grain (at the end of cycles), weighed and dried for 48 h at 70 °C to determine their dry weights. The total N content of the different parts of the plant was determined (Fison CHN elemental analyzer mod. EA 1108) for allowing the calculation of total N uptake (N content x biomass dry weight), as the sum of each part of the plant N uptake. Moreover, at the harvest (210 and 231 days after sowing for 2002 and 2004, respectively), plants were tested for wheat performance. Straw and grain yields were determined in the middle of each plot on an area of about 20 m^2, while on sub sample of grain the protein content was also calculated multiplying grain N concentration by 5.83 (Baker, 1979).

On the basis of these measurements, the following N efficiency indices were calculated:

1. pre anthesis N uptake (plant N uptake at anthesis, expressed in kg ha^{-1});
2. post anthesis N uptake (difference between plant N uptake at maturity and N uptake at anthesis, in kg ha^{-1});
3. nitrogen utilization efficiency "NUE", (ratio of grain weight to N uptake, in kg kg^{-1});
4. N harvest index "NHI" (ratio of grain N uptake to plant N uptake, in %);
5. nitrogen agronomic efficiency "NAE" as the ratio of (yield at Nx - control yield) to applied nitrogen (kg kg^{-1});
6. physiological efficiency "PE", as the ratio of (yield at Nx - control yield) to (N uptake at Nx - control N uptake) (kg kg^{-1});
7. apparent recovery fraction "ARF", as the ratio of (N uptake at Nx - control N uptake) to applied nitrogen (%).

8. N translocation (difference between plant N uptake at anthesis and straw N uptake, in kg ha^{-1});
9. N translocation efficiency (ratio of N translocation to plant N uptake at anthesis, in %).

These parameters were defined according to Delogu et al. (1998), Lòpez-Bellido et al. (2005) and Montemurro et al. (2006b) terminologies.

At the beginning (t0) and at the end (tf) of the experiment, on soil samples (0-40 cm layer), the total, extracted and humified organic carbon, the main chemical characteristics and the heavy metals content were determined. The soil samples were taken, air dried, ground to pass a 2-mm sieve and then analyzed using the same procedures applied for the characterization of the organic amendments, while the available P was determined by the Olsen and Sommers method, exchangeable K by the Thomas method and total N by the Kjeldahl method. These determinations were made in the unfertilized control (Contr) and in the Nmin and Ncomp treatments, which supplied plants the highest dose (100 kg ha^{-1}) of mineral or organic N (MSW compost).

Statistical Analysis

The data obtained in the two-year trial period were subjected to analysis of variance (ANOVA), using a randomized complete block design combined over years and the SAS procedures (SAS Institute, 1990). The differences among the experimental treatments were compared using the protected Least Significant Differences (LSD) and the Duncan Multiple Range Test (DMRT) tests, for two or more than two mean comparisons at the $P<0.05$ probability level. The Pearson correlation coefficients were used to correlate the wheat performance (grain, straw and protein content), grain and straw N uptake with the yields, N uptake and N utilization efficiency parameters. The same coefficients were used to correlate wheat performance with plant and soil N indicators. The differences found with the LSD and the DMRT tests for different main effect and interaction comparisons were calculated using the appropriate standard error term. Finally, the arcosin transformation of data was used to evaluate statistical differences among variables expressed as percentages.

Results and Discussion

Effects of Treatments on Wheat Yields, Protein Content and N Uptake

Durum wheat grain yield showed no significant difference between both the years and the soil tillage (table 1), even if the rainfall distribution during the wheat cropping cycles was substantially different (figure 2). Lòpez-Bellido et al. (2000) indicate that wheat yield in the wet years was lower under no-till than conventional soil tillage, while the opposite was found in the dry years, in which the effect of tillage was insignificant or less favorable with the deep plow. Conversely, our results pointed out the lack of differences between conventional and shallow tillage in grain production (4.87 and 4.67 t ha^{-1}, respectively), in agreement with the findings of Lòpez-Bellido et al. (1997).

Table 1. Grain and straw yields, protein content and N uptake (grain, straw, pre anthesis and post anthesis) as affected by years, soil tillage, crop rotations and N fertilization strategies.

Treatments	Parameters						
	Yield		Protein content	N uptake			
	Grain	Straw		Grain	Straw	Pre anthesis	Post anthesis
	t ha^{-1}	t ha^{-1}	%	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}
Years							
2002	4.81	6.21b	12.1a	101.3a	52.2a	109.0	44.5a
2004	4.74	7.28a	10.5b	88.4b	44.2b	117.4	15.1b
Soil tillage							
Conventional	4.87	6.70	11.5	98.7	50.2	112.8	36.2a
Shallow	4.67	6.79	11.1	91.0	46.2	113.6	23.5b
Crop rotations							
TW	4.74b	6.94a	10.6c	88.5b	37.1c	112.2	13.4c
SBW	5.05a	7.07a	11.2b	100.6a	57.8a	113.2	45.4a
SW	4.52b	6.22b	12.0a	95.4ab	49.6b	114.2	30.7b
N fertilizations							
Nmin	4.92b	6.99a	11.7	101.5a	53.9a	127.3a	28.1ab
Ncomp	4.44c	6.35b	11.1	86.6b	42.1c	97.5b	31.2ab
Nmix	5.29a	7.07a	11.5	107.0a	50.9ab	126.6a	31.2ab
Nslow	5.25ab	7.00a	11.2	103.8a	48.9ac	135.3a	17.4b
Contr	3.97c	6.31b	10.8	75.4c	45.1bc	79.3c	41.2a
Mean	47.7	6.74	11.3	94.8	48.2	113.2	29.8

Note: within years, soil tillage, crop rotations and N fertilization strategies, the values in each column followed by a different letter are significantly different according to LSD (two values) and DMRT (more than two values) at the $P \leq 0.05$ probability level.

Referring to the crop rotations tested, grain and straw yields decreased in the following sequence: SBW, TW and SW (5.05, 4.74 and 4.52 t ha^{-1} for grain, and 7.07, 6.94 and 6.22 t ha^{-1} for straw, respectively), that pointed out the worst productive values obtained by the wheat after sunflower crops, confirming the results found by Lòpez-Bellido et al. (1997). The responses obtained in our experiment indicated that the effect of sugar beet as preceding crop in achieving a high degree of N utilization may be attributed to its ability to exploit mineral N in the deepest soil horizons, part of this mineral N being restored to the soil and becoming available for the following crop. Among the N strategies, the highest yielding performance (5.29 t ha^{-1}) was obtained with the Nmix treatment, suggesting that multiple compost applications, when associated with mineral N fertilizer, are able to increase wheat yield.

Singer et al. (2004) also suggest that the distribution of organic materials could eliminate yielding differences between conventional and no-till systems. The same authors point out that N uptake alone may not be responsible of productive responses, but that N uptake efficiency, from improved soil physical, chemical and biological properties, may increase the yields in crop produced on compost-amended soil.

The significantly highest value of grain protein content was observed in 2002 trial year (12.1 vs. 10.5% of 2004), whereas no variation was found between the soil tillage depths. This qualitative parameter decreased in the following crop rotations sequence: SW, SBW and TW (12.0, 11.2 and 10.6%, respectively), always with significant differences. Therefore, wheat yield after sugar beet ensured the best grain yield and a good protein level (table 1). On the contrary, no significant difference was found among N treatments, even if Nmin and Nmix showed values higher of the 8.3 and 6.5% than the unfertilized control.

The main problem for a farmer is to ensure that a bread-wheat can achieve the required quality standards, without recourse to high use of N fertilizer. Lòpez-Bellido et al. (2004) reported that farmers growing for quality have no way of knowing or ensuring that a wheat crop will make the protein standard other than by applying extra fertilizer as insurance. The results of our study indicated that there is no difference in the grain protein when mineral N fertilizer is partially substituted by organic amendment, with the possibility to reduce the environmental risks associated with the high level of conventional N supply (table 1).

Lòpez-Bellido et al. (2004) indicate that a large proportion of the N in grain is remobilized from leaves and stems after anthesis, rather than being taken up from the soil. Accordingly, it is reasonable to expect that the N concentration of shoots or leaves at anthesis might be good indicators of the N concentration in grain at harvesting. Our results showed that whilst most of the N is remobilized at anthesis from shoots to grain (79.5% of pre anthesis N uptake of the total N uptake), this amount can be supplemented by post anthesis by 20.5% of the total N uptake (table 1). Furthermore, the pre anthesis N uptake in winter wheat was the highest share of total N uptake in the plant at harvesting and it was highly correlated with grain yield (table 2), according with the results of Heitholt et al. (1990) and of Montemurro et al. (2007). No significant difference was found for this parameter between years, soil tillage and crop rotations, whereas among the N treatments the significantly highest values were observed in Nmin, Nmix and Nslow, with 127.3, 126.6 and 135.3 kg ha^{-1}, respectively. The regression of pre anthesis N uptake and grain yield is reported in figure 3. In our study, significant and polynomial relationship ($R^2 = 0.5048$) was found between these parameters, confirming the importance of the first phases of winter wheat crop for the grain production. In addition, when the conditions of soil fertility (total N = 1.22 g kg^{-1} and organic matter = 20.7 g kg^{-1}) are good and the total annual rainfall is enough and consistent with long-term period (figure 1) as in this research, even the post anthesis N uptake is important because it is positively correlated with protein content (table 2). The unfertilized treatment (Contr) reached the highest value (41.2 kg ha^{-1}), confirming the results of Perez et al. (1983). The regression of post anthesis N uptake and protein content is reported in figure 4. In this study, a significant and polynomial relationship ($R^2 = 0.5537$) was found between these parameters, even if it is well known that the post anthesis N uptake is more influenced by environmental conditions (high temperature, heavy rainfall and soil moisture) than the pre anthesis N uptake (Papakosta and Gagianas, 1991).

Table 2. Correlation coefficients among grain and straw yields, protein content and N uptake (grain and straw) with N uptake and N utilization efficiency parameters.

	Grain yield	Straw yield	Protein content	Grain N uptake	Straw N uptake
Grain yield	-	0.459 ***	0.151 n.s.	0.784 ***	0.255 ***
Straw yield	0.459 ***	-	-0.165 *	0.218 **	0.332 ***
Protein content	0.151 n.s.	-0.165 *	-	0.720 ***	0.242 ***
Grain N uptake	0.784 ***	0.218 **	0.720 ***	-	0.334 ***
Straw N uptake	0.255 ***	0.332 ***	0.242 ***	0.334 ***	-
Pre anthesis N uptake	0.474 ***	0.339 ***	0.146 n.s.	0.431 ***	0.244 ***
Post anthesis N uptake	0.167 *	-0.026 n.s.	0.433 ***	0.383 ***	0.457 ***
N translocation	0.345 ***	0.172 *	0.025 n.s.	0.263 ***	-0.256 ***
Translocation efficiency	0.193 *	0.032 n.s.	-0.069 n.s.	0.099 n.s.	-0.566 ***
NUE	0.099 n.s.	-0.026 n.s.	-0.713 ***	-0.377 ***	-0.711 ***
NHI	0.331 ***	-0.193 **	0.303 ***	0.406 ***	-0.698 ***
NAE	0.502 ***	0.288 ***	0.006 n.s.	0.318 ***	0.147 n.s.
NPE	0.203 *	0.095 n.s.	-0.150 n.s.	0.026 n.s.	-0.051 n.s.
ARF	0.393 ***	0.261 ***	0.491 ***	0.596 ***	0.396 ***

*, **, *** = Significant at the P<0.05, 0.01 and 0.001 probability levels, respectively. n.s.=not significant.

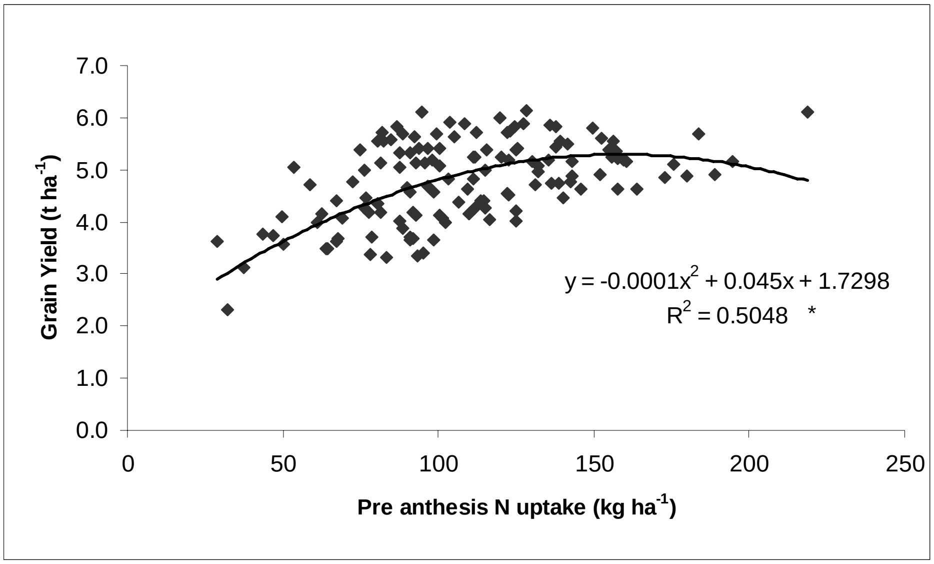

Figure 3. Regression coefficients between grain yield and pre anthesis N uptake.
* = Significant at the $P<0.05$ probability level.

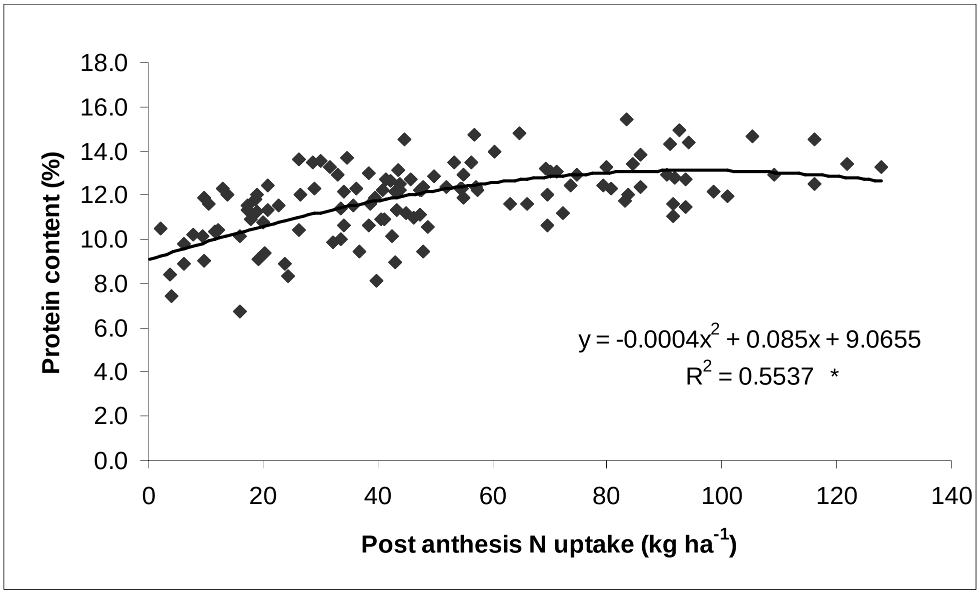

Figure 4. Regression coefficients between protein content and post anthesis N uptake.
* = Significant at the $P<0.05$ probability level.

Effects of Treatments on N Utilization Efficiency

Table 3 shows the N utilization parameters as affected by years, soil tillage, crop rotations and N fertilization strategies. No significant difference was found between years for NUE, NHI and ARF, while NAE, NPE, N translocation and translocation efficiency showed higher values in 2004 compared to 2002 year. Therefore, the higher amount of rains fell during wheat cycle in the second experimental year (339.0 and 557.1 mm for 2002 and 2004, respectively) (figure 2) seems to increase the N translocation and, as a consequence, the N agronomical and physiological efficiencies. The most important N efficiency parameters recorded in this research did not point out any significant difference between CT and ST. Conversely, Lòpez-Bellido and Lòpez-Bellido (2001) found that N use efficiency and N uptake were greater in conventional than in no tillage system. As mean value, the NUE parameter showed a low absolute level (33.4 kg kg^{-1}) and no correlation with grain and straw yields (table 2), reflecting a poor crop use of N fertilizer (Lòpez-Bellido and Lòpez-Bellido, 2001; Montemurro et al., 2007). This low value, consistent with other studies (Delogu et al., 1998; Lòpez-Bellido et al., 2005), indicated that durum wheat plants require high N rate to optimize yield and, as a consequence it could be difficult to obtain great performance and good agro ecosystem expectations with this crop in Mediterranean conditions. Furthermore, our results highlighted that there was no significant difference among the N treatments in NUE, indicating that the mineral N fertilizer, with the lowest NUE value (32.8 kg kg^{-1}), did not increase the efficiency of N translocation in yield, in comparison with the organic amendment. The Nmin and Nmix treatments showed no significant difference in the other N utilization efficiency parameters (NHI, NAE, NPE and ARF) (table 3), apart from in N uptake (grain, straw, pre and post anthesis) (table 1). In particular, the Nmix treatment reached the highest NAE value (13.3 kg kg^{-1}), which is an indicator of the amount of yield per unit of N fertilizer applied and it could be considered an index of plants ability related to N fertilization (Delogu et al., 1998). Our results have demonstrated the importance of this parameter in wheat performance, as confirmed by the high, positive and significant correlation with the grain yield (table 2). Besides, the NAE of Nmix treatment was significantly higher than Ncomp and greater of the 40.0% than Nmin. As a consequence, the partial substitution of mineral fertilizer with organic one increased N utilization and could also aid to understand the plant N response, thus optimizing the mineral N fertilization and reducing the risk of ground water pollution (Mahler et al., 1994). The first dose of N application, spread in the fall, one month before sowing (as organic) or during the seed bad preparation (as mineral), did not modify the N final utilization, because of no significant difference recorded between Nmin and Nmix treatments in all N utilization efficiency parameters. In fact, Lòpez-Bellido et al. (2005) report that a low fertilizer efficiency has been attributed only to the timing of application, especially when fertilizer N was applied in the fall. The decline in efficiency of N utilization and N uptake is indicative of substantial losses of mineral N, and justifies reduced N rates in the fall, particularly in situations of high residual N levels. Spring N application as a top dressing prior to stem elongation can increase fertilizer N recovery, maintain or enhance N utilization efficiency in comparison with the only fall application (Mossedaq and Smith, 1994; Sowers et al., 1994a). In addition, it should be borne in mind that the applied N rate used in the calculation of NAE and ARF was the total N fertilizer rate, i.e. 100 kg N ha^{-1}, without no difference between organic and mineral N supplies. As a consequence, considering that no significant difference in NHI, NAE, NPE and ARF was found for Nmin and Nmix treatments, it could be concluded that the partial substitution of mineral with organic N did not modify its efficiency. Conversely, the Ncomp treatment not only showed low values of total and grain N uptake (table 1) but also of N utilization efficiency parameters (table 3) when compared with Nmin and Nmix treatments. Therefore, the N applications split between fall and spring, as occurred in both Nmin and Nmix, increased yields, N use efficiency and N uptake efficiency in comparison with fall application in winter wheat under temperate conditions (Mahler et al., 1994; Sowers et al., 1994a). On the contrary, our results highlighted that the total substitution of mineral with organic N fertilizer (Ncomp treatment) decreased yields, N uptake and N efficiency.

Table 3. N utilization efficiency parameters as affected by years, soil tillage, crop rotations and N fertilization strategies.

Treatments	Parameters						
	NUE	NHI	NAE	NPE	ARF	N translocation	Translocation efficiency
	kg kg^{-1}	%	kg kg^{-1}	kg kg^{-1}	%	kg ha^{-1}	%
Years							
2002	32.4	67.1	8.1b	21.4b	29.3	56.8b	47.6b
2004	36.4	65.9	12.1a	35.9a	32.7	73.2a	58.9a
Soil tillage							
Conventional	33.9	66.6	8.3b	20.7b	30.1	62.5	50.6
Shallow	34.9	66.4	11.9a	36.6a	31.9	67.5	55.9
Crop rotations							
TW	38.5a	70.5a	7.0b	25.5	17.2c	75.0a	64.3a
SBW	32.4b	63.2b	8.8b	33.4	29.5b	55.4b	44.8b
SW	32.2b	65.8b	14.4a	27.0	46.2a	64.6ab	50.6b
N fertilizations							
Nmin	32.8	65.8ab	9.5a	28.1a	37.8a	73.4a	56.1a
Ncomp	35.4	67.2ab	4.7b	13.7b	15.0b	55.4b	54.1a
Nmix	34.2	68.0a	13.3a	35.7a	37.4a	75.7a	58.9a
Nslow	35.5	68.6a	12.8a	37.1a	33.9a	86.4a	60.8a
Contr	33.9	62.8b	-	-	-	34.2c	36.3b
Mean	34.4	66.5	10.1	28.6	31.0	65.0	53.2

Note: within years, soil tillage, crop rotations and N fertilization strategies, the values in each column followed by a different letter are significantly different according to LSD (two values) and DMRT (more than two values) at the $P \leq 0.05$ probability level.

Effects of the MSW Applications on the Main Soil Characteristics at the End of the Experiment

Averaging across years, crop rotations and soil tillage, the repeated applications of MSW compost increased both organic matter and mineral elements soil levels (table 4). In particular, the total and extracted organic carbon contents increased at the end of experiment in respect to the initial values (17.4 and 16.4%, respectively), whereas the humified carbon values reached similar levels. Conversely, the soil tillage depths did not influence the final levels of soil organic matter (total, extracted and humified), according to the results of Lòpez-Bellido et al. (1997). However, an increase of total organic carbon was found in Ncomp in respect to Nmin and Contr treatments when applied with the conventional soil tillage (14.68, 13.83 and 12.99 g kg^{-1}, respectively), while no substantial variation was observed for the shallow soil tillage. A similar and significant behavior was observed for the extracted (8.43, 6.81 and 7.01 g kg^{-1}) and humified (5.73, 4.26 and 4.66 g kg^{-1}) organic carbon. These findings pointed out the importance of the organic amendment incorporation in semiarid conditions, in which the high mineralization rate could decrease the soil organic matter in the shallow layer. Our results are in agreement with those of Silgram and Shepherd (1999) which suggest either that different tillage practices result in fundamentally different depth distributions of organic residues in soils, or that in minimum tillage systems it is possible an accumulation of organic carbon near the soil surface with a higher possibility of mineralization. Therefore, a deeper incorporation of MSW compost could ensure high soil fertility and minimize agricultural impact of cultivation on the environment through sequestration of organic carbon, reducing erosion and preserving soil biodiversity (Six et al., 2002).

Nitrate content was significantly different between shallow and conventional tillage (15.84 and 26.86 mg kg^{-1}, respectively) (table 4). This result confirmed the findings of Silgram and Shepherd (1999) which report differences in mineralization rates as function of the tillage system and, in particular, conventional tillage, being associated with a higher mineralization than reduced tillage. This behavior could be a consequence of a higher plowing occurred in conventional tillage which tends to decrease aggregate size and introduce a more oxidized environment, which will accelerate the organic matter mineralization. The high soil N mineral content recorded at the end of experiment when conventional tillage is adopted reveals the presence of plentiful N resources that should be borne in mind in establishing N fertilization schemes for crops under highly variable climatic conditions, including scant rainfall such as those of the Mediterranean region. Furthermore, in both soil tillage, significant higher values of nitrate were found in the Nmin treatment compared to the Ncomp and the unfertilized control, indicating that a share of mineral N supplied in Nmin remains unused in the soil at the end of wheat cropping cycle. According to Mcewen et al. (1989), ammonium content in the soil was scarcely affected by the tillage depths, while a significant increase was found only in the shallow tillage with the Ncomp treatment. However, two of the most pertinent questions in sustainable agriculture are the compatible application of the soil shallow tillage and the ultimate fate of the observed increases in ammonium. Silgram and Shepherd (1999) suggested that this amount could remain more labile and simply serve as a temporary store for a pool of ready mineralized N that may pose future environmental problems when it is remineralized once the soil is ploughed again.

Table 4. Total, extracted and humified organic carbon, chemical soil characteristics and potentially toxic elements at the beginning (t0) and at the end (tf) of the experiment divided by experimental treatments.

Chemical determinations	Mean				tf					
	t0	tf	tf		Shallow tillage			Conventional tillage		
			Shallow tillage	Conventional tillage	Control	Nmin	Ncomp	Control	Nmin	Ncomp
Total organic carbon (g kg^{-1})	11.84	13.90	13.96	13.83	14.32	13.32	14.25	12.99b	13.83ab	14.68a
Total extracted carbon (g kg^{-1})	6.52	7.59	7.76	7.42	7.37	7.75	8.17	7.01b	6.81b	8.43a
Humified organic carbon (g kg^{-1})	4.89	4.86	4.83	4.88	4.46	5.17	4.86	4.66b	4.26b	5.73a
Nitrate (mg kg^{-1})	14.24	21.35	15.84b	26.86a	9.32b	28.34a	9.85b	16.72b	41.15a	22.70b
Ammonium (mg kg^{-1})	2.29	1.47	1.46	1.48	1.16b	1.21b	2.01a	1.49	1.45	1.50
Available P (mg kg^{-1})	12.06	10.05	10.19	9.91	8.70b	10.00b	11.87a	8.66b	9.60b	11.47a
Exchangeable K (mg kg^{-1})	1648	1740	1761	1719	1637b	1786ab	1860a	1688b	1609b	1861a
Cu (mg kg^{-1})	27.58	28.73	29.24	28.21	29.41	28.32	29.99	28.23	27.55	28.85
Zn (mg kg^{-1})	65.19	68.70	69.57	67.84	68.34	68.52	71.84	65.81	67.09	70.62
Pb (mg kg^{-1})	16.49	19.09	20.47	17.71	19.84	22.59	18.98	16.04	18.59	18.49
Ni (mg kg^{-1})	23.04	23.44	23.61	23.27	23.84	23.32	23.67	23.67	22.91	23.23

Note: within soil tillage and N fertilization strategies, the values in each row followed by a different letter are significantly different according to LSD (two values) and DMRT (more than two values) at the $P \leq 0.05$ probability level.

The available P decreased from t0 and tf (12.06 and 10.05 mg kg^{-1}, respectively). This behavior was mainly due to the reduction of available P in the Contr and Nmin treatments, whereas its value in Ncomp was similar to the starting value in both shallow and conventional tillage (11.87 and 11.47 mg kg^{-1}, respectively). In particular, this higher level of available P in Ncomp treatment at the end of the experiment showed that the quantity of mineral fertilizer applied before sowing (50 kg P_2O_5 ha^{-1}) in each trial year was not enough to sustain wheat production. Therefore, the amount of this element in MSW compost was necessary to maintain the initial soil fertility.

As found for available P, no significant variation was observed for exchangeable K between both t0 and tf and the two soil tillage systems.

The highest values of exchangeable K were observed in the Ncomp treatment in both conventional and shallow tillage (1860 and 1861 mg kg^{-1}), confirming the possibility of using organic waste compost as fertilizer, as found in other crops (Montemurro et al., 2006a).

Notwithstanding the high starting value of heavy metals (Cu, Zn, Pd and Ni) present in the MSW compost used in our research, no significant difference was found for these potentially toxic elements in both shallow and conventional tillage among the fertilizing treatments, indicating the possible use of MSW compost in a short-term cropping system. The same results were found by Murillo et al. (1997) which pointed out that the increase in heavy metals concentration could only be present after repeated compost applications for a large number of years. This behavior was probably due to the dilution of the elements in the soil, as suggested by Montemurro et al. (2005). Conversely, although not significant, the Zn content increased in Ncomp by 5.1 and 7.3% in comparison with the unfertilized control, respectively, for the two soil tillage depths. Therefore, the results of this study showed that it is possible to sustain the wheat yield and increase soil fertility with MSW compost only for a limited number of applications (Montemurro et al., 2005; Montemurro et al., 2006a; Montemurro and Maiorana, 2007), without induce no risks on the environment, even if it is necessary to monitor over the years the levels of the potentially toxic elements in both soil and plants.

Use of Plant and Soil N Indicators to Save N Supply in Wheat Production

Table 5 reports the correlation coefficients among yields (grain and straw), protein content and N uptake (grain and straw) with plant and soil N indicators. The SPAD readings (at anthesis and as mean of the whole wheat cropping cycle) were highly correlated with grain yield and N uptake and, even if with lower absolute values, also with protein content. In figure 5 the relationship between the mean of SPAD readings with grain yield is presented, showing a significant linear relationship between these parameters ($R^2 = 0.6132$). Therefore, the positive correlation and the significant relationship between SPAD and wheat yield suggested that this N indicator could be used as a practical indicator to determine the optimum N fertilization. Subsequently, it could be possible also modify the N supply during plants growth, according to SPAD values, for optimizing both plant nutrition levels and application times of fertilization (Montemurro et al., 2007). The stem nitrate content at anthesis and as mean of wheat cycles showed a lower correlation with yields than SPAD readings and no correlation with protein content, grain and straw N uptake. These findings confirmed the results of Roth et al. (1989) who indicated that the stem nitrate test was sensitive to short term changes in the soil supply, and of Barraclough (1997) who suggested that the critical values of stem nitrate content during the wheat growing cycles can be large. As a consequence, plant nitrate concentration could depend on a wide range of environmental conditions, crop management and soil characteristics.

Table 5. Correlation coefficients among grain and straw yields, protein content and N uptake (grain and straw) with plant and soil N indicators.

	Grain yield	Straw yield	Protein content	Grain N uptake	Straw N uptake
SPAD at anthesis	0.517 ***	0.266 ***	0.245 ***	0.503 ***	0.410 ***
Mean SPAD	0.503 ***	0.251 ***	0.299 ***	0.525 ***	0.417 ***
Stem nitrate content at anthesis	0.228 **	0.365 ***	-0.103 n.s.	0.108 n.s.	-0.009 n.s.
Mean stem nitrate content	0.235 **	0.352 ***	-0.019 n.s.	0.127 n.s.	0.004 n.s.
Plant total N content at anthesis	0.350 ***	0.219 **	0.230 **	0.384 ***	0.243 ***
Leaf Area Index at anthesis	0.476 ***	0.598 ***	-0.109 n.s.	0.274 ***	-0.006 n.s.
Pre sowing soil mineral N	0.405 ***	0.391 ***	0.078 n.s.	0.328 ***	0.119 n.s.

*, **, *** = Significant at the P<0.05, 0.01 and 0.001 probability levels, respectively. n.s.=not significant.

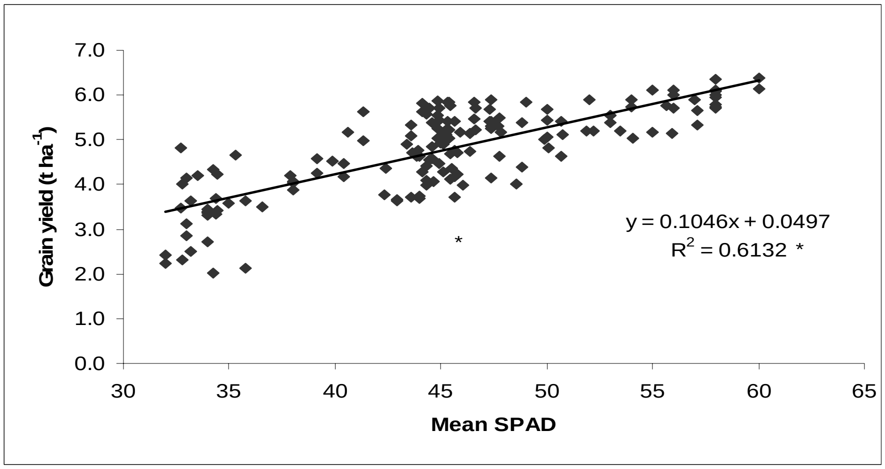

Figure 5. Regression coefficients between grain yield and mean SPAD recorded during wheat cycles.
* = Significant at the P<0.05 probability level.

Although plant total N content at anthesis showed positive correlations with yields, protein content, grain and straw N uptake, no significant relationship was obtained between this N status and wheat performance. This result was probably due to the plateau reached by the total N concentration in plants cropped with increasing N fertilization, therefore this parameter is not a suitable N indicator.

In general, the availability of soil mineral N affects the leaves initiation and plant development. Our results confirmed this statement, showing a positive correlation between pre sowing soil mineral N and yields (grain and straw), probably because these parameters are linked via nitrate uptake and, as a consequence, a higher value of pre sowing N increases the level of proteins and chlorophyll in the plants. Figure 6 shows the relationship among pre sowing soil mineral N and grain yield and points out the significant polynomial relationship between them ($R^2 = 0.5549$), confirming the results of Schröder et al. (2000) who suggested that the correlation between soil mineral N and nitrate concentration in crops is not linear. The positive correlation and the significant relationship between pre sowing soil mineral N and wheat yield could be due to the effect of the applications of organic fertilizer, which enhanced soil organic matter (table 4) and released N during the plants growing cycle. Therefore, our results highlighted that the levels of residual inorganic N in the root profile contribute to yielding performance and should be taken into account when formulating fertilizer recommendations for improving wheat performance (Sowers at al., 1994b).

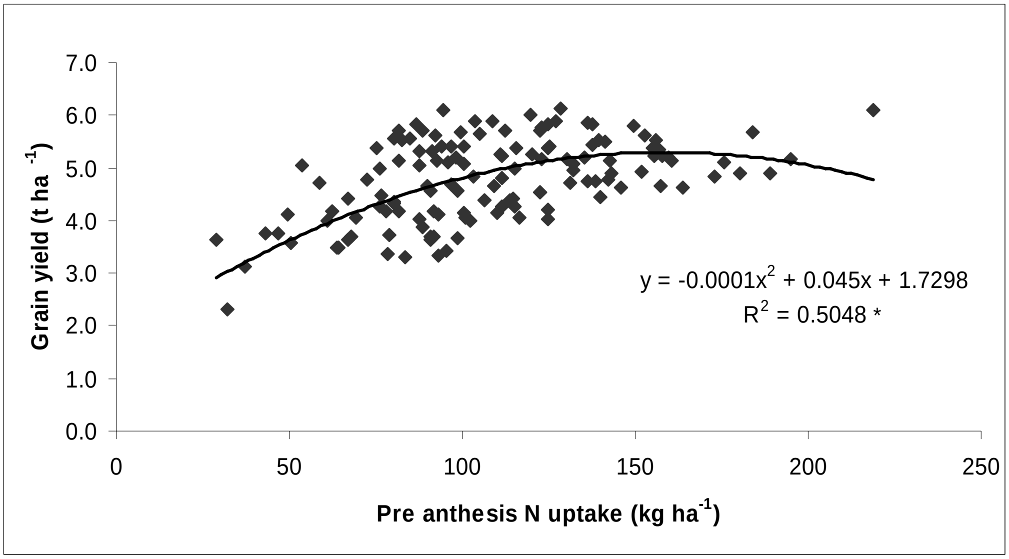

Figure 6. Regression coefficients between grain yield and pre anthesis N uptake.
* = Significant at the $P<0.05$ probability level.

On this matter, there is a long history of controversy if soil or plant N indicators provided a more suitable basis for making fertilizer recommendations. Both methods rely in a similar manner on calibration that is the determination of the relationship between their levels in soils or plants and the corresponding growth and yield response curves (Marschner, 1986). Chemical soil analysis indicates the potential availability of nutrients that roots may take up under favourable conditions for root growth and root activity. Plant analysis reflects only the actual nutritional status of plants. Our results highlighted that a combination of both methods in wheat cultivation provided a better basis for recommending fertilizer applications than one method alone. In fact, among the N indicators tested, the best responses were found for both plant (SPAD readings) and soil (pre sowing mineral N) indicators.

Conclusion

The bulk of evidence of this study pointed out little differences in durum wheat grain yield, protein content and N uptake between the soil tillage depths, thus showing the possibility to reduce the arable layer without compromise quantity and quality of production.

Among the crop rotations, wheat reached the highest grain yield and good protein content when cropped after sugar beet, while the worst results were obtained in rotation with industrial tomato.

The Nmix treatment application allowed the highest grain production and satisfactory protein content, thus pointing out a good balance between quantitative and qualitative wheat performances. Furthermore, in comparison with the Nmin treatment, it did not show any significant difference in the N uptake and in the N utilization efficiency parameters, confirming that the partial substitution of mineral N with organic one did not decrease N utilization and optimized the mineral fertilization, reducing the risk of ground water pollution. Conversely, Ncomp showed lower values of yield, N uptake and efficiency in comparison with Nmin and Nmix treatments, indicating that in the wheat grown in Mediterranean conditions the N fertilizer could not be applied only as organic N in one solution at the beginning of cropping cycles, but should be split in two times, in fall as organic and spring as mineral, as confirmed by the results found with the Nmix treatment.

The MSW compost applications improved soil organic matter (total, extracted and humified) and such effect was exacerbated when this fertilizing treatment was associated with the deepest soil tillage. No significant variation of heavy metals content was observed in the soil after the two-year MSW compost application, indicating the compatible environmental utilization of this organic material, although their concentrations in the compost always exceeded the limit values of Italian legislation. The highest dose of mineral N fertilizer applied with Nmin remained in the soil after cultivation with the leaching possibility.

The results also showed that the more reliable N indicator tests of wheat performance were the mean of SPAD readings recorded during plants growth and the pre sowing soil mineral N content, which presented a linear and polynomial relationships with grain yield, respectively.

On the basis of these results, effective strategies can be developed for establishing the N fertilizer methodologies applied to wheat, according to the rainfall, the preceding crop and the residual N in the soil, in order to optimize yield and reduce nitrate pollution of ground water. In fact, the traditional use of high rates of N fertilizer by farmers to meet crop requirements may be excessive, with greater N loss to denitrification, runoff and leaching during the periods of heavy winter rain. Consequently, in the rainy winter months, which characterize the Mediterranean environments, the mineral N fertilizer could be substitute with organic amendment, as emphasized by the our results.

As confirmed by the findings of this research, one possible solution that can be adopted by farmers is to determine pre sowing mineral N, to apply MSW compost before wheat sowing and then to monitor plants N status during cropping cycles. If the plant indicators did not show a good level of wheat N status, the second mineral N share will be applied to ensure a high qualitative and quantitative crops performance.

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Chapter VII

The Present Situation and the Future Improvement of Fertilizer Applications by Farmers in Rainfed Rice Culture in Northeast Thailand

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Abstract

Rainfed rice, which does not have any constructed irrigation facilities, occupies one third of all rice culture area in the world, and is generally characterized by low productivity. Northeast Thailand is a representative rainfed rice producing region. Rice yield there is as low as 1.7 t ha^{-1} on average, due to drought as well as low soil fertility. In order to improve productivity, we conducted an investigation of farmer's fields and improvement trials in experimental fields from 1995 to 2004. This manuscript summarizes the results in relation to fertilizer application.

Application rates of chemical fertilizer ranged from 0 to 65 kg N ha^{-1} among farmers, with an average of 24 kg N ha^{-1}. The agronomic efficiency of nitrogen (yield increase per unit applied nitrogen) was less than 20 kg kg^{-1}. Although the effect of N fertilizer was generally small, the effect was apparent in the field where rice biomass was small due to late transplanting or infertile soil. The simulation model we developed indicates that optimizing N fertilizer application could improve yield. The field experiment revealed that the N recovery efficiency (kg N absorbed / kg N applied) was 35% under frequent split application of 30 kg N ha^{-1} and increased up to 55% under 150 kg N ha^{-1}. Slow release fertilizer distinctly increased the N recovery efficiency (71%). Wood chip manure and green manure both increased yield, but only slightly.

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Incorporating pond sediments into fields increased rice yield over three seasons by increasing the N recovery efficiency. These results suggested that the proper use of N fertilizer and improving its recovery efficiency is the key to increasing the yield of rainfed rice in Northeast Thailand.

Introduction

Recent criticisms of world food security have recommended more production of staple cereals (Evans, 1999; Speth, 1998). The production of rice, which supports most of the population of Asia, also needs to be dramatically increased (IRRI, 1993). As cultivated areas are unlikely to be expanded, the increase of production in rice must be carried out by increasing productivity. Peng and Senadhira (1998) estimated that the target yield in 2030 should be 4.7 t ha^{-1} in the lowland ecosystem, while the present yield is 2.3 t ha^{-1}. Past increases in rice yield have occurred mainly in irrigated land, but the increase rate has been slowing according to yield leveling for intensive managements (Hossain, 1998). This indicates that improving the productivity of rainfed rice is important and urgent.

Rainfed rice occupies 27% of all rice culture area in the world and as much as 45% of that in Southeast Asia (FAO, 1996). The yield in these areas is generally low because of frequent droughts and low soil fertility (Garity et al., 1986; Wade, 1998). Northeast Thailand is a representative rainfed rice producing region of Southeast Asia. The production is characterized by low productivity (1.9 t ha^{-1} on average from 2000/2001 to 2002/2003, OAE, 2004) and is highly variable in time and space (Fukui, 1993; KKU-FORD cropping systems project, 1982). In order to improve and stabilize productivity, we conducted several research activities from 1995 to 2004, which included investigations of farmers' fields and experimental trials. This manuscript summarizes the results in relation to fertilizer application.

Geographic and agronomical features of Northeast Thailand

Northeast Thailand is bounded by the Phetchabun Mountain Range to the west, the Dongrek Mountains on the south and the Mekong River on the north and east (Figure 1). The area was almost called the Khorat Plateau, with an elevation from 100 to 200 m. Alluvial lowland, which is most suitable for paddy fields, occupies only 6% of the area, while mountainous land occupies 13% and the rest forms broad terraces. There are no great rivers here except for the Mun and Chi Rivers, tributaries to the Mekong, and no great lakes except for some large, but shallow lakes created by dams. A principal component of the topography of the Khorat Plateau is the mini-watersheds called *Nong* in Thai. Some mini-watersheds are open to rivers and others are closed; their dimensions are generally a few km^2 in area and a few m in depth. Numerous mini-watersheds are spread over the plateau; rice paddy fields extend on to them. Consequently, Northeast Thailand has a gently undulating topography, and is not suitable for developing large irrigation systems.

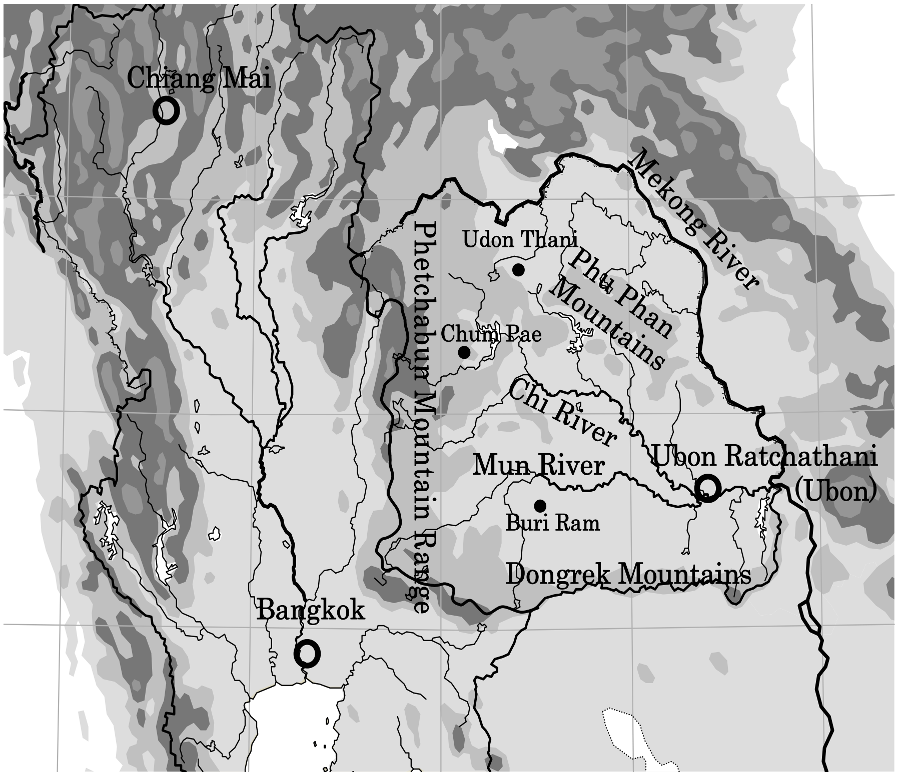

Figure 1. Map of Northeast Thailand.

Surface soils in Northeast Thailand are mostly highly weathered and sandy (Ragland et al., 1984; Mitsuchi, 1990). Besides soils originating from sandstone and siltstone, clay minerals in the soils are easily eroded by run-off and percolated due to their high dispersibility. The average clay content of surface soils in Northeast Thailand is only 6%. Since 97% of the sand fraction is quartz, mineral nutrients are extremely poor in the soils. In the distinct dry season, high air temperature and low clay content in the soils enhance the decomposition of organic matter in the soil. The average organic matter content is about 1%. The soils, which are poor in clay, organic matter, and mineral nutrients, cause not only a small supply capability for nutrients but also exhibit a low maintenance capacity for chemical fertilizers. Even if farmers applied large amounts of fertilizers, most of it would flow out and crops would uptake only a small part (Ragland and Boonpuckdee, 1987).

Annual precipitation in Northeast Thailand ranges from 1,000 to 2,000 mm, which is sufficient for rice production. However, annual pan-evaporation ranges from 2,000 to 3,000 mm and the precipitation pattern is erratic, producing a risk of drought. Most precipitation occurs in the rainy season, which starts in May and ends in October. There is a dry spell that generally occurs in July and continues for about 2 weeks, but sometimes exceeds 1 month. The representative drought types are (1) early-termination of rainy season, (2) dry spell after transplanting and (3) later-transplanting forced by dry spell (Chang et al., 1979; Fukai et al., 1998; Kono et al., 2001).

A farmhouse generally has a sequence of paddy fields from lower to upper in a mini-watershed (Miyagawa et al., 1985; Fukui, 1993; also see Figure 3). Farmers start plowing the fields and nursing seedlings at the beginning of the rainy season, in May. Rice seedlings are transplanted from June to August, and sometimes in September due to a rain shortage. Most traditional rice cultivars in the area head out at the end of the rainy season in October, and are harvested in November and December (Fukui 1993).

Farmers planted dozens of rice cultivars in 1980s, but only two cultivars, KDML 105 and its glutinous mutant RD 6, are commonly planted now. These two cultivars form about 80% of all rice planted area in Northeast Thailand (Miyagawa et al., 1999). Chemical fertilizer was rarely used for rice cultivation in the 1980s (Miyagawa and Kuroda, 1988), but was commonly used by 1993. The application rate of chemical fertilizer in the region was 153 kg ha^{-1} in 1993 and 198 kg ha^{-1} in 2003 on average for all crops (National Statistical Office, Thailand, 2005). Miyagawa et al. (1999) estimated that 90% of paddy fields received chemical fertilizer, at a rate of 20 to 30 kg N ha^{-1}. Previous studies indicated that rice production in Northeast Thailand is most severely limited by the application rate of nitrogen (N) fertilizer followed by phosphorus (P) fertilizer (Suriya-arunroj et al., 2000; Naklang et al., 2006).

Investigation of Farmer's Fields

Research sites

Research sites of the study were located in a rainfed rice culture area about 25 km northwest of the center of Ubon Ratchathani City; the area extends along Se Bai River, a branch of Moon River. The four mini-watersheds of Wang O, Kha Khom, Hua Don and Mak Phrik were selected as research sites (Figure 2). Rainfed rice has been cultivated for about 50 years at Wang O site and about 100 years at Kha Khom, Hua Don and Mak Phrik sites. Each site consists of traverse lines at each mini-watershed (*Nong*). The mini-watersheds of Wang O and Kha Khom sites are open to rivers, and those of Hua Don and Mak Phrik are closed. The traverse lines of Wang O and Mak Phrik are from top to bottom fields of the mini-watersheds, and those of Kha Khom and Hua Don extend from the top field on one side through bottom fields to the top on the opposite side in the mini-watershed. The lines of Wang O, Kha Khom, Hua Don and Mak Phrik sites are about 200, 500, 350 and 150 m in the length and 6.0, 1.5, 3.4 and 2.6 m in relative field elevation difference, respectively. The width of Hua Don, the widest site, is 250 m (Figure 3), and those of the others are about 50 m. The numbers of farmers farming the rice fields at Hua Don, Wang O, Kha Khom and Mak Phrik were 10, 1, 6 and 1, respectively. Toposequential positions of paddy fields are presented with field elevation relative to the lowest paddy field in each site.

According to soil map published by the Department of Land Development, Thailand (Changprai et al., 1971), soils in Wang O site are classified as Khorat series (USDA Taxonomy: Ustoxic Dystropept), those in Kha Khom are Ubon (Aquic Quartzipsamments) and Khorat series, those in Hua Don are Pimai (Vertic Tropaquept) and Ubon series, and those in Mak Phrik are Roi Et series (Aeric Paleaquults).

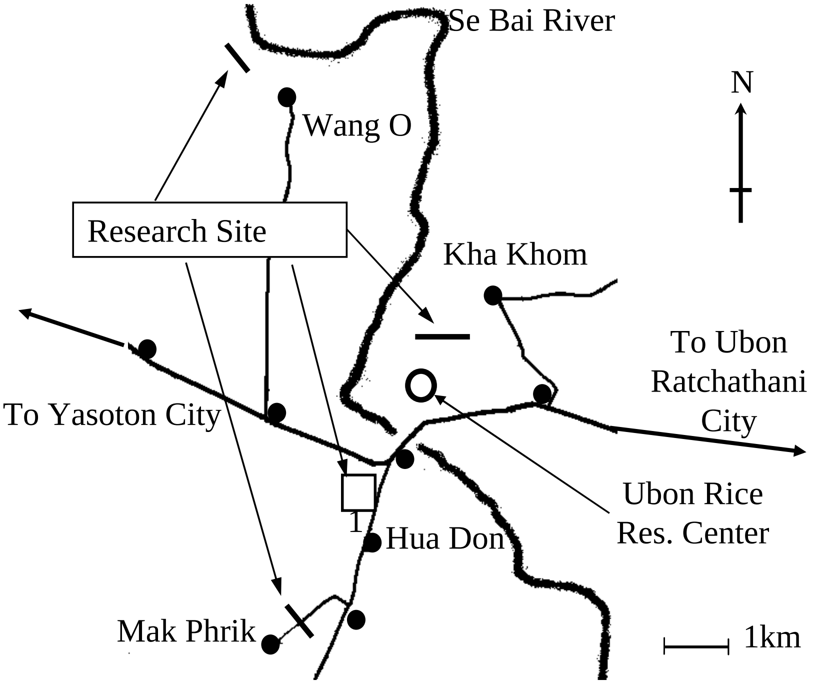

Figure 2. Map of research sites. J shows village and 1 the secondary grown woodland where soil samples were collected.

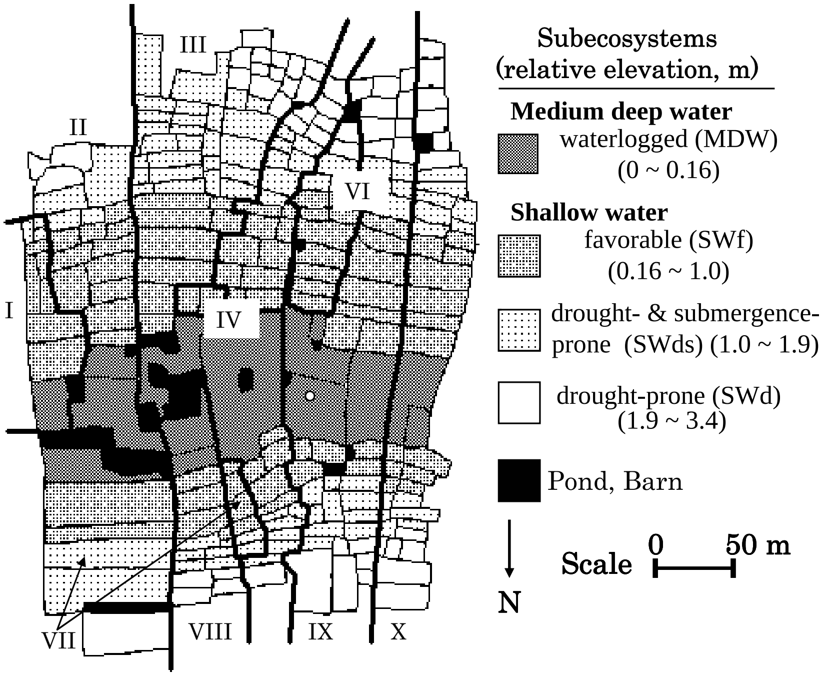

Figure 3. Map of investigated fields and their elevations relative to the lowest point (o) in the Hua Don research site. ▬ : boundaries between the fields of farmers I to X; — : boundaries between fields (adapted from Homma et al., 2004).

Toposequential distribution of soil fertility and water availability

Soil fertility, evaluated by phytometer with potted rice as a test plant under irrigated conditions, showed at most a 5 times difference, and was well indexed by soil organic carbon (SOC) content (Figure 4; Homma et al., 2003c). SOC content decreased with ascending relative elevation of the field in the mini-watersheds (Figure 5). Soils in the upper fields had about half of the SOC content as the secondary woodland adjacent to the Hua Don site. Clay content showed a steeper gradient than SOC content with relative elevation. Although the difference in clay content between soils in upper paddies and the secondary woodland adjacent to the Hua Don site was smaller than that in SOC content, the clay content in the woodland was 1.7 times as high as those in the uppermost fields (2 to 3 m) in Hua Don. This implies that soil was eroded from upper to lower by the change from forest to paddy field. According to the toposequential change in SOC and clay content, soil fertility closely and negatively correlates with the relative elevation of the field.

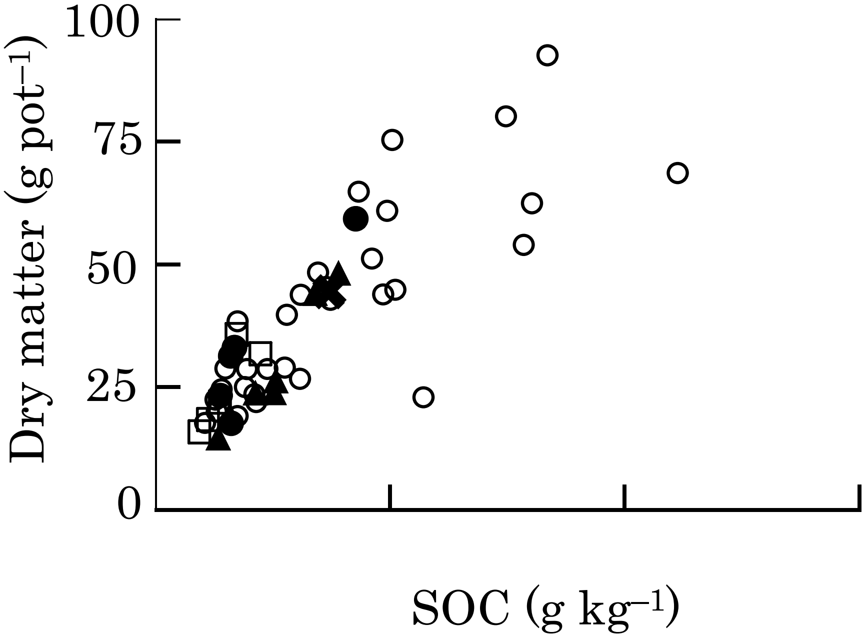

Figure 4. Relation between soil organic carbon (SOC) content and dry matter production at maturity in potted rice under unfertilized and irrigated conditions (Homma et al., 2003c). Soils were sampled from Wang O (G), Kha Khom (C), Hua Don (E), Mak Phrik (J) and secondary woodland adjacent to Hua Don (1).

The number of flooded days markedly decreased with ascending field elevation (Figure 6; Homma et al., 2004). Averaging all the fields, the number of flooded days was approximately half of the duration of the rainy season. However, since some fields never had standing water even though the soil was saturated, the number of flooded days did not accurately reflect water availability. Then, we used delay of heading date as an index of water in the region reach the heading stage on nearly the same day in the absence of water stress (Figure 7).

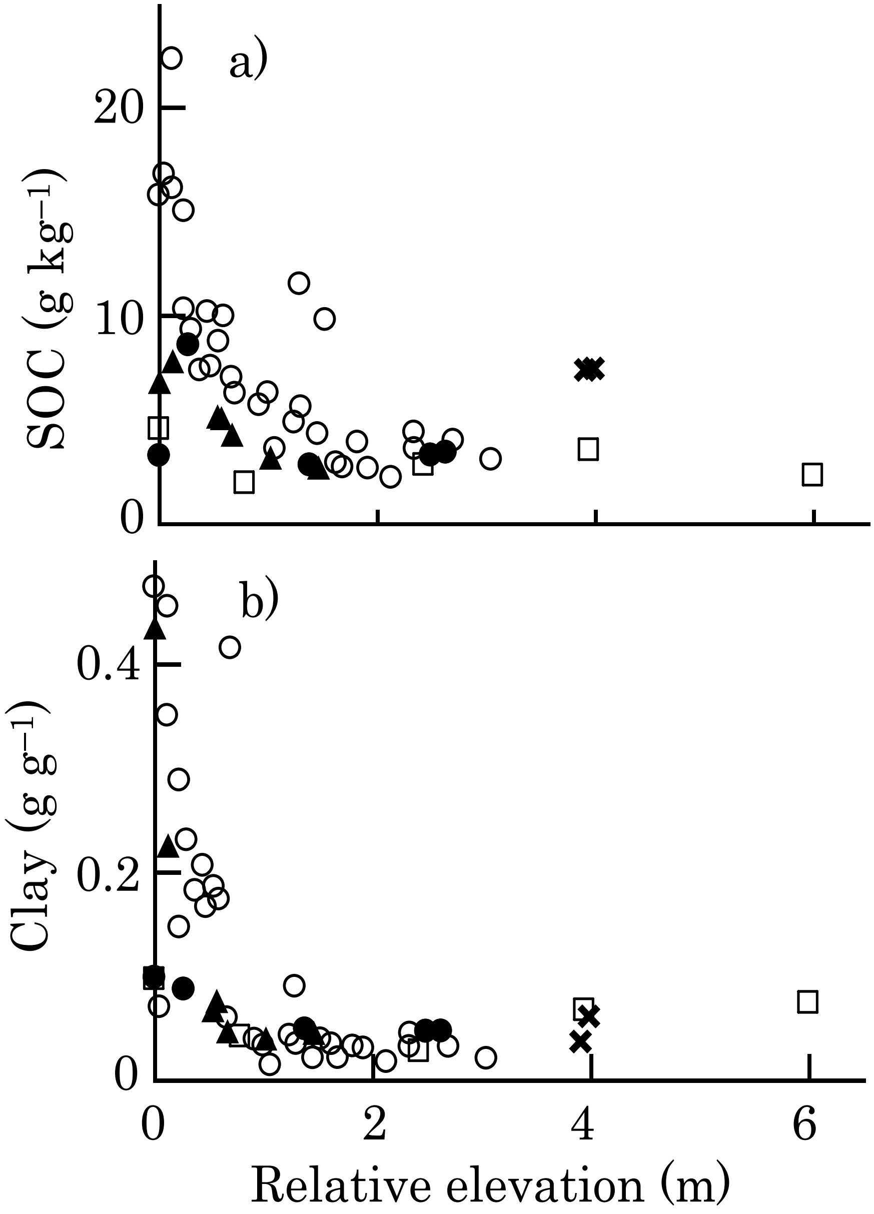

Figure 5. Soil organic carbon (SOC; a) and clay (b) content as a function of the relative elevation of each field in the research sites (Homma et al., 2003c). Symbols are the same as in Figure 4.

The heading date was delayed with ascending relative field elevation in the research sites (Figure 8). The earlier heading date in the lowermost field was probably affected by deep water, which sometimes exceeded 50 cm in depth. The fields with relative elevation less than 1 m in the Hua Don site had practically no water stress. The area of these fields exceeded more than a half of the research site (also see Figure 3). Delay of heading in the fields with relative elevation more than 1.9 m in the Hua Don site was approximately 10 days, indicating that the rice plant was damaged by severe water stress.

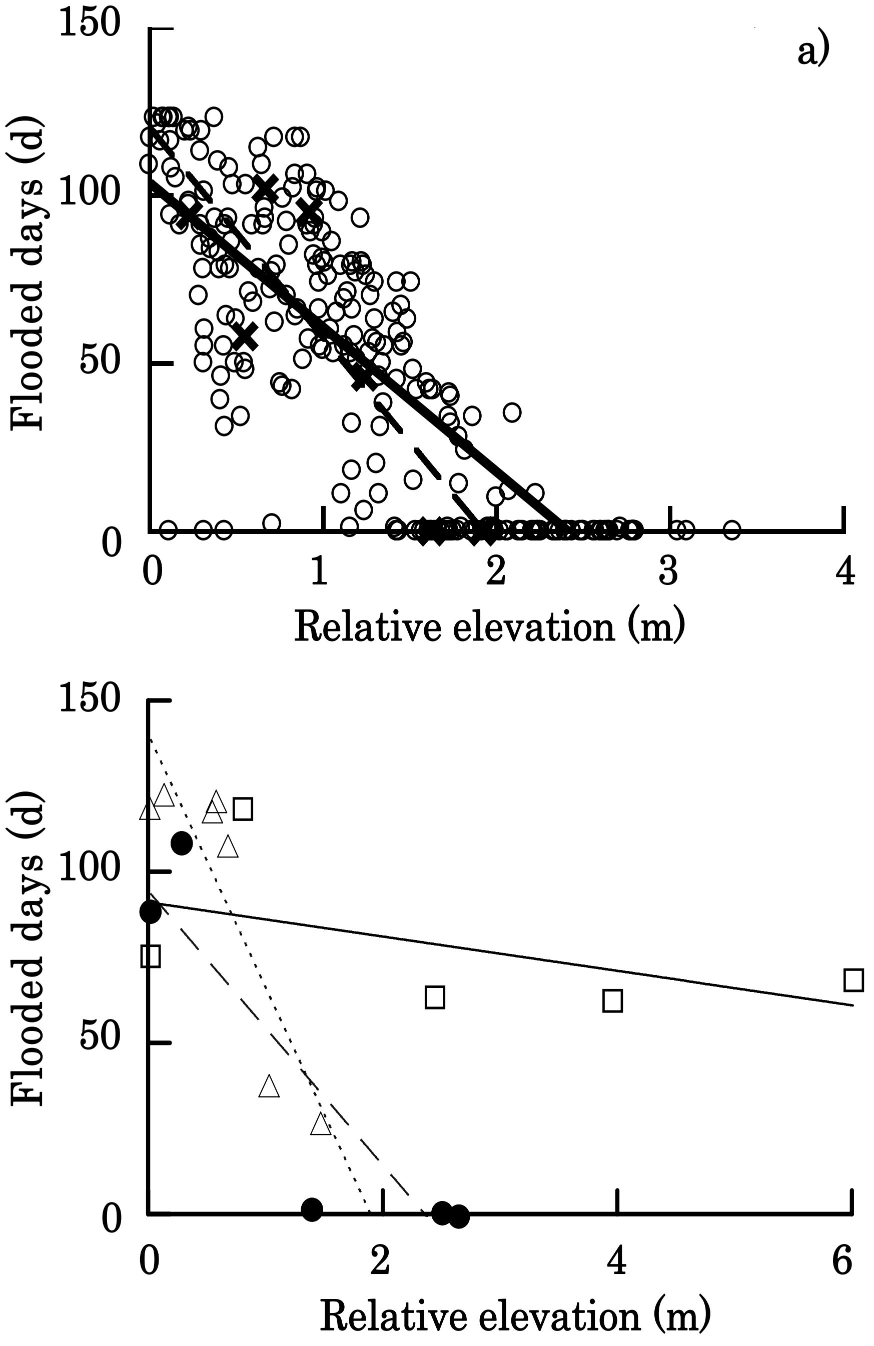

Figure 6. Number of flooded days as a function of relative field elevation in the research sites in the rainy season (1 July to 31 October) (Homma et al., 2004). (a) Hua Don site in 1997 (1, broken line) and 1998 (E, solid line); (b) Wang O (G, solid), Kha Khom (C, dotted) and Mak Phrik (J, broken) in 1998.

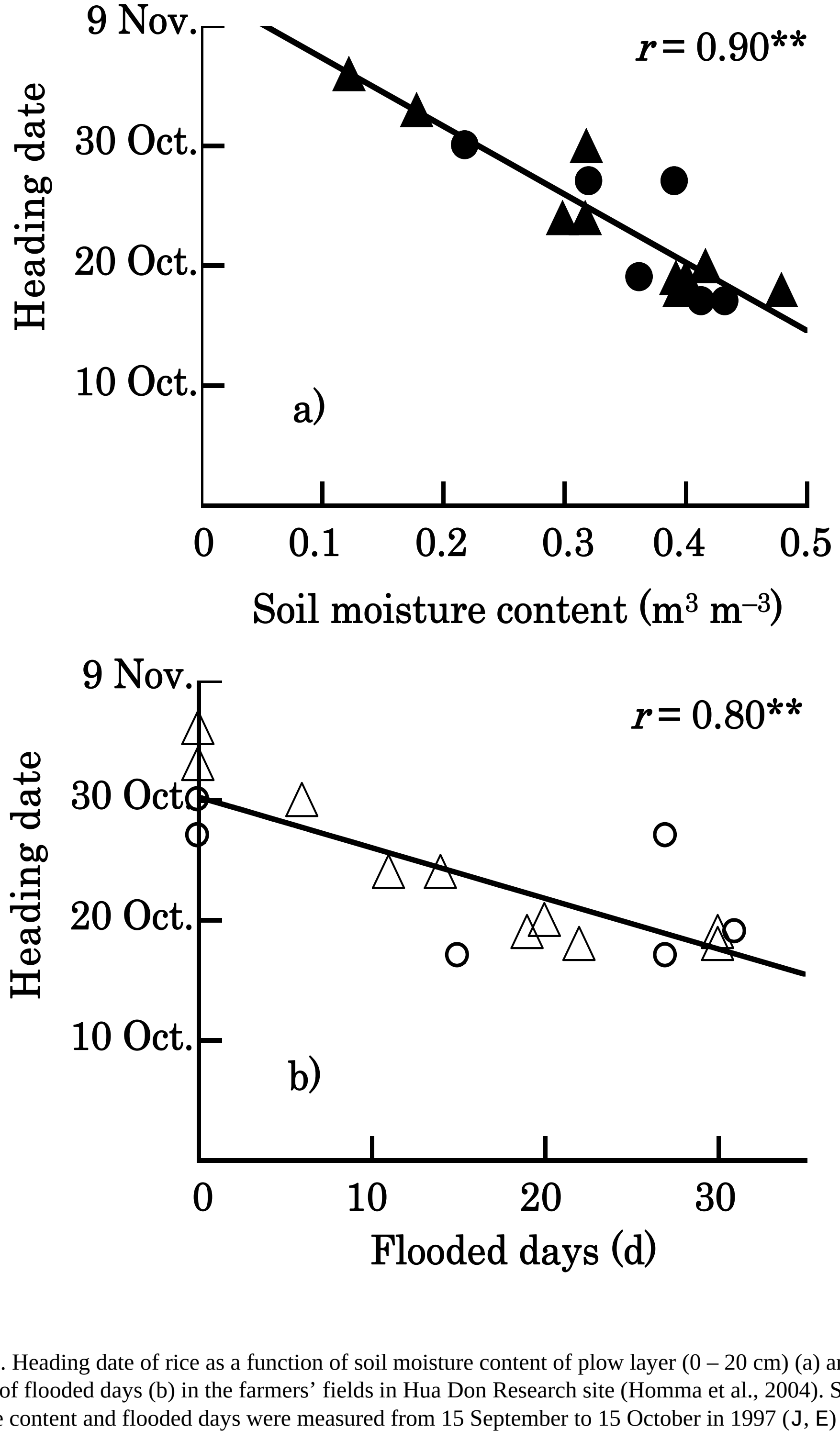

Figure 7. Heading date of rice as a function of soil moisture content of plow layer (0 – 20 cm) (a) and number of flooded days (b) in the farmers' fields in Hua Don Research site (Homma et al., 2004). Soil moisture content and flooded days were measured from 15 September to 15 October in 1997 (J, E) and 1998 (H,C).

Table 1. Area of fields, number of flooded days, soil organic carbon (SOC) content and clay content in the research sites and in each subecosystem in the Hua Don research site in 1998.

Site (Subecosystem)[1]	Flooded days	SOC ($g\ kg^{-1}$)	Clay ($g\ g^{-1}$)
Hua Don	49 ± 42	7.9 ± 5.0	0.13 ± 0.14
MDW	115 ± 9	17.7 ± 3.1	0.34 ± 0.19
SWf	81 ± 3	8.6 ± 2.6	0.18 ± 0.11
SWds	40 ± 3	5.5 ± 3.1	0.04 ± 0.02
SWd	1 ± 5	3.3 ± 0.8	0.03 ± 0.01
Wang O	78 ± 23	3.0 ± 1.0	0.06 ± 0.03
Kha Khom	105 ± 42	5.0 ± 1.8	0.13 ± 0.15
Mak Phrik	28 ± 54	4.3 ± 2.4	0.07 ± 0.03

± indicates standard deviation.

[1] MDW: Medium deep water, water logged; SW: Shallow water; f: favorable; ds: drought- and submergence-prone; d: drought-prone.

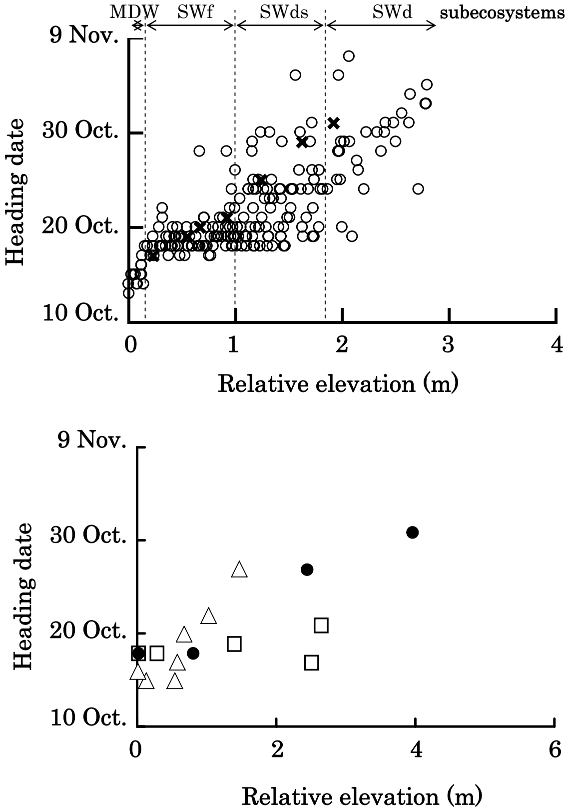

Figure 8. Relationship between heading date of rice and relative field elevation in the research sites (Homma et al., 2004). Symbols are the same as in Figure 6. Abbreviations of subecosystems are shown in Figure 3.

Farmer crop management

Farmers applied farmyard manure (FYM) at the end of the dry season with the amount related to their ownership of livestock, as shown in Table 2. In all the investigation sites, farmers started to plow paddy fields and make nursery beds in May after the rainy season started. Nursery beds were made at paddy fields located in the middle parts of the slopes of each mini-watershed. Transplanting was done from the end of June to the middle of August and from lower to upper fields in each mini-watershed, with one- to two-month-old seedlings. Some bottom fields were direct seeded at the Hua Don research site. In some direct seeded fields where farmers failed in seedling establishment, seedlings were re-transplanted. The fields used for nursery beds were either transplanted afterwards or unused. Most farmers in the research sites applied chemical fertilizer once or twice during rice growth: once after completing transplanting in all the fields, and sometimes once more in mid-September (about 30 days before heading). Since combined chemical fertilizer, 16-16-8 % (N-P_2O_5-K_2O) type, was the most popular, the amount of fertilizer is hereafter described by its nitrogen amount. Fields around the tops of the mini-watersheds did not have fertilizer applied because of the high risk of water shortage, while fields around the bottom also did not have it applied because of the high risk of flow out with flood. Hand weeding was conducted only once, at transplanting time. Pump water was also used for irrigation only at transplanting time, if needed. The heading times of KDML 105 and RD 6 were both usually in the middle of October, the end of the rainy season, provided no hard damage by water shortage (Homma et al., 2001). Rice was harvested successively from the bottom to the upper fields at each site during November.

To sum up, transplanting and harvesting time were usually determined by water availability. Thus, these practices differed between toposequential field positions, but not between farmers (Tables 2 and 3). Although fertilizer application was affected by the condition of standing water, it also depended on farmers' strategies for reducing economic risk and ensuring food security, resulting in large differences in the amount used by farmers. The application rate of chemical fertilizer ranged from 0 to 65 kg N ha^{-1} with an average of 24 kg N ha^{-1}. Since soil fertility and water availability were quite low at the Mak Phrik site, that farmer rarely took care of his paddy fields.

Paddy yield for each farmer ranged from 0.9 to 3.4 t ha^{-1}. Farmers who applied more fertilizer tended to have better paddy yields (Figure 9). The relationship between fertilizer application rate and yield ($r = 0.66$, $P < 0.01$) was firm despite difference in soil fertility and water availability. However, the agronomic efficiency of nitrogen (yield increase per unit applied nitrogen), 22.4 kg kg^{-1}, seems to be overestimated, compared with that experimentally obtained (6.3 ~ 15.8 kg kg^{-1} in Khunthasuvon et al., 1998; also see following sections).

Table 2. Rice acreage, management and livestock holdings by farmers at the research sites.

Site Farmer1)	Paddy field area (ha)	TP date	HV date	N fert. ($\mathrm{kg\ ha^{-1}}$)	FYM2)	Live-stock3)
Hua Don research site in 1997.4)						
II		15 July ~ 13	9 ~ 18 Nov.	35	O	Ca 1
Hua Don research site in 1998.						
I	0.3566	25 June ~ 12	6 ~ 10 Nov.	22.4	O	W 1
II	0.987	17 July ~ 12	6 ~ 18 Nov.	14.4	O	Ca 1
III	2.1803	25 June ~ 11	6 ~ 18 Nov.	17.6	O	W 3
IV	0.0618	6-Jul	9 Nov.	12.9	X	
V	0.5548	26 June ~ 12	7 ~ 18 Nov.	40.2	O	W 2
VI	0.4117	7 July ~ 6 Aug.	10 Nov.	45.3	X	
VII	1.1201	9 July ~ 25 July	7 ~ 9 Nov.	14.3	X	
VIII	0.4503	13 July ~ 17	9 Nov.	65	O	W 4
IX	1.8942	4 July ~ 15 Aug.	6 ~ 18 Nov.	13.4	O	Ch 200
X	1.2767	14 July ~ 13	6 ~ 18 Nov.	26.5	O	W 5
The other sites in 1998.4)						
Kha Khom		25 June ~ 30		16.1	1 / 6 6)	W 1
Wang O		1 July ~ 6 July		15	1 / 1 6)	Ca 6
Mak Phrik		23 July ~ 15		– 5)	0 / 1 6)	

1) Number of farmers, I ~ X, corresponding that in Figure 3.

2) FYM: Farm yard manure, O: applied and X: not applied.

3) Kinds and numbers of livestock as main source for FYM. W: water buffalo, Ca: caw and Ch: chicken. The numerals attached to livestock indicate the numbers of animals.

4) Values are averages of the fields in which research was conducted.

5) Very small amount of fertilizer.

6) The numerator is the number of farmers who applied FYM and the denominator is the number of farmers at the research site.

Table 3. Differences in farmers' managements among the subecosystems in Hua Don research site in 1998 (Homma et al., 2007).

Subecosystems[1)]	Transplant (DOY)[2)]	Harvest (DOY)[2)]	N fertilizer (kg ha^{-1})
MDW	183 ± 17 ^{a}	312 ± 1 ^{a}	0 ^{a}
SWf	195 ± 9 ^{b}	314 ± 2 ^{b}	37 ± 18 ^{b}
SWds	203 ± 13 ^{c}	315 ± 3 ^{c}	35 ± 24 ^{b}
SWd	217 ± 12 ^{d}	316 ± 4 ^{c}	0 ^{a}
Mean	200 ± 14	315 ± 3	29 ± 24

± indicates standard deviation.

Values within a column followed by the same letter are not significantly different at the 5% level.

1) Abbreviations are the same as in Table 1.

2) DOY: day of year, for example, 183th ± 17 means 2nd July ± 17 days.

Paddy yield for each farmer ranged from 0.9 to 3.4 t ha^{-1}. Farmers who applied more fertilizer tended to have better paddy yields (Figure 9). The relationship between fertilizer application rate and yield ($r = 0.66$, $P < 0.01$) was firm despite difference in soil fertility and water availability. However, the agronomic efficiency of nitrogen (yield increase per unit applied nitrogen), 22.4 kg kg^{-1}, seems to be overestimated, compared with that experimentally obtained (6.3 ~ 15.8 kg kg^{-1} in Khunthasuvon et al., 1998; also see following sections).

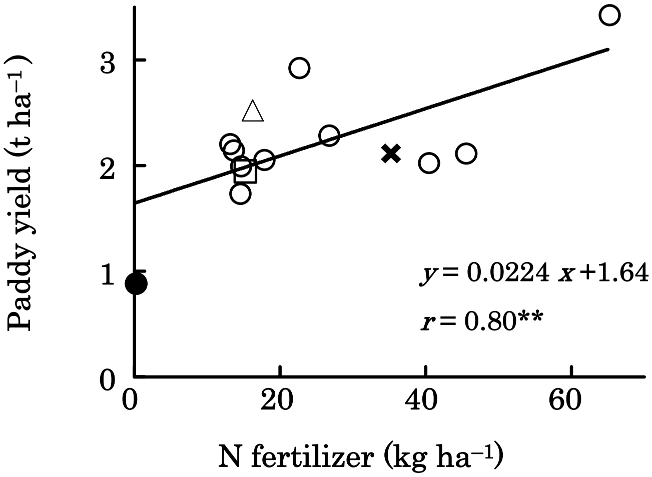

Figure 9. Paddy yield for each farmer as a function of application rate of chemical nitrogen (N) fertilizer at the research sites. Symbols are the same as in Figure 6. The value of the Kha Khom site is the average for 6 farmers.

Variability in paddy yield and its relation to fertilizer application

Since upper fields had more severe water stress, less fertile soil and later transplanting dates, rice biomass declined with increasing field elevation (Figure 10; Homma et al., 2007). Table 4 shows the differences in rice biomass and yield among the subecosystems in the Hua Don site in 1998. While rice biomass was the largest in the MDW field, yield was the highest at SWf because of the lower harvest index (HI) in the MDW field. Lodging in the MDW field seriously reduced HI. In the SWd field, rice biomass and paddy yield were extremely small, 2.68 and 0.53 t ha^{-1}, respectively.

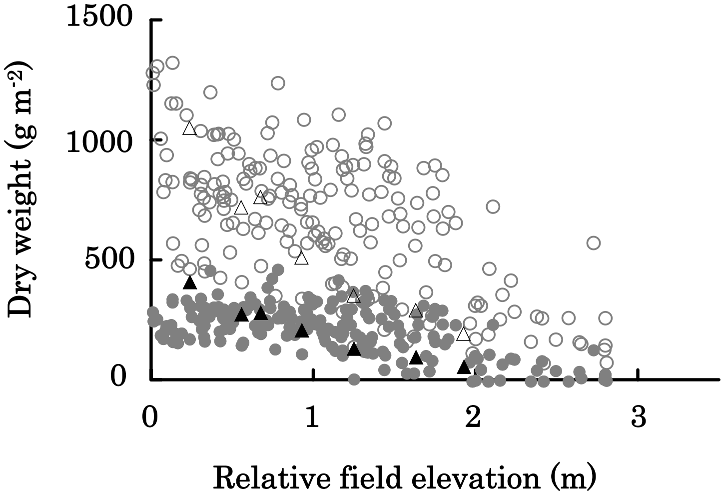

Figure 10. Variation with field elevation in above-ground biomass at maturity (C, E) and paddy yield (H, J) in Hua Don research site in 1997 (triangle) and 1998 (circle) (Homma et al., 2007).

Table 4. Differences in rice production among the subecosystems in Hua Don research site in 1998 (Homma et al., 2007).

Subecosystems[1)]	Biomass (t ha^{-1})	Yield (t ha^{-1})	HI[2)] (t t^{-1})
MDW	9.97 ± 2.80 [a]	2.34 ± 0.59 [b]	0.25 ± 0.06 [c]
SWf	7.78 ± 1.92 [b]	2.73 ± 0.67 [a]	0.35 ± 0.05 [a]
SWds	6.43 ± 2.46 [c]	2.15 ± 0.95 [b]	0.32 ± 0.06 [b]
SWd	2.68 ± 1.52 [d]	0.53 ± 0.56 [c]	0.16 ± 0.12 [d]
Mean	6.73 ± 2.84	2.19 ± 1.04	0.31 ± 0.09

± indicates standard deviation.

Values within a column followed by the same letter are not significantly different at the 5% level.

1) Abbreviations are the same as in Table 1.

2) HI: harvest index.

Table 5. Coefficients of correlation among relative elevation, transplanting date, N fertilizer application rate, rice biomass and yield in (a) the whole, (b) MDW, (c) SWf, (d) SWds and (e)SWd in the Hua Don research site in 1998 (Homma et al., 2007).

a) The whole

	Elevation	Transplanting	N fertilizer	Biomass	Yield
Relative elevation	1				
Transplanting date	0.61 **	1			
N fertilizer	-0.26 **	-0.2 *	1		
Biomass	-0.63 **	-0.65 **	0.38 **	1	
Yield	-0.60 **	-0.57 **	0.53 **	0.9 **	1
HI	-0.47 **	-0.31 **	0.48 **	0.4 **	0.69 **

b) MDW: medium deep water, waterlogged subecosystem

	Elevation	Transplanting	Biomass	Yield
Relative elevation	1			
Transplanting date	-0.18	1		
Biomass	-0.51	-0.30	1	
Yield	-0.19	-0.02	0.62 *	1
HI	0.47	-0.38	-0.61 *	0.22

c) SWf: shallow water, favorable subecosystem

	Elevation	Transplanting	N fertilizer	Biomass	Yield
Relative elevation	1				
Transplanting date	0.35 **	1			
N fertilizer	0.12	0.05	1		
Biomass	-0.05	-0.32 **	0.22 *	1	
Yield	-0.02	-0.19	0.27 *	0.85 **	1
HI	-0.05	0.29 **	0.04	-0.32 **	0.21

d) SWds: shallow water, drought- and submergence-prone subecosystem

	Elevation	Transplanting	N fertilizer	Biomass	Yield
Relative elevation	1				
Transplanting date	0.19	1			
N fertilizer	-0.15	-0.22	1		
Biomass	-0.12	-0.63 **	0.45 **	1	
Yield	-0.18	-0.55 **	0.43 **	0.96 **	1
HI	-0.24 *	-0.15	0.26 *	0.42 **	0.62 **

e) SWd: shallow water, drought-prone subecosystem

	Elevation	Transplanting	Biomass	Yield
Relative elevation	1			
Transplanting date	0.22	1		
Biomass	-0.28	-0.54 **	1	
Yield	-0.29	-0.49 *	0.90 **	1
HI	-0.29	-0.26	0.56 **	0.81 **

*, **: significantly different at 5 % and 1 % level, respectively.

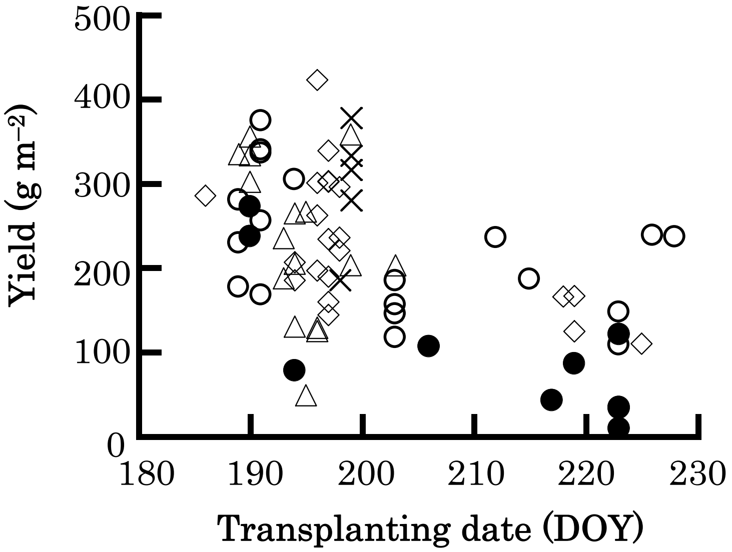

Figure 11. Effect of transplanting date and application rate of nitrogen (N) fertilizer on rice yield in shallow water, drought- and submergence-prone subecosystem (SWds) (Homma et al., 2007). N application rates were 0 (J), 2 ~ 3 (E), 3 ~ 4 (C), 4 ~ 5 (A) and 10 ~ 11 g m^{-2} (I).

Yield was severely restricted by HI like the low biomass production at SWd. Extremely low HI was caused by water stress (Homma et al., 2004). Even under such severe water stress, earlier transplanting significantly increased yield (Table 5e); the rate of increase was 2.2 g grain day^{-1}.

Consequently, the agronomic efficiency was around 10 to 17 kg kg^{-1}, similar to the values in Khunthasuvon et al. (1998). Applying fertilizer had a significant effect on paddy yield in SWds, where rice growth was restricted by infertile soil and drought. The fertilizer may increase root growth and enhance the capability to uptake soil nutrients and water. This implies that paddy yield may be increased with fertilizer application, even at SWd.

Optimization of fertilizer application predicted by rice simulation model

In order to evaluate variability in paddy yield in a mini-watershed, we developed a simulation model based on the results in the experimental fields (Ohnishi et al., 1999a) and the investigation of farmer’s fields (Homma et al., 2003a). Here, we broadly describe the model and show the result in relation to fertilizer applications.

The model consists of two major parts. One simulates the water condition and the other rice growth. The water condition simulation is schematically presented in Figure 12. Field water condition changes by water flux between field and ground water as well as precipitation and evapotranspiration. Calculating the amount and direction of the soil water flux depends on Fick's law, where moisture retention curve and hydraulic conductivity are determined by soil texture (Campbell, 1985).

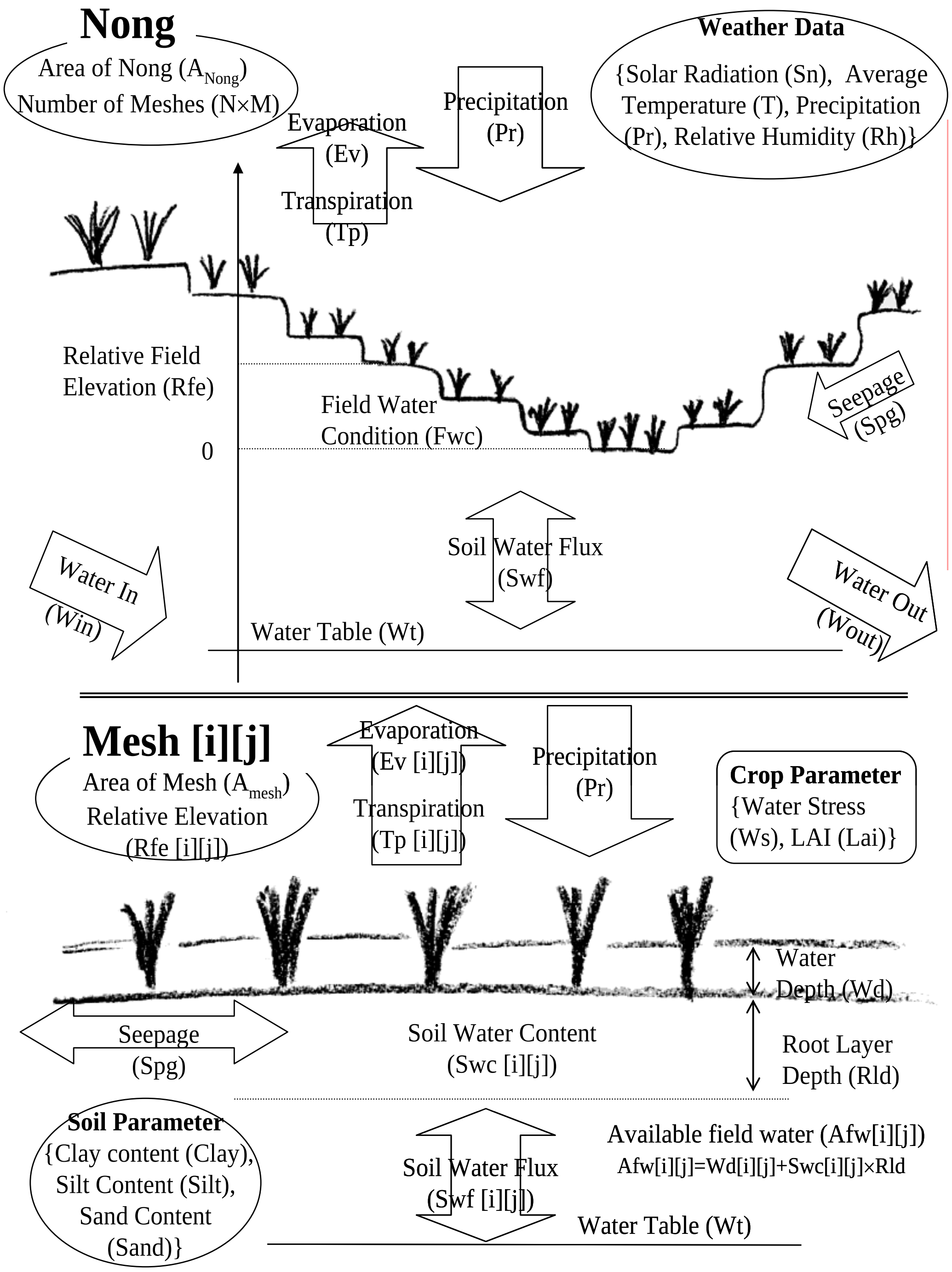

Figure 12. Concept of the model for simulating water budget for rainfed rice in a mini-watershed (*Nong*) (Homma et al., 2003a). Terms in ellipses are input variables. Crop parameters are derived from the sub-model for simulating rice growth shown in Figure 13.

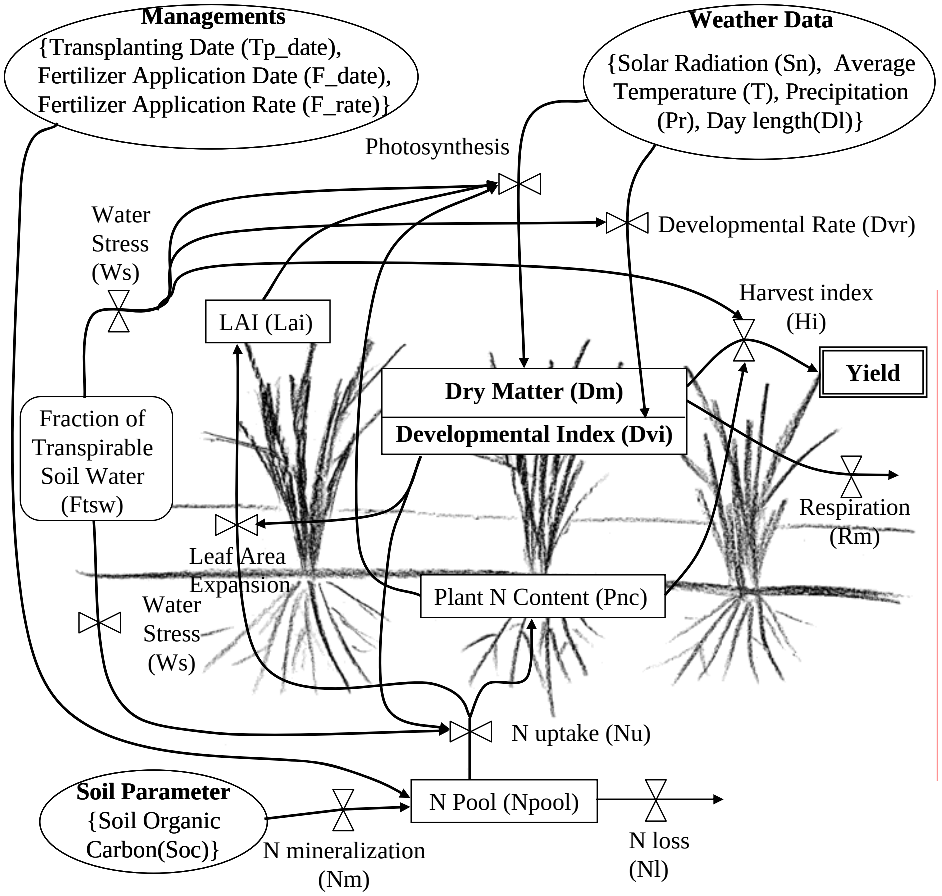

Figure 13. Concept of the model for simulating rice growth under rainfed conditions (Homma et al., 2003a). Terms in ellipses are input variables. Fraction of transpirable soil water is derived from the sub-model for simulating water budget shown in Figure 12.

The rice growth simulation involves phenological development, N uptake, LAI growth, dry matter production and yield formation (Figure 13). Phenological development is quantified by a variable called developmental index (Horie and Nakagawa, 1990). The nitrogen mineralization rate is a function of soil organic carbon (SOC) content, determined by a potted rice growth experiment (Homma et al., 1999). N mineralized and applied by chemical fertilizer is reserved in the soil N pool. The rate of N uptake by the rice plant and N loss are proportional to the amount of N in the pool. LAI growth is driven by N uptake (Ohnishi et al., 1997). Dry matter production is obtained by multiplying the intercepted radiation by the radiation conversion efficiency, and yield by multiplying dry matter by the harvest index (Horie et al., 1995). Water stress is proportional to the fraction of transpirable soil water (Meyer and Green, 1981; Loomis and Connor, 1992). Reductions of N uptake and dry matter production are proportional to water stress. Harvest index decreases in proportion to the accumulated water stress. Developmental rate decline should also be proportional to the water stress, but is assumed to be the accumulated water stress in this study.

The model was evaluated with the data obtained at the Hua Don research site in 1998 (Homma et al., 2003b). The values of parameters for rice growth of cultivar KDML 105 in this study were given by Ohnishi et al. (1999a), obtained by experimental results in URRC. SOC content and soil texture were expressed as functions of relative field elevation. Equilibrium values, which were repeatedly calculated under the condition in 1998, were used for initial water table, soil moisture content and standing water depth.

Although the simulation model effectively estimated time courses of soil water content and dry matter production of rice in some respective fields, the relation between measured and simulated paddy yields for fields in the Hua Don research site was relatively scattered (Figure 14; Homma et al., 2003c). The sparse correlation was partly caused with the estimation of SOC and soil texture, which were calculated as functions of relative field elevation. The estimation of farmers' fertilizer application rates also caused some errors because we obtained this rate by dividing the amount of a farmer's application by the acreage of the applied field without considering irregular management. Even though the result of the simulations contained errors for each field, the average value of simulated paddy yield for the all fields in the research site, 2.3 t ha^{-1}, agreed fairly well with that of measured paddy yield, 2.2 t ha^{-1}. The simulation model also estimated yearly change of paddy yield from 1997 to 2002 well (Homma et al., 2003c).

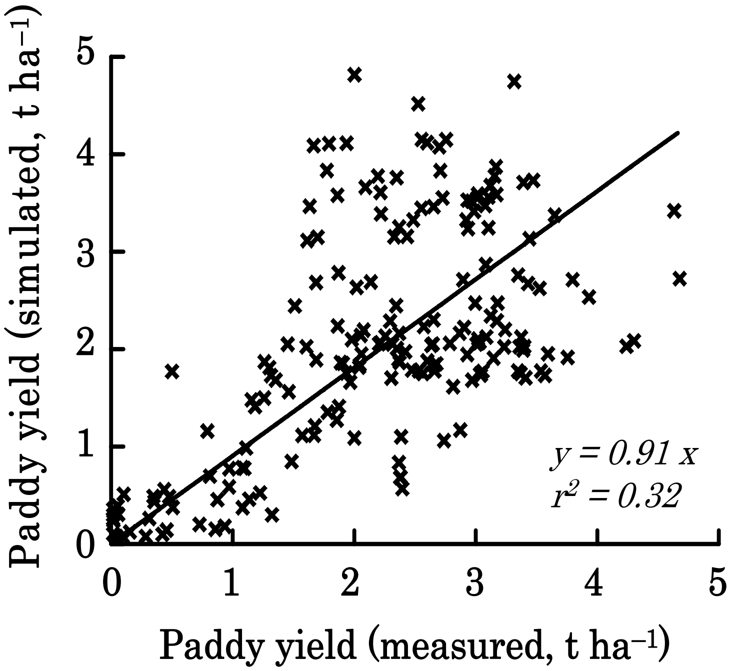

Figure 14. Relation between measured and simulated paddy yields for fields in Hua Don research site in 1998 (Homma et al., 2003 b).

Table 6. Simulated paddy yield (t ha^{-1}) and agronomic efficiency[1)] (values in parentheses, kg kg^{-1}). Simulation was carried out for the Hua Don research site in the case that all fields was transplanted at a selected date and had a selected rate of N fertilizer applied. The values in the table are averages and standard deviations for 5 years, simulated using weather date from 1997 to 2001.

TP date	N fertilizer rate		
	0 kg N ha^{-1}	25 kg N ha^{-1}	50 kg N ha^{-1}
15-Jun	3.39±0.16	3.76±0.19 (14.9±1.1)	3.87±0.19 (9.6±0.6)
15-Jul	2.18±0.12	2.77±0.2 (23.5±1.7)	3.2±0.2 (20.9±1.4)
15-Aug	1.1±0.1	1.7±0.2 (23.3±2.8)	2.3±0.2 (22.7±2.7)

1) Agronomic efficiency (yield increase per unit applied N fertilizer) = { (grain yield at 25 or 50 kg N ha^{-1}) – (grain yield at 0 kg N ha^{-1})} / 25 or 50, respectively.

The effect of transplanting date and fertilizer application rate on paddy yield was estimated with the simulation model (Table 6). In the simulation, we set three transplanting dates (15 June, 15 July and 15 August) and three application rates (0, 25 and 50 kg N ha^{-1}), and supposed that all fields in the research site were transplanted on the same day and had the same rate of fertilizer applied. One-month-earlier transplanting increased paddy yield by about 1 t ha^{-1}, corresponding to the result of statistical analysis (see previous section). The agronomic efficiency of N fertilizer (increment of yield per unit applied N) reached its maximum, 23.5 kg kg^{-1}, at an application rate of 25 kg N ha^{-1} and a transplanting date of 15 July. The efficiency was almost the same at the transplanting date of 15 August, but decreased to less than 15 kg kg^{-1} at the transplanting date of 15 June. These values also varied with years; for example, the efficiency at 25 kg N ha^{-1} and 15 July was 25.1 kg kg^{-1} under the weather conditions in 1997 and 20.9 kg kg^{-1} in 1998.

Although Table 6 shows that earlier transplanting and more fertilizer increases rice production, management was restricted by a farm budget, labor and so on. Therefore, we tested strategies of N fertilizer application, in which the amount of fertilizer applied by each farmer was unchanged, but the toposequential distribution of the application was varied (Figure 15). If fertilizer was applied one week after transplanting all at once, paddy yield was increased over the yield by the management observed in the Hua Don research site in 1998. However, since paddy yield increased slightly under double split application of N fertilizer, the increase under single split application was mostly derived from the application time of

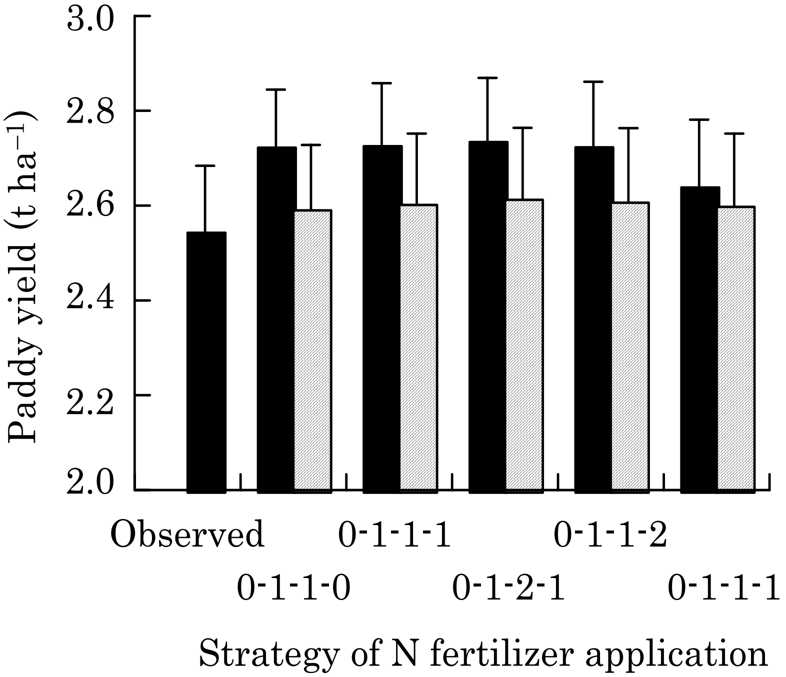

Figure 15. Simulated paddy yield for different strategy of N fertilizer application in Hua Don research site. Observed: the same management of farmers observed in 1998; four numbers indicate distribution rate for subecosystems, e.x. 0-1-1-2 means that the application rate of N fertilizer per unit area is 0 in MDW, those in SWf and SWds are the same, and that in SWd is twice that in SWds. In these strategies, the amount of fertilizer applied by each farmer is the same as that observed in 1998. The left gray bar for each strategy shows the value in the situation that fertilizer is applied all at once 1 week after transplanting and the right striped bar shows the value in the situation that half of the fertilizer is applied 1 week after transplanting and the remaining half is applied on the 15 September, around panicle initiation of the rice plant. These values and error bars are the averages for fields in the Hua Don research site and their standard deviations for 5 years, simulated using the weather from 1997 to 2001.

fertilizer, not the distribution. This suggests that early growth is important, as it increases the availability of nutrients and water. Although the effect was similar among the strategies, the 0-1-2-1 strategy was the best, in which the application rate of N fertilizer per unit area is 0 at MDW, those at SWf and SWd are the same, and that at SWds is twice of that at SWf.

Improvement Trials in Experimental Field

Several experiments were conducted at the Ubon Rice Research Center, Department of Agriculture, Thailand (URRC; 15°20’N and 104°04’E, 123 m in altitude) to improve rainfed rice productivity. Here, we explain four of the results: (1) fertilizer response of local leading cultivar, KDML 105; (2) cultivar difference in N uptake rate and its physiological efficiency; (3) green manure trial with rice-*Stylosanthes guianensis* (stylo) relay-intercropping; and (4) incorporation of pond sediments to improve rice productivity.

Fertilizer response of local leading cultivar, KDML 105

Since rice production in Northeast Thailand is most severely restricted by nitrogen (N) supply (Wade et al., 1999; Naklang et al., 2006), one of the leading cultivars in the region, KDML 105, was grown under different N management methods (Ohnishi et al., 1999b). N management included different N rates in frequent split urea application (FSU), FSU with organic matter (FSU+OM) application and slow release fertilizer (SRF). In SRF management, we used 140-day release type as basal (LP coat 140, Chisso Corp., Japan) and 60-day release type (LP coat 60, Chisso Corp., Japan) as a top dressing at 24 days before heading. Both types of SRFs contained the same amounts of N, P_2O_5 and K_2O. LP coat 140 releases 80% of N in the 140 days after application under Japanese growing conditions. The OM used in this study was a mixture of faeces of water buffalo and wood chips in a 1:2 ratio. Although this experiment was conducted under both irrigated and rainfed conditions, apparent water stress was not observed for the rainfed condition.

Apparent N uptake from applied N was calculated by subtracting the plant N content in no N application from that under fertilized treatments. Figure 16 shows the relationships between applied N and apparent uptake at panicle initiation, heading and maturity in both years. The ratio between apparent N uptake and applied N is referred to as fertilizer N recovery efficiency. The fertilizer N recovery efficiencies at panicle initiation were not significantly different among applied N levels, but increased with the N level at heading and maturity. The efficiency at maturity in the urea applications varied from 35.4% to 55.5% for application rates of 30 kg N ha^{-1} and 150 kg N ha^{-1}, respectively, with an average of 43.9%. The fertilizer N recovery efficiency in SRF application was superior to that in FSU treatment. The efficiencies were 57.1%, 68.2% and 71.2% at panicle initiation, heading and maturity, respectively. However, the plant N uptake patterns in SRF treatment suggested that LP coat 140 released almost all N within 50 days of the application under high temperature conditions in Northeast Thailand. The application of OM also increased the recovery efficiency of N fertilizer, but the increase might be associated with nitrogen supply from OM.

The responses of LAI and dry weight at panicle initiation and harvest index and yield at maturity to N uptake at panicle initiation are shown in Figure 17. Physiological efficiencies (LAI, dry weight, harvest index and yield per unit N uptake) were obtained from these relations. The LAI and dry weight at panicle initiation were closely related to N uptake at this stage. Harvest indices at maturity decreased linearly with increases in N uptake at panicle initiation. Low harvest indices for application rate of 150 kg N ha^{-1} of both urea and SRF treatments were due to lodging. The maximum yield of 4 t ha^{-1} was attained at about 40 kg ha^{-1} of N uptake at panicle initiation. The physiological efficiencies were not significantly different among different N management. The dry matter, harvest index and yield responses to N uptake at both heading and maturity were similar to those at panicle initiation. Figure 18 show the relationship at maturity and those obtained in the cultivar experiments (see following section). The maximum yield was attained at a N uptake of about 80 and 90 kg N ha^{-1} at heading and maturity, respectively, when various N management treatments were combined.

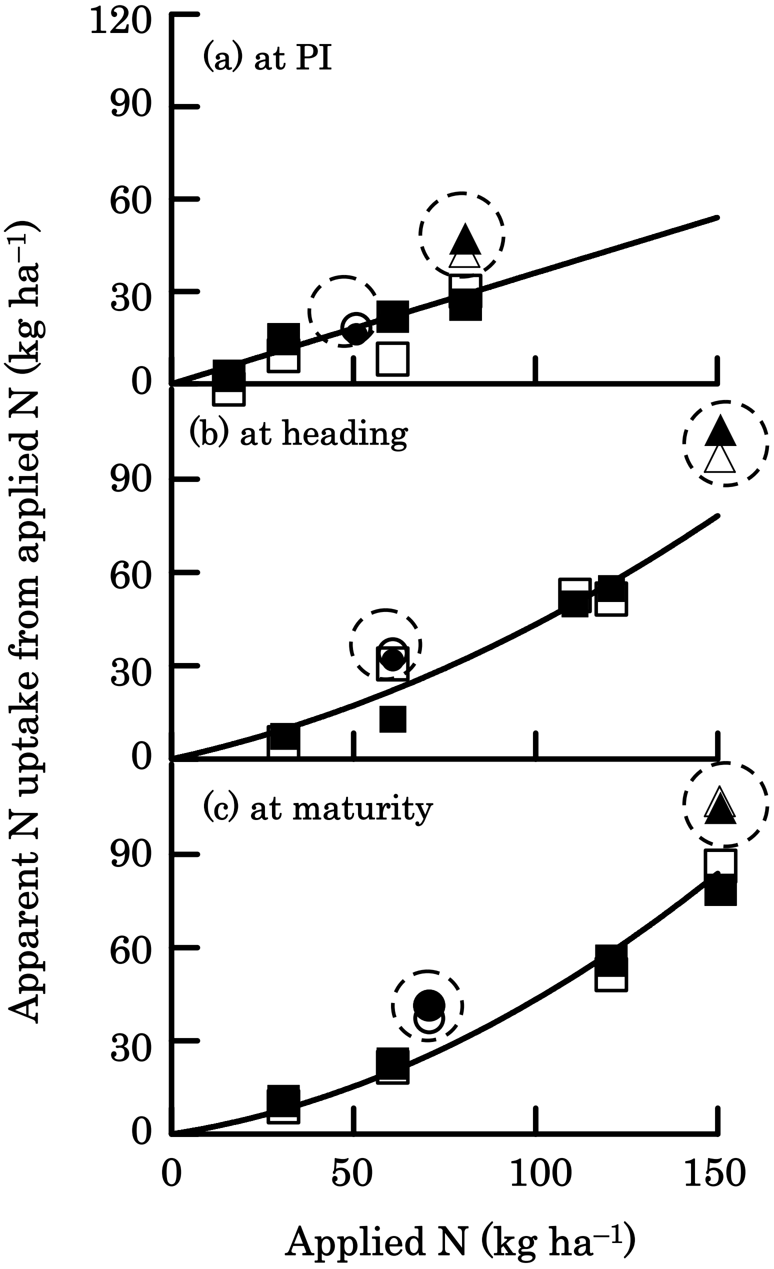

Figure 16. Apparent N uptake and applied fertilizer N: at (a) panicle initiation (PI), (b) heading and (c) maturity of KDML105 treated with different N management conditions under irrigated (filled) and rainfed conditions (open) (adapted from Ohnishi et al., 1999b). Apparent N uptake was calculated by subtracting the plant N content in no N application from that in fertilized treatments in the same water management. N managements were frequent split urea (FSU, square), slow release fertilizer (SRF, triangle), and FSU with organic matter (FSU+OM, circle) applications. Regression lines were calculated only for FSU.

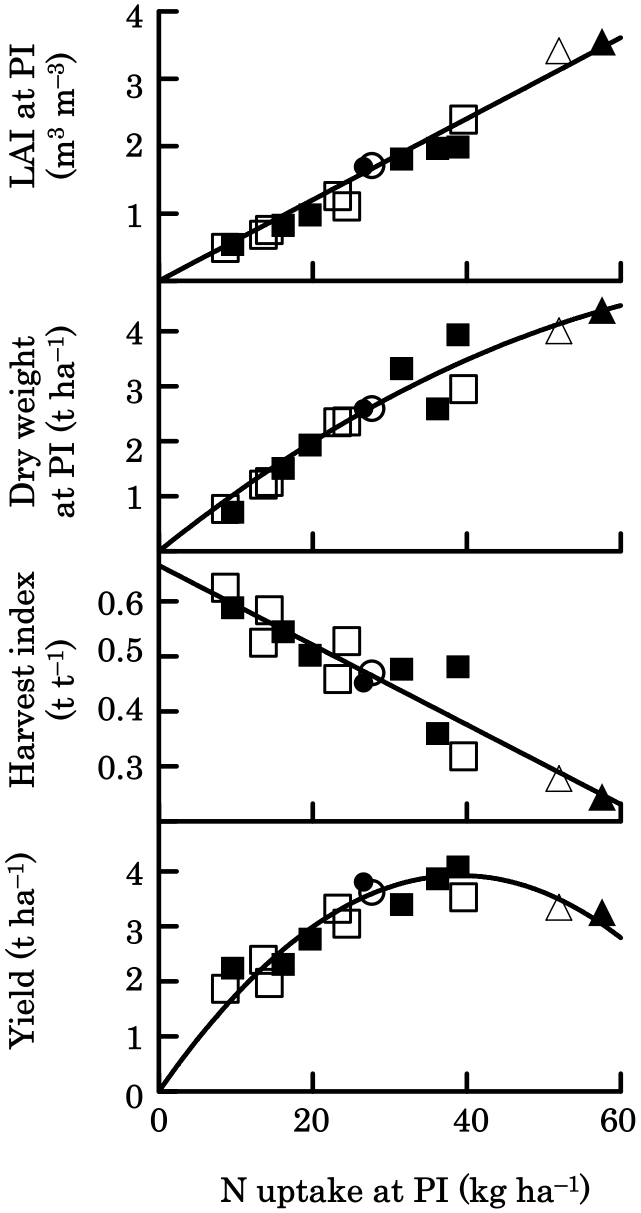

Figure 17. Response of LAI and dry weight at panicle initiation (PI), and harvest index and yield at maturity, to N uptake at PI of KDML 105 (Ohnishi et al., 1999b). Symbols are the same as in Figure 16.

The agronomic efficiency (increment of yield per unit applied N) varied from 18.0 to 8.3 kg kg^{-1} at 60 kg N ha^{-1} of FSU and 150 kg N ha^{-1} of SRF application, respectively. The lower agronomic efficiency under higher fertilizer application was due to lodging.

Farmers in Northeast Thailand usually apply fertilizer once or twice during rice cultivation. Khunthasuvon et al. (1998) obtained the nitrogen fertilizer recovery efficiency in single split application (25% to 41%). Nakalang et al. (2006) estimated that the fertilizer recovery efficiency was 33% in double split application. Thus, FSU application in this study increased fertilizer N recovery efficiency by 10%. However, the frequent application did not substantially increase agronomic efficiency of KDML 105 compared with that in farmers' fields (see previous section). SRF also increased N recovery efficiency, but did not increase agronomic efficiency. This also corresponds to the simulation results, where double split application did not increase paddy yield (Figure 15). These results may imply that KDML 105 is prone to produce dry matter with fertilizer application, but not grain, and that the application time of fertilizer is important to increase paddy yield.

Cultivar difference in N uptake rate and its physiological efficiency

The result of the experiment for the cultivar KDML 105 shows the limitation of productivity potential of KDML 105 under well fertilized condition. In order to explore ways to improve rice productivity in the region, cultivars of different origins were tested under irrigated and well fertilized conditions in URRC (Ohnishi et al., 1999b).

Table 7. Days to maturity, N uptake and its rate of rice cultivars under irrigated and well-fertilized conditions in Ubon Rice Research Center (URRC) (adapted from Ohnishi et al., 1999b).

Cultivar	Origin	no.	Days to heading	N uptake	
				Amount ($kg\ N\ ha^{-1}$)	Rate ($kg\ N\ ha^{-1}\ day^{-1}$)
Yamadanishiki	Japan	1	68	52.8	0.78
Amaroo	Australia	2	68	68.9	1.01
Echuca	Australia	3	68	57.0	0.84
Toyonishiki	Japan	4	81	76.7	0.95
Urumamochi	Japan	5	82	90.4	1.10
Takanari	Japan	6	90	98.2	1.09
Taichung 65	Taiwan	7	95	64.1	0.67
Silewa	Indonesia	8	95	94.2	0.99
Tainung 9	Taiwan	9	98	60.7	0.62
Kaoshung 141	Taiwan	10	98	33.7	0.34
IR 72	Philippines	11	98	121.6	1.24
Tainoh 5	Taiwan	12	98	105.3	1.07
Tainoh 70	Taiwan	13	100	27.3	0.27
Banten	Indonesia	14	108	108.0	1.00
KDML105	Thailand	0	132	138.0	1.05

The total N uptake of all cultivars tended to decrease with shortening growth duration (Table 7). When expressed as N uptake rate per day, the differences among cultivars were smaller, but still large. IR 72 had the largest N uptake rate (1.24 kg N ha^{-1} day^{-1}) and Tainoh 5 had the lowest (0.28 kg N ha^{-1} day^{-1}). The difference might be associated with fertilizer N uptake ability of plant root in sandy soil with a low holding capacity for nutrients.

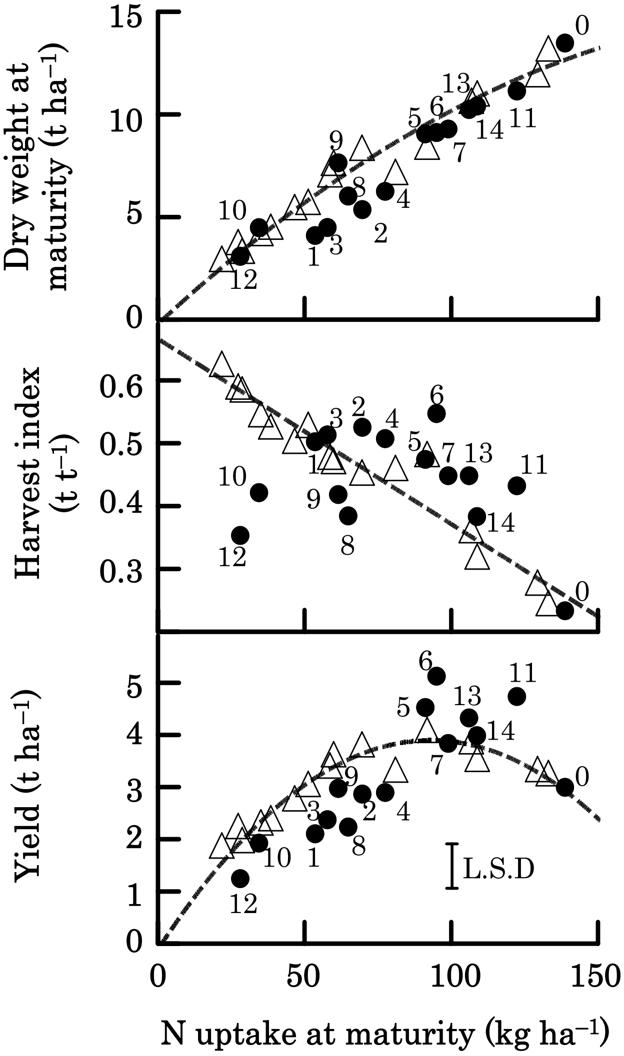

Figure 18. Responses of dry weight, harvest index and yield to N uptake at maturity of 18 cultivars grown under irrigated and well-fertilized condition (adapted from Ohnishi et al., 1999b). Triangles and dotted line indicate the relationship for KDML105 obtained with different N management (see Figure 17). Numbers in the figure denote cultivar (see Table 7).

Figure 18 shows responses of crop dry weight, harvest index and yield to N uptake at maturity. In this figure, cultivar differences are compared with the relationships obtained for KDML 105. The dry weight responses to N uptake in all cultivars were similar to that of KDML 105. For a given N uptake level at maturity, some cultivars tended to have a higher harvest index than KDML 105; Takanari had the maximum harvest index. Yield per unit N uptake in Taichung65, Takanari, IR72 and Taino70 were larger than that in KDML 105.

These results indicate that it is possible to increase rice productivity by improving characteristics of the cultivar in terms of N uptake rate and N use efficiency, as proposed by Fukai (1998).

Green manure trial with rice-*Stylosanthes guianensis* (stylo) relay-intercropping

Since one production constraint in rainfed rice culture in the region is low soil fertility related to low soil organic matter (Homma et al., 2003; Willet, 1995; also see previous section), many investigators have incorporated organic materials, like farmyard manure, compost and green manure, into soil and concluded that these effectively increase soil fertility and rice productivity (Supapoj et al., 1998; Whitbread et al., 1999). Since the availability of farmyard manure and compost is often limited, green manure is recognized as a promising application (Garity and Becker, 1994). However, since the management of green manure still has agronomic constraints involving crop establishment and incorporation practices (Palaniappan and Budhar, 1994), it is not common for farmers in Northeast Thailand. Therefore, we developed a rice-stylo relay intercropping system to produce stylo as green manure and increase rice productivity (Homma et al., 2008).

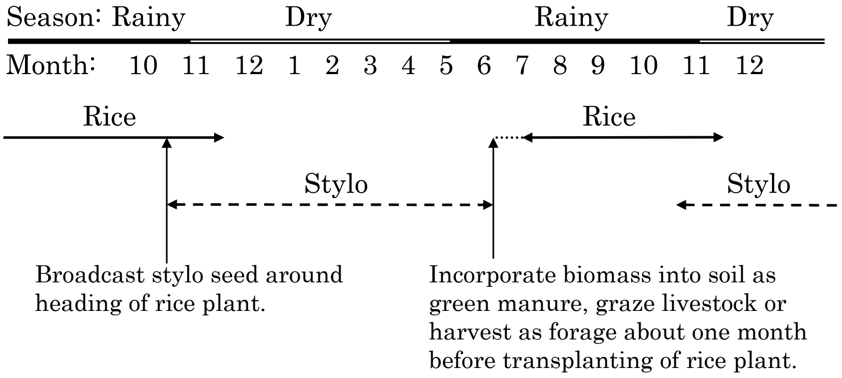

Figure 19. Cropping calendar of rice-*Stylosanthes guianensis* (stylo) relay intercropping system.

Figure 19 shows our rice-stylo relay intercropping system. Seeds of the stylo are broadcast before rice harvesting, if possible, around heading of rice. Stylo endures the dry season under non-irrigation and non-fertilizer application, utilizing residual soil moisture and nutrients after the rice crop. At the end of the dry season, stylo starts to grow rapidly as precipitation increases. The above-ground biomass of stylo is harvested and incorporated into soil as green manure about one month before rice transplanting. Since stylo is a forage crop, it can be incorporated after grazing by livestock or composting through digestion by livestock.

Above ground biomass in early June exceeded 9 t ha^{-1} at its highest even though irrigation water and chemical fertilizer were not applied for stylo production. Although the production was not stable in terms of fields and years, the average production was 3.5 t ha^{-1}. Incorporating biomass into the soil generally increased rice production. The increase rate (paddy yield in relay intercrop / that in rice monocrop – 1 %) was 51% at its highest, as obtained by incorporating the maximum stylo production. The average increase rate was 15% (Figure 20).

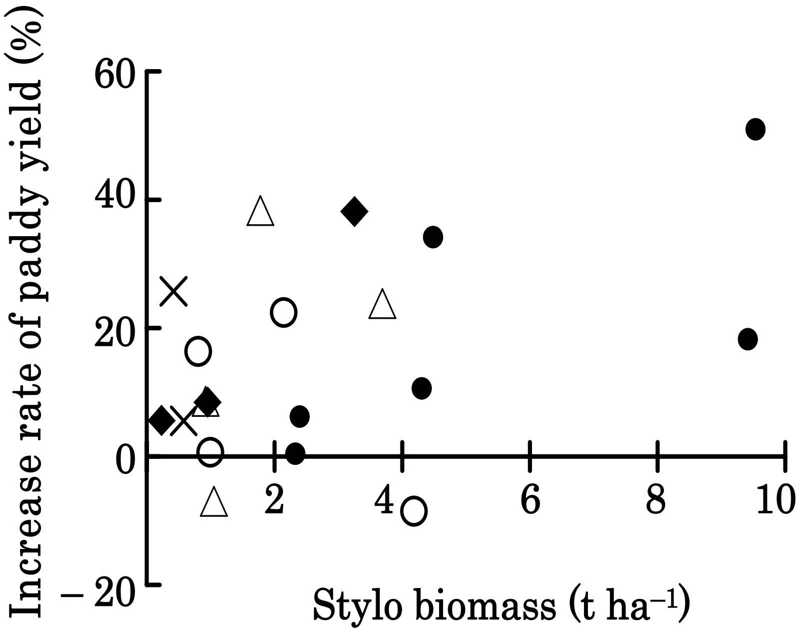

Figure 20. Relationship between above-ground biomass of stylo in early June and increase rate of paddy yield (paddy yield in the relay-intercrop / that in rice monocrop – 1, %) (Homma et al., 2008). `E`, `J`: experimental fields at upper and lower toposequence in a mini-watershed in Ubon Rice Research Center, respectively; `C`, `F`, `I`: farmers' fields in Udon Thani, Chum Pae and Buri Ram, respectivery.

We also tried this system in farmers' fields at 3 locations in Northeast Thailand (Udon Thani, Chum Pae and Buri Ram, see Figure 1). Farmers managed the system for themselves, although stylo seed was supplied. One farmer used stylo biomass as green manure as expected (Chum Pae), but another cut and brought out the biomass for forage (Udon Thani), and the other grazed water buffalo (Buri Ram). The average above-ground biomass in early June was 1.4 t ha^{-1}, 40% of that obtained in the experimental field. The increase rate of paddy yield was 13% on average, similar to the experimental value.

Since the increase rate of paddy yield is significant but not apparent, the appeal of this system to farmers is not adequate to spread it among farmers. However, an increase in meat consumption has expanded the demand for cattle and buffalo in Thailand, causing a shortage of forage. Consequently, a rice-stylo-grazing system or rice-stylo for forage production system is suitable for Northeast Thailand.

Incorporation of pond sediments to improve rice productivity

As mentioned above, organic matter and clay are generally lacking in soils in Northeast Thailand, although these accumulate in the bottoms of mini-watersheds (Figure 4). We collected pond sediments at the bottom of a mini-watershed in URRC and incorporated it into the paddy fields.

Table 8. Soil textures in the pond sediment soil incorporated and non-incorporated plots in July 2001 (before first season rice cultivation) and November 2003 (after three seasons of rice cultivation) (Mochizuki et al., 2006).

	Soil	Sand (kg kg^{-1})	Silt (kg kg^{-1})	Clay (kg kg^{-1})
2001 July	Incorporated	0.61^{a*}	$0.20^{n.s.}$	0.19^{a**}
	Non-incorporated	0.72^{b}	0.19	0.09^{b}
2003 November	Incorporated	0.62^{a}	0.23	0.16^{a}
	Non-incorporated	0.72^{b}	0.19	0.10^{b}

*, ** and n. s.: significance at 5 %, 1 % level and non-significance, respectively.

Table 9. Soil organic carbon (SOC) content, anaerobic nitrogen mineralization (NH_3-N) and cation exchange capacity (CEC) in the soil incorporated and non-incorporated plots in July 2001 (before first season rice cultivation) and November 2003 (after three seasons of rice cultivation) (Mochizuki et al., 2006).

Soil		SOC (g kg^{-1})	NH_3-N (mg kg^{-1})	CEC (cmol kg^{-1})	
				pH 7.0	pH 5.0
2001 July	Incorporated	5.7^{a*}	$13^{n.s.}$	7.1^{a**}	6.4^{a**}
	Non-incorporated	4.6^{b}	11	5.0^{b}	4.7^{b}
2003 July	Incorporated	5.8^{a}	20	6.7^{a}	5.9^{a}
	Non-incorporated	4.6^{b}	16	4.7^{b}	4.4^{b}

*, ** and n. s.: significance at 5 %, 1 % level and non-significance, respectively.

Incorporating pond sediments increased soil organic carbon (SOC) and clay content, an increase that was maintained for 3 years (Tables 8 and 9). Although this also increased the nutrient supply capacity, rice yield did not increase under non-fertilized conditions (Table 10). When chemical fertilizer application was combined with pond sediment incorporation, rice yield increased by 28% on average over three seasons. The increase in rice yield was associated with increased fertilizer-N recovery efficiency, which was ascribed to an increase in cation exchange capacity (CEC). The agronomic efficiency of N fertilizer, 13.6 kg kg^{-1}, in the non-incorporated conditions increased by 30.8 kg kg^{-1} in the incorporated conditin. The relatively higher agronomic efficiency in both conditions may be associated with low soil fertility and drought, as mentioned in the previous section.

Since incorporating of pond sediments is inexpensive and has long-lasting effects on paddy yield, the technology is suitable to rain-fed paddy culture in Northeast Thailand.

Table 10. Paddy yield, N recovery efficiency and agronomic efficiency of N fertilizer for three-season rice crops in the soil incorporated and non-incorporated plots with and without chemical fertilizer applications (Mochizuki et al., 2006).

	Soil	Chemical fertilizer	2001	2002	2003	Average
Grain yield (t ha^{-1})	Incorporated	Fertilized	2.86	3.37	1.52	2.58 ^{a*}
		Non-fertilized	1.8	2.6	1.04	1.81 ^{b}
	Non-incorporated	Fertilized	2.39	2.62	1.04	2.02 ^{b}
		Non-fertilized	1.88	2.17	0.98	1.68 ^{b}
N uptake (kg N ha^{-1})	Incorporated	Fertilized	45.90	56.50	29.40	43.90 ^{a*}
		Non-fertilized	34.90	35.50	17.80	29.40 ^{b}
	Non-incorporated	Fertilized	36.20	36.30	18.30	30.20 ^{b}
		Non-fertilized	29.10	30.00	14.30	24.30 ^{b}
N recovery efficiency[1] (kg kg^{-1})	Incorporated		0.44	0.84	0.57	0.61 ^{a*}
	Non-incorporated		0.28	0.27	0.12	0.23 ^{b}

*: numerals followed by a common letter are not significantly different at 5 % level.

[1] N recovery efficiency = {(N uptake in N fertilized plot) – (N uptake in N unfertilized plot)} / (amount of N fertilizer applied)

Conclusion

Low productivity in rainfed rice culture in Northeast Thailand is caused by drought and low soil fertility. The low soil fertility is associated with low organic matter and clay content in soil, which also produce low recovery efficiency of chemical fertilizer.

Investigations on farmers' fields revealed that application rates of chemical fertilizers varies by farmer and by the toposequential positions of fields. The linear relationship between paddy yield and fertilizer application rate in terms of nitrogen (N) indicates that more fertilizer application caused more paddy yield. However, the cost of 1 kg N in combined-fertilizer, 16-16-8 % (N-P_2O_5-K_2O) type, which was most popular in the region, was equal to about 6 kg paddy in 2000. Since the agronomic efficiency of nitrogen (yield increase per unit applied nitrogen) was 10 to 17 kg kg^{-1}, the risk of drought and flood may discourage farmers from applying more fertilizer. However, even though farmers do not buy more fertilizer, proper application of fertilizer will improve rice production.

Frequent split fertilizer application or slow release fertilizer improved fertilizer recovery efficiency. The increased paddy yield in the experiment with pond sediments incorporation was caused by increased fertilizer recovery efficiency. Although the frequent split fertilizer application and the slow release fertilizer did not substantially improve agronomic efficiency, the strong responsiveness to N uptake of KDML 105, one of the leading cultivars in the region, indicates that fertilizer recovery efficiency is the key to improving rice productivity. Since the maximum yield of KDML 105 was attained at a plant N uptake of 90 kg N ha^{-1}, it is important to control N supply from the soil, fertilizer, and uptake. The results of this study also suggest the importance of early growth, which increases the availability of water and nutrients in the soil. The prominent result in the pond sediment incorporation experiment suggests that improving fertilizer holding capacity is important for improving rice productivity, which might also be achieved with a soil improvement agent like bentonite or vermiculite.

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Chapter VIII

Optimization of N Fertilization Management in Citrus Trees: ^{15}N as a Tool in NUE Improvement Studies

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Abstract

In agricultural systems, excess application of N-fertilizer may result in NO_3^- displacement to deeper soil layers that may eventually end up in the groundwater. Increases in nitrate concentrations above the drinking water quality standards of the World Health Organization (50 mg L^{-1}) is a common concerning in most agricultural areas. Along the Mediterranean coast of Spain, where the cultivation of citrus fruits predominates, a severe increase in contamination by leaching of the nitrate-ion has been observed within the last two decades. Nowadays, efforts are being directed to understand the large number of processes in which N is involved in the plant-soil system, in order to reduce N rate and N losses, which may result in surface and ground water pollution, maintaining crop productivity. Thus, several trials relying on ^{15}N techniques are being conducted to investigate the improvement of nitrogen uptake efficiency (NUE) of citrus trees. The use of ^{15}N tracer opens the possibility to follow and quantify this plant nutrient in different compartments of the system under study. This article gathers the results of several studies based on ^{15}N techniques, carried out by the authors, in order to reevaluate current fertilization programs, organized according to different management practices: (1) Irrigation system: the dose of N and water commonly applied to commercial citrus orchards (up to 240 $kgN \cdot ha^{-1}yr^{-1}$ and 5000 $m^3 \cdot ha^{-1}yr^{-1}$) can be markedly reduced (roughly 15%) with the use of drip irrigated systems. (2) Time of N application: the N recovered by plants is 20% higher for summer N applications than for spring N when applied in flood irrigation trees. In drip irrigation, the highest NUE is obtained when N rate is applied following a monthly distribution in accordance with a seasonal absorption curve of N in which the maximum rates are supplied during summer. (3) N form and use of nitrification inhibitors (NI): nitrate-N fertilizers are absorbed more efficiently than ammonium-N by citrus plants, however ammonium fertilizers are recommended during the rainfall period. The addition of NI to ammonium-N fertilizers increases NUE (16%), resulting in lower N-NO_3^- content in the soil (10%) and in water drainage (36%).(4) Split N application: several split N applications result in greater fertilizer use efficiency and smaller accumulations of residual nitrates in the soil. (5) Soil type: N uptake efficiency is slightly lower in loamy than in sandy soils when N is applied both as nitrate and ammonium form; on the contrary, N retained in the organic and mineral fractions is higher in loamy soils that could be used in the next growing cycle. In addition to nitrogen and irrigation management improvement, plant tissue, soil and water N content must be considered in order to adjust N dose to plant demand, since rates exceeding N needs result in lower NUE.

Introduction

The purpose of nitrogenous fertilization is to increase the natural fertility of the soil in order to improve the nutritional status of crop plants. Citrus trees demand high-amounts of nitrogenous fertilizers as nitrogen (N) has a greater influence on growth and production than other nutrients (Smith, 1966). In citrus orchards, farmers have applied excesive dosages of nitrogen because of poor fertilizing criteria and slight enhances found in fruit yield when increasing the N dosage. This has resulted in a deterioration in the commercial quality of the fruit (Chapman, 1968), a reduction in the profitability of the citrus crops (Wild, 1992), a NO_3^- displacement to deeper soil layers. Many studies have shown direct relationships between this addition of N in areas of intensive agriculture and the alarming increase of NO_3^- concentration in groundwater (Singh and Kanehiro, 1969; Bingham et al., 1971; Burkart and Stoner, 2002; Babiker et al., 2004; de Paz and Ramos, 2004). Monitoring of drinking water and superficial groundwater revealed increases in nitrate concentrations above the drinking water quality standards of 10 mg NO_3-N L^{-1} (U.S. Environmental Protection Agency, 1994) in citrus-producing regions in central Florida (Alva et al., 1998; Lamb et al., 1999). Along the Mediterranean coast of Spain, where the cultivation of citrus fruits predominates, a severe increase in contamination by leaching of the nitrate-ion has also been observed in subterranean waters, above the limit of the World Health Organization (WHO) guideline (WHO, 2004), of 50 mg L^{-1} as nitrate (Sanchís, 1991; Fernández et al., 1998).

Nowadays, efforts are being directed to understand the large number of processes in which N is involved in the plant-soil system, like irrigation management, N application frequency, timing of application, as well as soil processes such as nitrification, denitrification, immobilization, volatilization and leaching, in order to reduce N rates and thus N losses, which may result in surface and ground water pollution, maintaining crop productivity. Studies of N-dynamics in laboratory soil columns (Vanden Heuvel et al., 1991; Esala and Leppänen, 1998), and in assays under greenhouse or field conditions (Westerman and Tucker, 1979; Mansell et al., 1986; Recous et al., 1988; Bengtsson and Bergwall, 2000) show important discrepancies which can be explained by the difference in experimental practicalities. However, the use of ^{15}N tracer opens the possibility to follow and accurately quantify this plant nutrient in different compartments of the system under study. Some authors have used this technique to determine N requirements of citrus trees and hence develop N fertilizer recommendations (Legaz et al., 1982; Kato et al., 1981; Mooney and Richardson, 1994). Nevertheless, these information is very specific and has its application in a limited number of cultivation conditions. More general guidelines based on the improvement of nitrogen uptake efficiency (NUE) of citrus trees would allow producers to realize similar yields while reducing overall N-fertilizer use.

This article compiles the results of several studies based on ^{15}N techniques, organized according to different management practices, carried out by the authors with the aim of reevaluating current fertilization programs. This information is necessary to deeply understand NUE and thus advance towards Best Management Practices for citrus crops.

Experimental Description

Experiment 1. Effect of Irrigation System and Fertilization Management on NUE

Assay conditions

The trial was carried out using 24 uniform orange trees (*Citrus sinensis*) c.v. Navelina grafted onto Carrizo citrange (*Citrus sinensis* x *Poncirus trifoliata*). In October 1992, 2-yr-old trees were planted in hexagonal drainage concrete lysimeters about 3.5 m^2 in area, 1 m deep, and containing about 3.5 m^3 of soil. In order to isolate soil from air temperatures, lysimeters walls were 15 cm thick. The study started 6 years later (in March 1998), and at that moment, all trees were essentially the same height (170 cm on average). At the bottom of each tank, drainage tubes were connected to cylinders used to collect the surplus drainage water to determine potential nitrate leaching. The trees were cultivated on a loam sandy-clay soil (67.4% sand, 10.8% silt, 21.8% clay; pH 7.9; organic matter content 0.6%; no $CaCO_3$ and a density of 1.35 kg m^{-3}).

Fertilization and Irrigation Scheduling

The N-fertilizer rate for citrus plants was calculated based on criteria established by Legaz and Primo-Millo (1988) for a 2.3 meter canopy diameter. The N requirement was assumed to be 175 g N tree^{-1} year^{-1}, of which 125 g were supplied as labelled KNO_3 with an isotopic enrichment of 7 atom % ^{15}N. The N-remainder was provided by typical irrigation water in the mediterranean area which contained 40 mg NO_3^- L^{-1}. The quantity of N contributed by the irrigation water was calculated using the formula described by Martínez et al. (2002).

Flood or drip system was used for irrigation. For the flood irrigation treatment, labelled-N fertilizer was splitted in two (applied on 26 March and 23 June) or five (applied on 26 March, 8 May, 23 Jun, 7 August and 16 September) equal doses. These application rates were within the range commonly used in the fertilization of flood irrigated citrus trees in Mediterranean area. In the case of drip-irrigated trees, ^{15}N enriched KNO_3 was applied following two different monthly distributions based on seasonal N demand and seasonal evapotranspiration (ET). In drip treatment by N demand, the dosage was divided into a monthly distribution percentage of total N in accordance with the seasonal absorption curve of N established in citrus plants (Legaz *et al*., 1983): in March (5%), April (10%), May (15%), June (22%), July (18%), August (15%), September (10%) and October (5%). In drip treatment by ET demand, the dosage was divided setting a constant N quantity per litre of water applied. In this case, the percentage of monthly distribution depended on evapotranspiration demand. The soil containers used for both irrigation systems were randomized across the experimental area and analyzed individually.

The amount of water applied to each tree was equivalent to the total seasonal crop evapotranspiration (ETc) (Doorenbos and Pruitt 1977). For drip-irrigated treatments, the weekly applied volume of water to each tree was calculated using the following expression: $ETc = ET_o * K_c$; where ET_c is crop evapotranspiration; ET_o is reference crop evapotranspiration under standard conditions and K_c is crop coefficient. The crop coefficient (K_c) accounts for crop-specific effects on overall crop water requirements and is a function of canopy size and leaf properties. The ET_o values were determined using the Penman-Monteith approach (Allen et al., 1998). The K_c values were based on guidelines provided by Castel and Buj (1994). For flood irrigation treatments, the volume of water applied was 15% higher compared to drip irrigation treatments. This difference was due to the different application efficiency for each irrigation system, which was reported to be between 12% (Manjunatha et al., 2000) and 21% (Tekinel et al., 1989) higher in drip irrigation. Irrigation water requirements (6498 and 5649 L $tree^{-1}$ under flood and drip irrigation, respectively) were met by the effective rainfall of the entire year (1235 L $tree^{-1}$) plus irrigation water. Water was applied weekly or fortnightly in the flood-irrigated trees with 25 applications of 260 L per tree (68.4 L m^{-2}) during the overall growth cycle (from January until December 1998). The volume of water supplied by drip irrigation was divided into 66 drip irrigation applications. Trees were irrigated 1-3 times per week using 4 commercial emitters per tree (4 L h^{-1}) resulting in a 33% wetting area (Keller and Karmelli, 1974).

Therefore, the assay consisted of four treatments with three replications, one tree each (Figure 1) in which different fertilizer managements were compared: Low frequency N application under flood irrigation, with two (FI-2) or five N applications (FI-5) versus high frequency N application under drip irrigation based on **N** demand (DI-N) or ET demand criteria (DI-ET).

Soil and Plant Tissue Sampling

Soil from the 0-15, 15-30, 30-45, 45-60 and 60-90 cm soil layers was sampled using a 4 cm diameter soil auger. In the flood-treated trees, three soil samples cores were taken mid-way between trunk and lysimeter wall, 7, 35 and 80 days after first and second N application. In the drip-irrigated trees, three soil samples were taken monthly in the wetting zone about mid-way between the emitter and the periphery of the wetting front. At the end of the experiment, three soil samples per tree were also taken for both treatments at all soil depths, following the same procedure explained above. The weight of each soil layer was about 700 kg. Each sample hole was refilled with the same kind of soil to prevent preferential movement of water and fertilizer.

In order to quantify biomass and N losses associated with senescing tree parts, tree litter was caught with a screen of 6 m^2 positioned around each container. The litter (petals, calyces, ovaries, young fruits and old leaves) were gathered fortnightly from the beginning of April to the end of June (from onset of flowering until the end of fruit setting).

In order to determine the NUE, plants were removed in December 1998. Trees were segregated into mature fruit, leaves and twigs of new shoots, old leaves (from previous years), old twigs (separated by diameter: <1, 1-3 and 3-7 cm), trunk (7-12 cm) and roots (separated into: fibrous roots (off-white) while all other roots were sorted based on diameter < 1, 1-3, and 3-12 cm).

Treatment	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
FI-2 split[1]	50 (1)			50 (1)					
FI-5 split	20 (1)		20 (1)	20 (1)		20 (1)	20 (1)		
DI-N demand	4.2 (2)	10.8 (5)	15.1 (4)	21.8 (9)	17.9 (14)	14.9 (13)	10.3 (11)	5.0 (8)	
DI-ET demand	2.5 (2)	8.4 (5)	6.2 (4)	13.0 (9)	21.3 (14)	19.9 (13)	16.2 (11)	12.5 (8)	
Flood irrigation[3]	388[2] (2) (2)	472 (2)	359 (2)	731 (3)	1170 (4)	1104 (4)	909 (4)	707 (3)	428 (2)
Drip irrigation[4]	337(5)	410 (5)	312 (4)	636 (9)	1017(14)	960 (13)	790 (11)	615 (8)	372 (5)

[1] Percentages of N applied at the end of each month in each treatment (in brackets the number of N applications).

[2] Litres of irrigation water applied at the end of each month (in brackets the number of irrigations).

[3] Total water applied in flood irrigation = 6498 litres (includes 230 litres applied in January).

[4] Total water applied in drip irrigation = 5649 litres (includes 200 litres applied in January).

Figure 1. Seasonal distribution of N dosage and irrigation water using flood or drip irrigation system in citrus trees

All soil layers were excavated and large and small roots were separated by hand. In order to estimate the fibrous roots remaining in the soil, three soil samples were taken in each layer and sifted through a 2 mm mesh sieve to remove the rest of the fibrous roots. All tree components were weighted in the field and representative subsamples of these portions were collected and weighted.

Experiment 2. Effect of Timing of N Application, N Form and Soil Texture On NUE

Assay Conditions

The experiment was carried out in 42 Valencia late orange trees (Citrus sinensis (L.) Osbeck), grafted on Troyer citrange (Citrus sinensis x Poncirus trifoliata) rootstock, three-year-old, which were cultivated outdoors individually in 103 L containers. At the bottom of each tank, drainage tubes were connected to cylinders used to collect the surplus drainage water and to determine potential nitrate leaching. Two soils were utilized for the experiment: a sandy soil (pH= 8.0, 93.9% sand, 3.3 % silt, 2.8 % clay and 0.6% organic matter) and a loamy soil (pH=8.2, 37.1% sand, 40.8% silt, 22.1% clay and 1.44% organic matter).

Fertilization and Irrigation Scheduling

Four blocks of uniform trees were fertilized in a single application with 30 g N.tree^{-1}, according to recommendations stablished by Legaz and Primo-Millo (1988) four young trees. This nitrogen was supplied as labelled $(NH_4)_2SO_4$ or KNO_3 with an isotopic enrichment of 8.5 atom % ^{15}N from beginning of spring flush (26 March). Other two blocks were fertilizer with enriched KNO_3 (8.5 atom % ^{15}N in excess) from beginning of summer flush (24 July). At dormancy, plants were distructively harvested.

The amount of water applied to each tree was equivalent to the total seasonal crop evapotranspiration (ETc) (Doorenbos and Pruitt 1977). Flood system was used for irrigation and water was applied fortnightly. The volume of water supplied from the beginning of the assay until dormancy was around 650 and 735 L.tree^{-1} for trees cultivated in sandy and loamy soil, respectively. The water used for irrigation came from a well and contained 25 mg N-NO_3^- L^{-1}. This nitrogen was also used as fertilizer according to the formula described by Martínez et al. (2002).

Six treatments (N fertilizer managements) with seven replications (one tree each) were studied (Figure 2): Ammonium Sulphate applied in Spring flush to citrus cultivated in Sandy soil (AS-Sand Sp) and Loamy soil (AS-Loam Sp), Potassium Nitrate applied in Spring flush to citrus cultivated in Sandy soil (PN-Sand Sp) and Loamy soil (PN-Loam Sp) and Potassium Nitrate applied in summer flush to citrus cultivated in Sandy soil (PN-Sand Su) and Loamy soil (PN-Loam Su).

Soil and Plant Tissue Sampling

In order to quantify biomass and N losses associated with senescing tree parts, tree litter was caught with a screen positioned around each container. The litter (petals, calyces, ovaries, young fruits and old leaves) were gathered fortnightly from the beginning of April to the end of June (from onset of flowering until the end of fruit setting).

At dormancy (November), two labelled plants were removed from each treatment; the different parts of the plant were fractionated in order to determine NUE. Trees were segregated into mature petals, ovaries, fruits, leaves and twigs of new shoots, old leaves (from previous years), old twigs, trunk (7-12 cm) and fibrous and large roots (Figure 2).

In all harvest period, soil samples were taken mid-way between trunk and container wall at different depths: 0-15, 15-30, 30-45, 45-60, 60-75 and 75-110 cm, using a 4 cm diameter soil auger.

Experiment 3. Effect of N Form by Usingthe Nitrification Inhibitor (DMPP) on NUE

Assay conditions

The assay was carried out on 12 uniform 10-year-old trees of Navelina (*Citrus sinensis*) grafted on Carrizo citrange (*Citrus sinensis* x *Poncirus trifoliata*) rootstock distributed at random in 4 similar blocks of 3 replicate trees. The study started once Experiment 1 was finished, with the same assay conditions described above.

Treatment[1]	Labelling (days)	N applied (g.tree^{-1})	Water applied (L)
AS-Sand Sp	243	50.5	657
AS-Loam Sp	243	54.2	741
PN-Sand Sp	243	50.5	657
PN-Loam Sp	243	54.2	741
PN-Sand Su	116	50.1	642
PN-Loam Su	116	53.9	732

Figure 2. Length of labelling period, N and water amount applied to different treatments.

Fertilization and Irrigation Scheduling

The N-fertilizer rate for citrus plants was calculated based on criteria established by Legaz and Primo-Millo (1988). The N requirement was assumed to be, therefore, 220 g N tree^{-1} supplied to six trees as labelled $(^{15}NH_4)_2SO_4$ with an isotopic enrichment of 4 atom % ^{15}N, either without or with nitrification inhibitor of 0.5%. Other three trees were fertilizer with $^{15}NH_4NO_3$ and another three received a $K^{15}NO_3$ plus $Ca(NO_3)_2$, with the same isotopic enrichment. This dosage was supplied into 66 aplications through drip irrigation with a monthly distribution according to the seasonal N absorption curve (Legaz et al., 1983).

The volume of water applied weekly to each tree was calculated using the following expression: ETc = ETo x Kc; ETo was determined according to the previous experiment (Allen et al., 1998). The volume of water (7061 L.tree^{-}1) supplied by drip irrigation was divided into 66 applications. Trees were irrigated 1-3 times per week using 4 commercial emitters per tree (4 L h^{-1}) resulting in a 33% wetting area (Keller and Karmelli, 1974).

Therefore, the assay consisted of four treatments (N form) with three replications (one tree each): **A**monium **S**ulphate (AS) and **A**monium **S**ulphate with **N**itrification **I**nhibitor (AS+NI), Ammonium **N**itrate (AN) and **P**otassium **N**itrate plus **Ca**lcium **N**itrate (PN+CaN).

Soil and Plant Tissue Sampling

In order to determine the NUE, the trees were removed and soil sampled were extracted at the end of the growth cycle following the methodology explained in above experiments.

Optimal Control of Irrigation Scheduling

In all studies, within each container, 1 or 2 clusters of tensiometers were installed at a soil depth of 15 and 45 cm. Tensiometers were used to monitor the soil water tension in order to schedule irrigation events. The tensiometers were read every 2 days, and irrigation was scheduled when the matric potential at 30 cm depth attained -10 kPa in drip irrigated treatment and -20 kPa in flood irrigated trees (Smajstrla et al., 1987; Parsons, 1989).

Treatment of Plant and Soil Samples

All the vegetal samples were washed in non ionic detergent solution followed by several rinses in distilled water and then frozen in liquid-N_2 and freeze-dried. Vegetative samples were ground to pass trough a sieve with 0.3-mm diam. holes using a water-refrigerated mill and stored at –4ºC until further analysis.

All soil samples were air-dried, sieved through a 2 mm screen and stored at room temperature (22ºC) until subsequent analysis.

Mineral Nutrient Analysis

Total nitrogen (mineral-N and organic-N) content of both plant tissue material and soil samples was determined using the Semi Micro-Kjeldahl method described by Bremner (1965). The analysis of the mineral nitrogen of the soil (as NO3-N and NH4-N) was measured with Flow Injection Analysis (Aquatec 5400, Foss Tecator, Höganäs, Sweden) following the methodology described by Raigon et al. (1992). Soil organic nitrogen content was calculated by the difference between total and mineral N contents (method Kjeldahl described by Bremner, 1996). In the first three assays, isotopic composition (14N/15N) of mineral and total N (Hauck and Bremner, 1976) was evaluated with an emission spectrometer, N-15 analyzer (Jasco-15 Spectroscopic, Tokyo, Japan) following procedures outlined by Yamamuro (1981). In the last experiment, total N and 15N concentrations were determined with an Elemental analyzer (EA NC 2500, Thermo Finigan) coupled to an Isotope Mass Spectrometer (Delta Plus, Thermo Finigan).

^{15}n Calculations

Atom %^{15}N excess was obtained by subtracting $^{15}\text{N}_{\text{air natural abundance}}$ (0.366 atom %^{15}N) from the ^{15}N enrichment determined by the emission spectrometer (Junk and Svec 1958).

NO_3-^{15}N or organic-^{15}N concentration in soil (mg ^{15}N kg soil^{-1}) was calculated as NO_3-N or organic-N concentration (mg N kg soil^{-1}) multiplying by atom %^{15}N excess and divided by 100.

Nitrogen absorbed from fertilizer (Naff) is the ratio of N absorbed in whole tree and the ^{15}N applied from fertilizer. This index was calculated using the following formula:

$$\text{Naff} = \frac{^{15}\text{N}_{\text{tissue}}\ \text{content(mg)}}{^{15}\text{N}_{\text{whole tree}}\ \text{content(mg)}} \times 100$$

Nitrogen retained from fertilizer (Nrff) is the ratio of N retained in the soil profile and the N applied from fertilizer. This index was calculated using the following formula:

$$\text{Nrff} = \frac{^{15}\text{N}_{\text{fracction}}\ \text{retain(mg)}}{^{15}\text{N}_{\text{soil profile}}\ \text{retain (mg)}} \times 100$$

In order to determine the N recovery we used the following procedures:

- For soil N calculations, NO_3-^{15}N or organic-^{15}N content (mg) were calculated by multiplying NO_3-^{15}N or organic-^{15}N concentration (mg kg soil^{-1}) by soil layer weight (kg).

- For plant N calculations, ^{15}N-total content (mg) was calculated by multiplying total N concentration (mg N g^{-1}) by dry biomass weight (g), by atom %^{15}N excess divided by 100.
- N-fertilizer recovery was based on ^{15}N content (mg in soil and plant fraction) divided by the ^{15}N excess content of the applied fertilizer.

Statistical Analysis

Results of different trials were analyzed using standard analysis of variance techniques (ANOVA). Treatment means separation was determined using LSD-Fisher-test ($P \leq 0.05$) on all the parameters.

Results

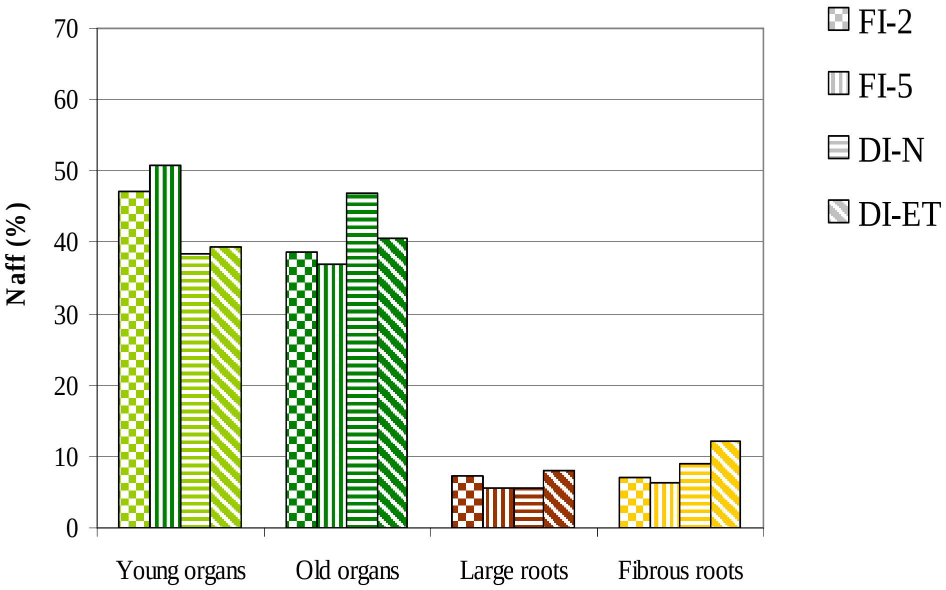
Experiment 1. Effect of Irrigation Systemand Fertilization Managementon NUE

N Absorbed from the Fertilizer and its Relative Distribution

The N absorbed from fertilizer (Naff) by the entire tree was significantly greater in trees using drip irrigation (93.8 and 88.4% in DI-N and DI-ET treatments, respectively) than those under flood irrigation (78.2 and 79.1% in FI-2 and FI-5 treatments, respectively). In young organs, the N accumulated in fruit oscillated between 26-29% with flood irrigation and 22-24% with drip irrigation. These relative values were greater than the N absorbed by the leaves plus twigs of new shoots in all treatments. Among old organs, old leaves presented the higher percentages. The new flush leaves and young organs accumulated significantly higher relative percentages with flood irrigation than drip irrigation. However, the percentages of Naff for the fibrous roots were significantly higher with drip irrigation. The frequency of split application did not significantly affect the percentage of N accumulated in any of the organs in flood treatments. In monthly distribution of the N dosage, the percentages Naff by old organs and by the tree top in DI-N was significantly greater than in DI-ET(Figure 3).

In treatments with conventional irrigation, young organs stored a higher average of the total absorbed (47.2 and 50.9%) than old organs (38.7 and 37.0%, in FI-2 and FI-5 treatments, respectively). In contrast, this tendency is the reversed with drip irrigation, where young organs accumulated a 38.5 and 39.4 % of the total absorbed and the old organs a 47.0 and 40.5%, DI-N and DI-ET, respectively (Figure 3). Fibrous roots, despite their low percentages of dry weight (data not shown), retained quantities of labelled N equal to or greater than those of large and small roots (Figure 3). The root system accounted for around 14% of the total absorbed in FI-2, FI-5 and DI-N, whilst in DI-ET, it was 20%.

These results show that, the differing seasonal amount of the N dosage greatly influenced the relative distribution in the tree of the N absorbed from the fertiliser (Naff), regardless of the irrigation system employed. Thus, the N applied up to the end of June (100 and 60% of the total N dosage in FI-2 and FI-5, respectively) was accumulated in greater percentages in young organs. These results are in line with those of Kubota et al. (1976a) and Akao et al. (1978) who observed that 70-75% of the NO_3^--^{15}N applied to 9- or 15-yr-old "Satsuma" trees in March was translocated to the top parts of the trees and partitioned preferentially to newly developing organs in spring. Martínez et al. (2002) and Quiñones et al. (2005) also found a greater ^{15}N recovery in the new organs (55% and 49% of de ^{15}N applied, respectively) of citrus trees when the N was applied from March to June. However, when 55% N dosage (DI-N treatment) was applied in periods of greater N absorption from the beginning of June till the end of August the greater proportions of Naff were stored in old organs (47%); while a similar percentage of N was applied later from the beginning of July till the end of September (DI-ET treatment) higher values were accumulated in root system (20%). Akao *et al*. (1978), Legaz *et al*. (1983) and Kato *et al*. (1987) observed a greater tendency of N accumulation in the roots on applying N in autumn. The different distribution of N stored in the above organs could no affect on the N remobilization towards the development of new tissues in the next year, because all the reserve organs (leaves, branches and roots) can export part of their stored N (Legaz *et al*.1995) and the timing of N supply do not affect to the N remobilization in the following spring growth (Quartieri and *et al*., 2002).



[1]Significant effects of factors are given at *P*>0,05 (N.S.), *P*≤0,05 (*), *P*≤0,01 (**) and *P*≤0,001 (***)
[2]Significant differences between treatments due to irrigation system (IS)
[3]Significant differences between treatments with flood irrigation due to frequency of application (FA (FI))[4]Significant differences between treatments with drip irrigation due to monthly distribution (MD (DI))

Figure 3. Effects of different N fertilizer management on N absorbed from fertilizer (Naff) among different organs of citrus trees of the Experiment 1.

N Retained from the Fertilizer and its Relative Distribution

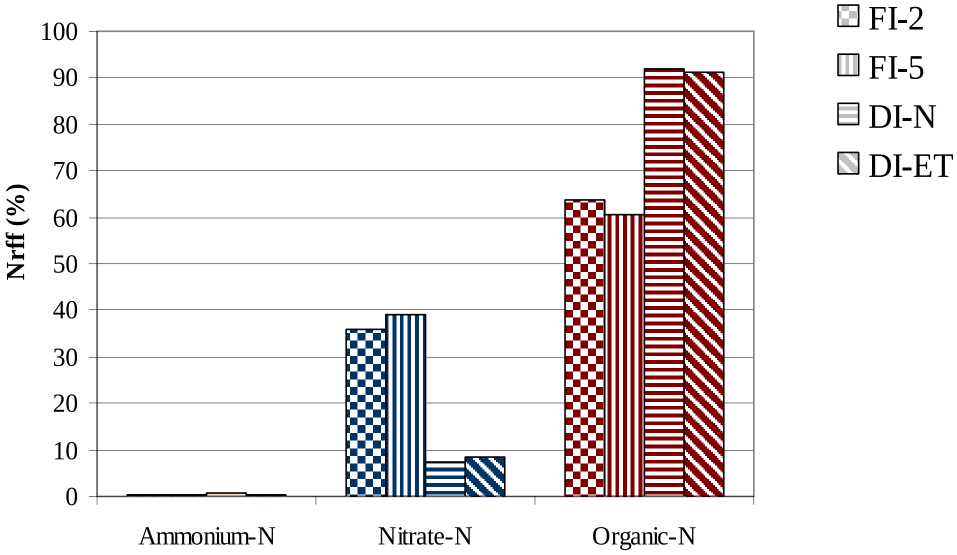
The ^{15}N recovered from the fertilizer in the different N soil fractions showed that the percentages retained as NO_3^--N were significantly higher for the flood irrigated (around 38% of the N retain) than for the Drip irrigated trees (8%). This lower residual NO_3^--N accumulation under fertigation is advantageous in comparison with flood irrigation from an environmental point of view. As discussed above, the main source of groundwater pollution is NO_3^- leached from intensive agricultural areas (Bingham et al. 1971; Burkart and Stoner 2002; Babiker et al., 2004; de Paz and Ramos, 2004). Nevertheless, no significant differences appeared in the amount of organic-^{15}N for both treatments. In the literature, Feigenbaum et al. (1987) found low nitrate recovery values (0.6%) between 0 and 150 cm depth, whereas in the upper 15 cm of the soil profile, 4.3% of the ^{15}N was partitioned to the organic soil fraction, 9 months after N-fertigation in a sandy to sandy loam Hamra soil. Similarly, Kee Kwong et al. (1986) found that the percentage of fertilizer N immobilized as organic-^{15}N ranged from 22 to 25%, 10 months after N application with an organic matter content of 7.2%. Recous et al. (1988) observed that 17 and 1% of the applied ^{15}N accumulated in the organic and inorganic soil N pools, respectively, 154 days after N application with an organic matter content of 2.1%.

For both treatments, it is worth mentioning that only 6 days after the N application a small fraction of the ^{15}N occurred as ammonium (0.7%^{15}N excess in all soil layers and 0.05%^{15}N excess in two first layers in FI and DI treatments, respectively). This can be due to the process of fertilizer nitrate inmobilization and later mineralization of labeled soil organic matter. Davidson et al. (1991) found a rapid turnover of a small NO_3^- pool in intact soil cores due to a rapid phase of immobilization immediately following the addition of ^{15}N tracers to soils. A gross mineralization rate took place about 3 to 4 days after immobilization (Barraclough, 1995). The labeled ammonium-N medium found at the end of the trial accounted only for the 0.1% of applied-N for treatments (Figure 4).

Nitrogen Recovered from the Fertilizer

The drip irrigation system significantly increased the efficiency of N absorption by the whole tree and the root system (Figure 5). The percentages of N recovered from the whole tree were 75 and 71% for DI-N and DI-ET treatments, respectively, as opposed to 63 and 63% with flood irrigation (FI-2 and FI-5, respectively). This data are similar to those of Syvertsen and Smith (1996) who found a NUE value for lysimeter-grown citrus trees on the order of 61 to 68%. Further improve NUE by citrus with fertigation compared with dry granular fertilizer was also reported by Dasberg et al. (1988), Alva and Paramasivam (1998), Alva et al. (1998) and Quiñones et al. (2005). More frequent application of dilute N solutions doubled NUE compared with less frequent application of more concentrated N solutions (Scholberg et al. 2002). In another study, Alva et al. (2006) demonstrated a slight increase in N uptake efficiency as a result of better management practices associated with N placement, timing of application, and optimal irrigation scheduling when comparing fertigation (FRT-15 N applications) versus water soluble granular (WSG-4 N applications). These values were 2.3 kg N for the WSG and 2.2 kg N for the FRT as kg of N per 1 Mg (1 metric tone) of fruit. Similar increases in NUE were obtained by other authors expressed as increment in fruit yield. Boman (1996) reported a greater NUE (9% greater fruit yield) in grapefruit trees receiving a combination of one dry granular broadcast application (33% of the annual rate) and 18 fertigations at 2-wk intervals compared to trees that received 3 applications of dry fertilizer. Alva et al. (2003) evaluated different combinations of irrigation and nitrogen management. Fruit yield of 36-yr-old Valencia orange trees was greater with the application of N as fertigation compared to that of the trees which received a similar rate of N as 3 broadcast applications of granular product. Previous studies showed a lack of response to fertigation frequency in fruit yield. Koo (1980) also reported that two dry fertilizer N applications resulted in similar fruit yields as 10 fertigation applications. Alva and Paramasivam (1998) have showed similar results in two years of a three-year study. Koo (1986) and Boman (1993) also found no difference in yield when comparing two applications of dry soluble fertilizer with controlled release N sources.

The grade of dividing up the fertiliser did not produce significant differences in flood-irrigated trees (FI-2 and FI-5). The monthly distribution of N with drip irrigation (DI-N and DI-ET) gave rise to significant differences in the tree top of the tree. Therefore, the tree top of DI-N (64%) took up more N from the fertiliser than those of DI-ET (57%). The values found in the literature on N recovered from the fertiliser by the plant are closely related to timing of N application, the labelling period and the irrigation system used. Kubota *et al.* (1976a) found an efficiency of 25.0% (118 days after beginning of labelling) in satsumas mandarin “Sugiyama” with a single N supply in March by flood irrigation.



[1]Significant effects of factors are given at P>0,05 (N.S.), P≤0,05 (*), P≤0,01 (**) and P≤0,001 (***)

[2]Significant differences between treatments due to irrigation system (IS)

[3]Significant differences between treatments with flood irrigation due to frequency of application (FA (FI))

[4]Significant differences between treatments with drip irrigation due to monthly distribution (MD (DI))

Figure 4. Effects of different N fertilizer management on N retained from fertilizer (Nrff) in different fractions of soil of the Experiment 1.

	mg ^{15}N (placement x 100/fertilizer[6])				ANOVA[2]		
Placement[1]	FI-2	FI-5	DI-N	DI-ET	IS[3]	FA FI[4]	M D[5]
NUE	62,6	63,2	75,0	70,7	**	N.S.	N.S.
Fallen organs	2,9	2,3	1,6	1,6	**	N.S.	N.S.
Total soil	19,7	23,1	12,6	13,2	***	*	N.S.
Drainage	0,1	0,1	0,0	0,0	**	N.S.	N.S.
Recovery	86,4	89,8	90,1	86,4	N.S.	N.S.	N.S.

[1] Means of three trees replications.

[2] Significant effects of factors are given at $P>0,05$ (N.S.), $P\leq 0,05$ (*), $P\leq 0,01$ (**) and $P\leq 0,001$ (***).

[3] Significant differences between treatments due to irrigation system (IS)

[4] Significant differences between treatments with flood irrigation due to frequency of application (FA (FI)).

[5] Significant differences between treatments with drip irrigation due to monthly distribution (MD (DI))

[6] mg ^{15}N content in fertilizer = 8750 mg.

Figure 5. Effects of different N fertilizer management on recovery of ^{15}N fertilizer (%) in whole tree (NUE), drainage water and soil profile at harvesting time of the Experiment 1[1].

In the same conditions, the recovery was higher (61.0%) when the N was supplied in June and trees were removed 181 days later (Kubota *et al.*,1976b). Martínez *et al.* (2002) obtained a recovery of 38% in young oranges “Valencia Late”, 243 days after a single application of N in March by flood irrigation. Feigenbaum *et al.* (1987) found an efficiency of 56.6% in mature oranges “Shamouti”, 112 days after the latest supply (25 applications from April to August) in drip irrigation.

The N-percentages absorbed and shed by tree litter were significantly higher in flood-irrigated trees (2.6% of N applied) than those under drip irrigation (1.6% of N applied), and depended on the N-dose applied during the litter drop season which was 100, 60, 51 and 30% for FI-2, FI-5, DI-N and DI-ET treatments, respectively.

With flood irrigation (FI-2 and FI-5), a greater mean value in the form of nitrate and in the total fraction (nitrate plus organic) was retained as opposed to the values with drip irrigation (DI-N and DI-ET) (Figure 5). The degree of dividing up the N dosage also significantly increased in FI-5 over FI-2. The time lapse between the last N application (June) and the sampling of the soil in FI-2 was notably greater than in FI-5, which was carried out in September (Figure 1). Therefore, the nitrate had more time to be absorbed, and this fact conducted to a decreasing in the nitrate and total N fractions in the soil. The irrigation methods and seasonal N distribution and soil type can affect the N retained from the fertiliser in the soil profile. Mansell *et al.* (1986) obtained that 2% of the N applied remained in the form of nitrate (0-100 cm depth) at 134 days from N application in citrus plants irrigated by sprinkler system. Feigenbaum *et al.* (1987) found low values in nitrate recuperation (0.6%) between 0 and 150 cm depth, whereas at between 0 and 15 cm, they recovered 4.3% as organic N at 120 days of applying N. Martínez *et al.* (2002) obtained values of 4.2 and 18.9% for the N retained in nitrate and organic forms, respectively, at 243 days from N application. Similar results were obtained using another crop, Recous *et al.* (1988) found that 17% of the N applied was retained in organic N and 1% in nitrate form at 154 days from N application in cultivating wheat.

Throughout the trial, depth percolation only occurred for the flood irrigation treatment and even in this case only of 0.1% of the applied-N was lost due to leaching which was related to irrigation being based on actual crop water requirements and the lack of leaching rainfall events.

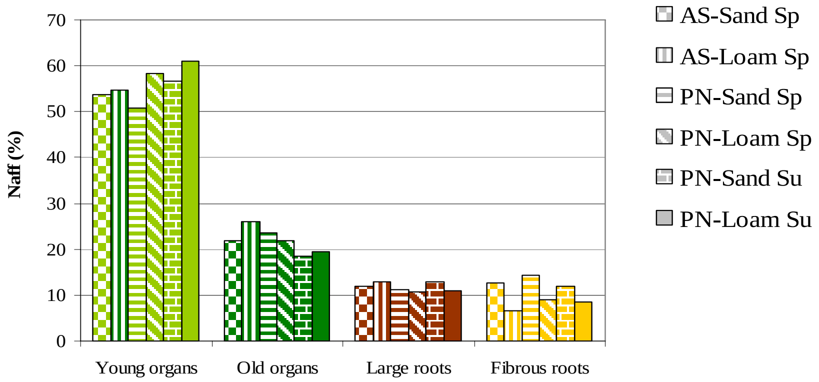
The variables used did not significantly affect the N recovered from fertiliser by the plant-soil-water system (86.4, 89.8, 90.1 and 86.4% in Fl 2, Fl 5, DI N and DI ET, respectively) but we found higher recoveries, lower losses from the fallen organs and from nitrate residual in soil, and less supply of water using drip irrigation than those under flood irrigation for the same labelling periods. The best behaviour of the drip system is a consequence that N and water supplies are coincident with plants seasonal requirements and N and water losses are minimised.

Under field conditions higher differences between both irrigation managements could be expected since water and N requirements are usually overestimated in flood irrigated areas. This could lead to even lower N uptake efficiency and higher N leaching below the active root zone.

Experiment 2. Effect of Timing of N Application, N Form and Soil Texture on NUE

N Absorbed from the Fertilizer and its Relative Distribution

The greatest ^{15}N absorbed occurred in the new growing organs (flowers, fruits, new flush leaves and twigs) in all the treatment studied (Figure 6).



[1] Significant effects of factors are given at *P*>0,05 (N.S.), *P*≤0,05 (*), *P*≤0,01 (**) and *P*≤0,001 (***)

[2] Significant differences between treatments with spring application due to fertilizer applied (F)

[3] Significant differences between treatments with spring application due to type of soil (S)

[4] Significant differences between treatments with spring application due to fertilizer applied vs. Soil type (FxS)

[5] Significant differences between treatments with potassium nitrate due to timing of N application (T)

[6] Significant differences between treatments with potassium nitrate due to type of soil (S)

[7] Significant differences between treatments with potassium nitrate due to timing of N application vs. type of soil (TxS)

Figure 6. Effects of different N fertilizer management on N absorbed from fertilizer (Naff) among different organs of citrus trees of the Experiment 2.

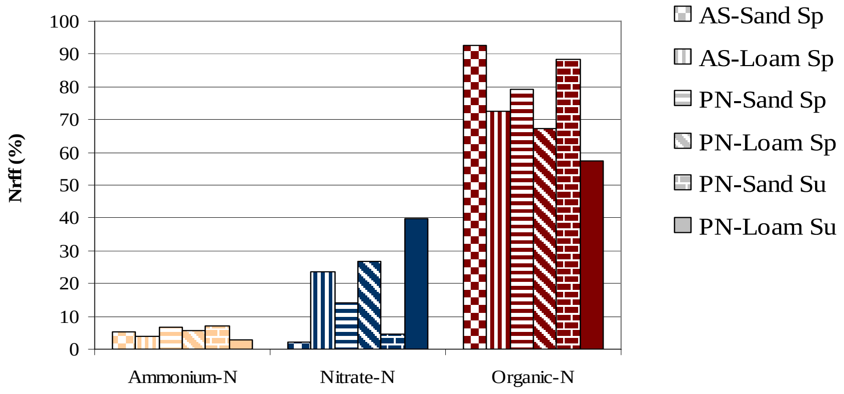
Our results are in agreement with other works carried out with citrus (Kubota et al., 1976a; Legaz et al., 1982), pear (Sanchez et al., 1992), and peach (Huett and Stewart, 1999). In young oranges trees, where vegetative vs. reproductive development predominates, Legaz *et al.* (1983b) and Legaz and Primo-Millo (1987) observed higher %Nadf in young than in old organs at the end of the growing cycle; while the opposite was found in adult trees (Feigenbaum *et al.*, 1987; Mooney y Richardson, 1994; Lea-Cox *et al.*, 2001, Quiñones, 2002).

In treatments with spring application, the N form in the fertilizer only affected to N absorbed from fertilizer in fibrous roots. The Naff by fibrous roots was significantly greater in trees fertilized with potassium nitrate (14.3 and 9.1% in PN-Sand and PN-Loam, respectively) than those under ammonium fertilization (12.7 and 6.5% in AS-Sand and AS-Loam treatments, respectively). In these treatments, young organs stored a higher average of the total absorbed (52%) in citrus cultivated in sandy soil than in loam soil (57%). Legaz and Primo-Millo (1988) found an increase of 4.5 fold in young *citrus* trees grown in inert sand during a similar period time. In contrast, this tendency is the reversed with the N absorbed by fibrous roots, where citrus developed in sandy soil absorbed 12 and 14 % of AS-Sand Sp and NP-Sand Sp treatments, respectively, and in loam soil this percentage descended to 7 and 9% for AS-Loam Sp and NP-Loam Sp, respectively (Figure 3). The trees grown in sandy soil showed higher percentages of absorbed N in total roots, as a result of higher N values also found in fibrous roots (data not shown).

In the citrus fertilized with potassium nitrate, the timing of N application affected to N acumulated in young and old organs and fibrous roots. When the N application was in spring, Naff in young organs acumulated a 54% of the N absorbed for the entire tree, whereas a 59% of the N absorbed was found in trees fertilized in summer. The opposite tendency was found in old organs and fibrous roots. Overall percentage of N allocations were similar to those reported by Lea-Cox et al. (2001) for 4-yr-old “Redblush” grapefruit trees supplied with a single application of $^{15}NH_4{}^{15}NO_3$ in late April. Newly developing spring leaves and fruit formed dominant competitive sinks for ^{15}N, representing between 40% and 70% of the total ^{15}N. However, when N dosage was applied in periods of greater N absorption (summer), the greater proportions of Naff were stored in old organs (Kato *et al.*, 1987; Quiñones et al., 2005). On the other hand, a similar pattern in the summer application was found, to that reported for spring application, regarding soil texture.

N Retained from the Fertilizer and its Relative Distribution

For both N applications (spring and summer), the ^{15}N recovered from the fertilizer in the different N soil fractions showed that the percentages retained as NO_3^--N were significantly higher for the trees cultivated in sandy soil (around 7% of the totan N retain) than those developed in losmy soil (30%), due to the greater water holding capacity of sandy soils (Figure 7).



F (Sp)[2]	N.S.[1]	N.S.	N.S.
S (Sp)[3]	**	**	**
F x S (Sp)[4]	N.S.	N.S.	N.S.
T (PN)[5]	N.S.	N.S.	N.S.
S (PN)[6]	*	***	**
T x S (PN)[7]	N.S.	N.S.	N.S.

[1] Significant effects of factors are given at $P>0,05$ (N.S.), $P\leq 0,05$ (*), $P\leq 0,01$ (**) and $P\leq 0,001$ (***)

[2] Significant differences between treatments with spring application due to fertilizer applied (F)

[3] Significant differences between treatments with spring application due to type of soil (S)

[4] Significant differences between treatments with spring application due to fertilizer applied vs. Soil type (FxS)

[5] Significant differences between treatments with potassium nitrate due to timing of N application (T)

[6] Significant differences between treatments with potassium nitrate due to type of soil (S)

[7] Significant differences between treatments with potassium nitrate due to timing of N application vs. type of soil (TxS)

Figure 7. Effects of different N fertilizer management on N retained from fertilizer (Nrff) in different fractions of soilof the Experiment 2.

Nitrogen Recovered from the Fertiliser

In all treatments, N recovery from fertilizer in whole tree was lower than 66% of N applied. Similar works based on N labelling differ from this assay in NUE values, since this parameter directly depends on the adjustment between N supply and plant demand, soil texture, timing of N applications and length of labelling period. In this way, Feigenbaum *et al.* (1987) found NUE of 56-61% when supplying high and low potassium nitrate doses respectively to “Shamouti” orange trees under flood irrigation. Kubota *et al.* (1972a) obtained NUE values of 22% in satsuma mandarin trees, labelled in a single calcium nitrate application in sand culture.

The N recovery by whole tree in spring treatments was significantly greater in trees fertilized with potassium nitrate (40.1 and 37.0% in PN-Sand and PN-Loam, respectively) than those under ammonium fertilization (37.9 and 33.9% in AS-Sand and AS-Loam treatments, respectively). Moreover, the trees grown in sandy soil showed higher percentages of N recovery in whole tree for trees fertilized in spring and summer (Figure 5). In trees feed with potassium nitrate, ^{15}N recovery in whole tree was higher in summer treatments (59.0 and 51.5% PN-Sand Su and PN-Loam Su, respectively) than in spring (40.1 and 37.0% in PN-

Sand Sp and PN-Loam Su, respectively). This However, when N dosage was applied in periods of greater N absorption (summer).

In both timing of application (spring and summer), a greater mean value in the total fraction was retained in loamy soil as opposed to the values with sandy soil (Figure 5). Soil texture affected the retention of N for the different soils as the sand, which had the coarsest texture and lowest Cationic Exchange Capacity, retained the least amount of nutrients (Powell and Gaines, 1994).

Experiment 3. Effect of N Form by Using the Nitrification Inhibitor (DMPP) on NUE

N Absorbed from the Fertilizer and its Relative Distribution

N form used significantly affected the Naff in young organs and large roots. In these organs, the lowest N content was found in SA+NI treatment, because N was mainly acumulated in large roots (Figure 9).

	mg ^{15}N (placement x 100/fertilizer[9])				ANOVA[2]		
Placement	AS-Sand Sp	As-Loam Sp	PN-Sand Sp	PN-Loam Sp	F (Sp)[3]	S (Sp)[4]	T x S (Sp)[5]
NUE	37.9	33.9	40.1	37.0	*	*	N.S.
Fallen organs	4.3	2.5	5.7	0.0	N.S.	***	N.S.
Total soil	4.6	13.8	4.7	10.9	N.S.	***	N.S.
Drainage	0.03	---	0.0002	---			
Recovery	46.9	50.2	50.5	48.7	N.S.	N.S.	N.S.

		mg ^{15}N (placement x 100/fertilizer[9])				
		PN-Sand Su	PN-Loam Su	T PN[6]	S PN[7]	T x S PN[8]
NUE		59.0	51.5	**	N.S.	N.S.
Fallen organs		---	---			
Total soil		3.8	14.9	N.S:	***	N.S.
Drainage		---	---			
Recovery		62.8	66.4	*	N.S.	N.S.

[1] Means of two trees replications.

[2] Significant effects of factors are given at *P*>0,05 (N.S.), *P*≤0,05 (*), *P*≤0,01 (**) and *P*≤0,001 (***).

[3] Significant differences between treatments with spring application due to fertilizer applied (F).

[4] Significant differences between treatments with spring application due to type of soil (S)

[5] Significant differences between treatments with spring application due to fertilizer applied vs. Soil type (FxS).

[6] Significant differences between treatments with potassium nitrate due to timing of N application (T)

[7] Significant differences between treatments with potassium nitrate due to type of soil (S)

[8] Significant differences between treatments with potassium nitrate due to timing of N application vs type of soil (TxS).

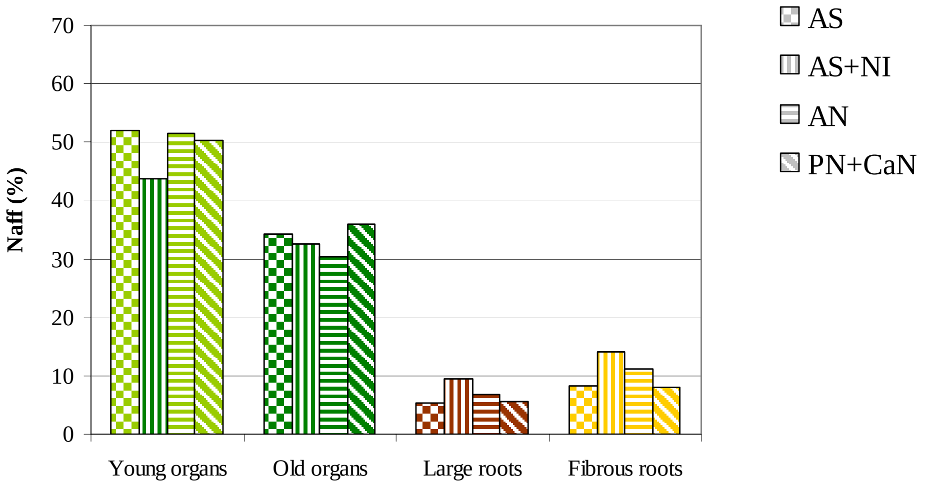
[9] mg ^{15}N content in fertilizer = 2550 mg.

Figure 8. Effects of different N fertilizer management on recovery of ^{15}N fertilizer (%) in whole tree (NUE), drainage water and soil profile at harvesting time of the Experiment 2[1].

In presence of the NI, the relative distribution of uptaken N was lower in young and old organs (34 and 33% respectively, in AS+NI treatment) than in AS treatment (52 and 44%, respectively), while the opposite tendency was observed in the different fracctions of the root system. Serna et al. (2000) found that the DMPP added to ammonium sulphate nitrate resulted in higher uptake of the N fertilizer by *Citrus* plants. These authors also found that this inhibitor led to higher NH_4^+ levels in the soil, this fact can favour N uptake by plants, because citrus plants are able to absorb NH_4^+ from nutrient solutions at higher rates than NO_3^- (Serna et al.,1992). Samplig along the growth cycle showed a greater NH_4^+ concentration in soil profile when the inhibitor was added (data not shown).

N Retained from the Fertilizer and its Relative Distribution

The N retain in ammonium form was lower than 2% of the Ntotal retain in the soil profile, moreover, the application of DMPP did not affected to this variable.



	Young organs	Old organs	Large roots	Fibrous roots
F[2]	*	N.S.	*	**
NI[3]	**[1]	*	**	**

[1] Significant effects of factors are given at $P>0,05$ (N.S.), $P \leq 0,05$ (*), $P \leq 0,01$ (**) and $P \leq 0,001$ (***)
[2] Significant differences between treatments due to fertilizer applied (F)
[3] Significant differences between treatments with Amomonium sulphate due to nitrification inhibitor NI)

Figure 9. Effects of different N fertilizer management on N absorbed from fertilizer (Naff) among different organs of citrus trees of the Experiment 3.

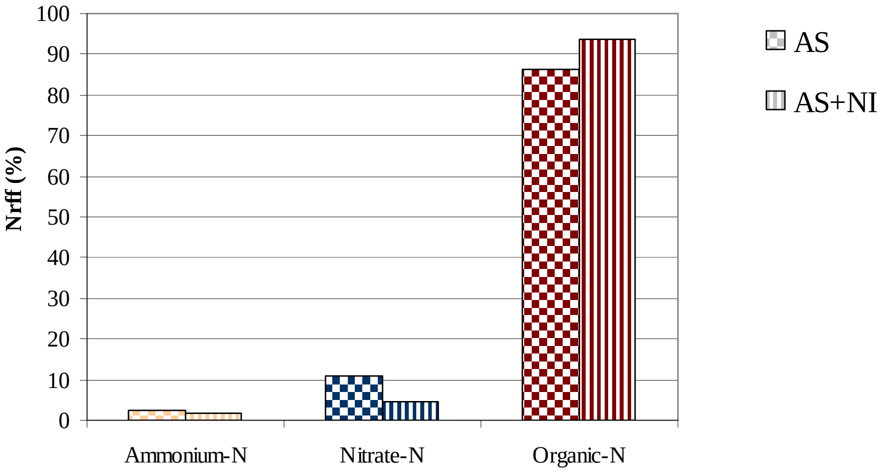
Variations in the NO_3-^{15}N and organic-^{15}N retain are shown in figure 10, a lower residual concentration of labelled NO_3-^{15}N was observed in the soil profile was observed when the nitrification inhibitor (DMPP) was added to Ammonium sulphate fertilizer. According to several authors, the use of DMPP in conventional N fertilization originated lower NO_3-N content in soil (Serna et al.2000; Zerulla et al., 2001; Bañuls et al., 2001; Bañuls et al. 2004 (Ver citas y añadir algo más, artículo DMPP). This could reduce the potential risk of the N losses. However, the N immobilized in the organic form followed the opposite pattern. It appears that elevated NH_4^+ levels in soil may increase immobilization of N because the micro-organisms responsible for immobilization prefer NH_4^+ than NO_3^- (Broadbent and Tyler, 1962). This fact probably favoured the higher N organic recovery value in presence of the DMPP. Additionally, by using the nitrification inhibitor, less N fertilizer was lost by leaching, enabling more N to be available for immobilization.

Nitrogen Recovered from the Fertilizer

Ammonium sulphate treatment resulted in the lowest N values (Figure 11). Legaz et al. (1994) also found the highest values in trees treated with nitrate fertilizer in 7 year-old tres of clementine mandarin assay. However, the addition of NI to AS resulted in a greater amount of NUE in whole tree, reaching the NUE values achieved with nitrate treatments (AN and PN+CaN).

Increases in N uptake through the use of nitrification inhibitor have been reported by Rodgers and Ashworth (1982) in wheat, Somda et al, (1991) in tomato, Rochester et al (1996) in cotton and Carrasco and Villar (2001) in maize (using DMPP).

The N recovered in whole tree and in the total system was significantly higher when NI was added; meanwhile N losses in fallen organs and drainage water were significantly lower for this treatment. Serna et al, 2000, Bañuls et al., 2001, Carrasco and Villar (2001) also found greater NUE when the DMPP was used. Regarding to N content in drainage water, Bañuls et al. (2001) found that the total amount of N leached as nitrate was much lower in the AS+DMPP treatment in comparison with AS alone.



NI[2]	N.S.[1]	**	*

[1] Significant effects of factors are given at $P>0,05$ (N.S.), $P\leq0,05$ (*), $P\leq0,01$ (**) and $P\leq0,001$ (***)

[2] Significant differences between treatments with Amomonium sulphate due to nitrification inhibitor (NI)

Figure 10. Effects of different N fertilizer management on N retained from fertilizer (Nrff) in different fractions of soil of the Experiment 3.

	mg ^{15}N (placement x 100/fertilizer[4])				ANOVA[2]
Placement[1]	SA	SA+NI	AN	PN+CaN	NI[3]
NUE	48.9	64.9	59.3	58.1	**
Fallen organs	4.9	1.8			**
Total soil	23.6	26.3			NS
Drainage	8.7	3.2			**
Recovery	86.1	96.2			N.S.

[1] Means of three trees replications.

[2] Significant effects of factors are given at $P>0,05$ (N.S.), $P\leq 0,05$ (*), $P\leq 0,01$ (**) and $P\leq 0,001$ (***).

[3] Significant differences between treatments with Amomonium sulphate due to nitrification inhibitor (NI)

[4] mg ^{15}N content in fertilizer = 8800 mg.

Figure 11. Effects of different N fertilizer management on recovery of ^{15}N fertilizer (%) in whole tree (NUE), drainage water and soil profile at harvesting time of the Experiment 3[1].

Conclusions

Practical implications of our findings are:

1) Irrigation system: the dose of N and water commonly applied to commercial citrus orchards (up to 240 kgN·ha^{-1}yr^{-1} and 5000 m^3·ha^{-1}yr^{-1}) can be markedly reduced (roughly 15%) with the use of drip irrigated systems.
2) Time of N application: the N recovered by plants is 20% higher for summer N applications than for spring N when applied in flood irrigation trees. In drip irrigation, the highest NUE is obtained when N rate is applied following a monthly distribution in accordance with a seasonal absorption curve of N in which the maximum rates are supplied during summer.
3) N form and use of nitrification inhibitors (NI): nitrate-N fertilizers are absorbed more efficiently than ammonium-N by citrus plants, however ammonium fertilizers are recommended during the rainfall period. The addition of NI to ammonium-N fertilizers increases NUE (16%), resulting in lower N-NO$_3^-$ content in the soil (10%) and in water drainage (36%).
4) Split N application: several split N applications result in greater fertilizer use efficiency and smaller accumulations of residual nitrates in the soil.
5) Soil texture: N uptake efficiency is slightly lower in loamy than in sandy soils when N is applied both as nitrate and ammonium form; on the contrary, N retained in the organic and mineral fractions is higher in loamy soils that could be used in the next growing cycle.

In addition to nitrogen and irrigation management improvement, plant tissue, soil and water N content must be considered in order to adjust N dose to plant demand, since rates exceeding N needs result in lower NUE.

These findings could be used to reduce markedly the N and water doses that would advance towards BMP.

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Chapter IX

A New Analytical System for Remotely Monitoring Fertilizer Ions: The Potentiometric Electronic Tongue

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Abstract

The use of an electronic tongue is proposed for remote monitoring of the nutrient solution composition produced by a horticultural closed soilless system. This new approach in chemical analysis consists of an array of non-specific sensors coupled with a multivariate calibration tool. For the studied case, the proposed system was formed by a sensor array of 10 potentiometric sensors based on polymeric (PVC) membranes with cross selectivity and one sensor based on Ag/AgCl for chloride ion. The subsequent cross-response processing was based on a multilayer artificial neural network (ANN) model. The primary data combined the potentials supplied by the sensor array plus acquired temperature, in order to correct its effect. With the optimized model, the concentration levels of ammonium, potassium and nitrate fertilizer ions, and the undesired saline sodium and chloride ions were monitored directly in the recirculated nutrient solution for more than two weeks. The approach appears as a feasible method for the on-line assessment of nutrients and undesired compounds in fertigation solutions, where temperature and drift effects could be compensated. The implemented radio transmission worked robustly during all the experiment, thus demonstrating the viability of the proposed system for automated remote applications.

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Introduction

Electrochemical sensors, which are widely available from about 40 years ago, emerged as an alternative to the atomic absorption spectrometry and chromatography traditionally used to analyze the chemical composition of aqueous solutions (Minoia and Caroli, 1992; Haddad and Jackson, 1990). Today, electrochemical sensors still compete favorably with the most advanced analytical techniques (i.e., ion chromatography), especially in continuous monitoring. Therefore, sensor systems are gaining interest in the measurement of pollutant ions in drinking water, natural water, industrial wastewater, etc.

The key components of an ion-selective system are:

a. An electrode and a reference electrode, used separately or in ensemble .
b. A data acquisition system to measure the difference of potential between these two electrodes and
c. A solution containing the ion which concentration we need to know.

Ion-selective electrodes (ISEs) allow making direct measurements of concentration for over 20 chemical species present in different solutions. Within these species, inorganic ions closely related to the environment are found: ammonium (NH_4^+), cadmium (Cd^{2+}), calcium (Ca^{2+}), cyanide (CN^-), chloride (Cl^-), copper (Cu^{2+}), nitrate (NO_3^-), nitrite (NO_2-), lead (Pb^{2+}), potassium (K^+) and sodium (Na^+). In addition, using a whole of ISEs, which is called sensor array, the number of species that can be determined simultaneously increases. However, the number of chemical species with which to work is still small, due to some interfering effects from one determination into the others, which are normally controlled in the laboratory through executions of specialized discrimination stages, but are difficult to perform in on-line applications.

Additional drawbacks of ISEs when they are working in continuous mode are related to the need of their periodical recalibration, the slow speed of response in some situations, the lack of stability of the electrical signal in some cases, the lack of robustness or mechanical resistance with some electrodes and ISE durability and cost.

Crystalline electrodes are a kind of ISEs that have a solid membrane that connects the solution and the electrode. This type of membrane lends a great robustness and durability to the electrode. There are crystalline electrodes to determine Cl^-, Br^- and F^-. ISEs based on crystal membranes containing an ionic reference solution inside a plastic body. The same configuration was later applied with the use of polymeric membranes, which supported an ion-exchange ligand-based equilibrium. The ion, which concentration is intended to determine, is exchanged through the membrane and creates a potential difference that can be measured electrically. The potential difference generated depends on the ionic concentration of the solution in which it is submerged. The exchange membrane can also be used directly placed onto a solid contact without internal reference solution. This configuration is called all-solid-state ISE.

Recently, potentiometric sensors have appeared that take profit of the electric field generated by the membrane potential caused by presence of specific ions; the electric field modulates current in a field effect transistor, in this way these sensors are known as Ion-

Selective Field Effect Transistors (ISFETs). ISFET sensors have a great future in continuous monitoring, given that they are able to determine ions at very low concentrations and they can be massively produced using microelectronic technology, although their use is just in the beginning.

In closed soilless systems with recirculation, aqueous flow, operation and environmental conditions are quite aggressive. Therefore it is interesting to have robust and cheap ionic sensors that provide real-time information of the ionic composition of the nutrient solution, although accuracy is not excessively high. Closed soilless systems are techniques implemented in modern horticulture in order to improve the efficiency in the use of water and fertilizers and to preserve the environment. In few words, in this technique, plants grow on artificial substrates which substitute natural soil. A fertilizer supply unit provides nutrients and the solution not used by the plants is collected and regenerated to be reused several cycles. The addition of new fertilizer ions (ammonium, potassium, nitrate, phosphate) and tap water is controlled by the value of electrical conductivity (EC) and pH signals. However, this protocol can only give a qualitative control over these species given ion's uptake by the plants may vary. Another disadvantage of the closed soilless systems is that non-essential ions such as sodium and chloride accumulate in nutrient solution causing an increase in the overall EC and consequently a decrease of the concentration of the nutrient ions if the conductivity is maintained at a fixed value. Therefore, the measurement of the concentration of each individual ion in the nutrient solution in continuous mode, and in real-time can be a clear improvement to normal use in this area, and can lead to fine control of fertilizer dosage adapted to each plant stage.

There are numerous antecedents in the literature for the use of on-line sensors for monitoring and managing crops without soil (Bailey et al., 1988; Hashimoto et al., 1989; Van den Vlekkert et al., 1992; Durán and Navas, 2000; Gieling et al., 2005), but in practice, farmers have not been provided with ion sensors that are both economic and robust. On the contrary, ionic sensors available have been expensive in relation to its lifetime and have sensitivity problems towards temperature and interfering ions as mentioned above (Kläring, 2001).

A novel strategy to solve these problems is the use of electronic tongues. This novel concept in the chemical sensors field entails the use of an array of sensors with partially selective response plus a multivariate calibration model to develop qualitative (identification) or quantitative (multidetermination) applications in liquid media (Vlasov et al., 2005). This approach receives the biomimetic qualifier, because it is inspired in the sense of taste in animals and follows the former electronic nose concept, used for gas analysis. Basically, this proposal represents an alternative to the ideal sensor with a very high selectivity, moving the complexity of the system to the data processing stage. Among the different types of sensors that can be used in an electronic tongue, potentiometric electrodes require relatively simple measuring devices and provide wide variety of sensing elements. Of those, ISEs are the most used because they present cross-response to different ions in solution. An advantage of using ISE arrays is that this allows us not only to obtain the concentration of the primary ion, but also the concentration of the interfering ions without the need of eliminating them.

Our reseach group proposed for the first time the use of an electronic tongue in the monitoring of the composition of the nutrient solution produced by a closed soilless culture

(Gutiérrez et al., 2007a). This system was formed by a sensor array of eight ISEs based on poly(vinyl chloride) (PVC) membranes and the subsequent multicomponent calibration model was based on an Artificial Neural Network (ANN). ANNs are advanced and powerful modeling tools, taken from the artificial intelligence knowledge area, as they mimic the ability for information processing of human brain (Cartwright, 1993). In this way, a constructed model predicted the concentration values of the fertilizer ions ammonium, potassium and nitrate, plus the undesired sodium and chloride compounds, compensating the temperature effect. These promising results encouraged us up to develop new experiments focused to study the effect of response drifts to the sensor array, how to correct the strong interferences and to incorporate phosphate ion to the model. The presented chapter will summarize knowledge accumulated along approximately two years working on this topic. In summary, it will present how the use of traditional ISEs as the electronic tongue concept improve their performance, specifically concerning interference effects of neighbor ions or temperature variations (Gallardo et al., 2003a; Gallardo et al. 2005, Gutiérrez et al., 2008). It will also present how the information derived can be used to monitor and control the concentration levels of fertilizer ions in fertigation applications. To demonstrate remote use, portability and applicability, the specific case described has employed a radio link to communicate a base station just with the sensors and related instrumentation, to a central unit performing final calculations.

Experimental

Reagents and solutions

The ion-selective poly(vinyl chloride) (PVC) membranes were prepared from high-molecular weight PVC (Fluka, Buchs, Switzerland), using bis(1-butylpentyl)adipate (BPA), dioctylsebacate (DOS), 2-nitrophenyloctylether (NPOE), dibutylsebacate (DBS) and dibutylphtalate (DBP) (all from Fluka) as plasticizers. The recognition elements employed to formulate the potentiometric membranes were the ionophores nonactin (nonactin from Streptomyces, Fluka), valinomycin (potassium ionophore I, Fluka) and bis[(12-crown-4)methyl]-2-dodecyl-2-methylmalonate (CMDMM, Dojindo, Kumamoto, Japan), and the charged carrier tetraoctylammonium nitrate (TOAN, Fluka). Additionally, three recognition elements with generic response were used, dibenzo-18-crown-6 and lasalocide, both for cations, and tetraoctylammonium bromide (TOAB) for anions (all from Fluka). Potassium tetrakis(4-chlorophenyl)borate (Fluka) was used when necessary for a correct potentiometric response. All the components of the membrane were dissolved in tetrahydrofuran (THF, Fluka).

Silver foil (Ag, Aldrich, Milwaukee, USA) of 99.9 % purity and 0.5 mm thick was used to prepare a Ag/AgCl based sensor for chloride.

The materials used to prepare the solid electrical contact were the epoxy resin components Araldite M and Hardener HR (both from Uneco, Barcelona, Spain), and graphite powder (50 μm, BDH Laboratory Supplies, Poole, UK) as conducting filler.

All other reagents used for the preparation of the training and testing solutions were of high purity, analytical grade, pro analysis or equivalent.

Sensor array

The used sensors were all-solid-state ISEs with a solid electrical contact made from a conductive epoxy composite. The basic response of this type of potentiometric sensors is described by the Nikolskii-Eisenmann equation (Cattrall, 1997).

$$E_{ISE} = K + \left(RT / z_x F\right)\ln\left(a_x + \sum_{j \neq x}^{N} K_{x,j}^{pot} a_j^{z_x / z_j}\right) \tag{1}$$

where E_{ISE} is the measured potential, and K entails constant factors, such as the reference electrode potential. R is the gas constant, T the absolute temperature, F is Faraday constant, a_x and z_x are the activity and the charge of the ion being monitored, respectively, a_j and z_j are the activity and the charge of interfering ions, and finally $K_{x,j}^{pot}$ is the potentiometric selectivity coefficient of j (interfering ion) over x (primary ion).

Figure 1 depicts schematically the construction procedure of one of these sensors. These are of general use in our laboratories (Alegret and Martínez-Fábregas, 1989). The sensor was formed by filling a plastic tube (6 mm internal diameter) having a Cu electrical contact (A,B) with a homogeneous mixture of 35.7 % Araldite M, 14.3 % HR hardener, and 50 % graphite powder, and cured for 12 h at 40 ºC (C). Next, a 0.3 mm depth cavity is formed on the top of the constructed body (D). Membranes are formed by solvent casting on this cavity using eight or nine drops of PVC membrane cocktail (1 ml per each 20 mg PVC) while their final thickness is ca. 0.3 mm (E). Once formed, membranes were conditioned in a 0.1 M solution of their primary ion for 24 h (F). The formulation of the different membranes used is outlined in Table 1.

The used sensor array was constituted by 11 sensors: two ISEs for ammonium, two for potassium, two for sodium, one for nitrate, one for chloride, plus three generic membrane formulations, two for alkaline ions employing the electroactive elements dibenzo-18-crown-6 and lasalocide, and other for anions employing TOAB. Sensors for alkaline ions were duplicated because this experiment was mainly focused in the ammonium fertilizer ion and to correct the alkaline interferences in its determination. One sensor based on Ag/AgCl was incorporated in order to improve the response to chloride ion since this sensor does not show nitrate interference. The latter is characteristic when PVC membrane carrier-based sensors are used with samples having high levels of nitrate (Umezawa, 1990). This chloride sensor was formed by AgCl electrodeposition on a disc of clean Ag foil, 5 mm diameter. To obtain an homogeneous deposition, 0.1 mA were passed through the electrolysis cell containing 0.1 M NaCl during 1 hour.

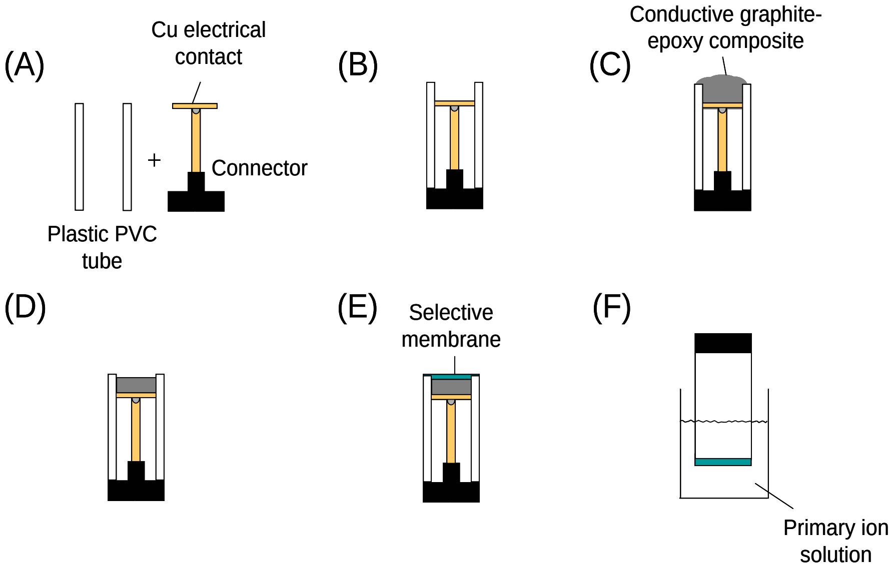

Figure 1. Schematic diagram of the fabrication process of the PVC-membrane all-solid-state potentiometric sensors, based on the graphite-epoxy composite.

Table 1. Formulation of the ion selective membranes employed in the construction of the potentiometric sensor array.

Sensor	PVC (%)	Plasticizer (%)	Recognition element (%)	Reference
NH_4^+	33	BPA (66)	Nonactin (1)	(Davies et al., 1988)
K^+	30	DOS (66)	Valinomycin (3)[a]	(Shen et al., 1998)
Na^+	22	NPOE (70)	CMDMM (6)[a]	(Tamura et al., 1982)
Generic 1	29	DOS (67)	Dibenzo-18-crown-6 (4)	(Umezawa, 1990)
Generic 2	27	DBS (70)	Lasalocide (3)	(Suzuki et al., 1988)
NO_3^-	30	DBP (67)	TOAN (3)	(Pérez-Olmos et al., 2001)
Generic 3	29	DBP (65)	TOAB (4)	(Isildak and Asan, 1999)

[a] The formulation includes potassium tetrakis(4-chlorophenyl)borate as additive.

Apparatus

Potentiometric measurements were performed with a multichannel potentiometer developed in the laboratory. Each channel has a conditioning stage using an INA116 (Burr Brown) instrumentation amplifier for adapting the impedance of each sensor. Measurements were differential versus the reference electrode (double junction Ag/AgCl electrode, Orion 90-02-00) and grounded with an extra connection in contact with the solution through a stainless steel wire. All channels were noise-shielded with their signal guard while each amplifier output was passed through a second order active low-pass filter with -3 dB, 2 Hz cutoff frequency, using a UAF42 (Burr Brown) universal filter. These filtered outputs were connected to a MPC506 (Burr Brown) 16 channel analog multiplexer. Digitalisation was performed by an A/D converter ADS7804 (Burr Brown). The complete data acquisition system was controlled using an AT90S8515 (Atmel) microcontroller, that also provided the RS-232-C serial communication. This microcontroller was programmed making use of the interface ImageCraft Development Tools that employed language C. The program's main tasks were to control the multiplexer operation in selecting each channel, the data acquisition with the analogical to digital converter and the transmission/reception of words as much of control as of data. This instrumental system has been recently applied to different environmental applications (Gutiérrez et al., 2007b).

For telemetry tests, physical communication channel was replaced with a pair of wireless radio modems (Data-Linc Group) model SRM6100, that operate at 2.4-2.4835 GHz license-free band employing advanced spectrum frequency hopping and error detection technology. To obtain the best communication performance, they used a data transmission rate of 57600 bauds. This speed of transmission allowed to reach a distance up to 15 miles in optimal conditions with line-of-sight between radios, according to the manufacturer.

For the construction of the chloride sensor, an Autolab PGSTAT (Eco Chemie, Utrecht, The Netherlands) was used for the AgCl electrodeposition.

Training and measurement procedure

In order to verify the correct functioning of the prepared sensors, these were calibrated to their corresponding primary ion in 25 ml of doubly distilled water by sequential additions of standard solutions. The generic response sensors were calibrated with potassium and nitrate, respectively.

Before any application, the response of the system had to be assessed employing an ANN model. ANN is a modelling tool specially useful for nonlinear systems like this. Measurements for training were done with solutions with a defined background. In order to compensate the matrix effect, the background has to be as similar as possible to the real sample. In this way, we used a 1/3 (v/v) mixture of nutrient solution from the greenhouse and doubly distilled water as base solution instead of generating it in the laboratory due to the complexity of the real conditions.

Using this background, different mixtures were prepared by additions of stock solutions of the considered ions accordingly to a statistical experimental design. In this case, 27 solutions were defined from a fractional factorial design with three levels of concentration and five factors (the five considered ions, 3^{5-2}). The ranges of variation of the concentration for the analytes in these solutions, which correspond to expected variations, are summarized on Table 2.

Table 2. Ranges of variation of the concentration of the analytes in the solutions used for the training process.

Species	Variation (M)
Ammonium	0.00053 – 0.005
Potassium	0.002 – 0.015
Sodium	0.0005 – 0.01
Chloride	0.0006 – 0.01
Nitrate	0.0027 – 0.015

In order to correct possible sensor drifts, the inputs in the neural network were relative measurements of each sensor with respect to a reference solution periodically checked. The composition of this reference solution was 10^{-4} M for ammonium and 10^{-3} M for the rest of the considered ions (potassium, sodium, chloride and nitrate). These are the minimum concentrations present in the nutrient solution. The used model also included as input the solution temperature in order to compensate any influence in the response of these potentiometric sensors. Therefore, a laboratory-made temperature probe based on a LM35 integrated circuit (National Semiconductor, Santa Clara, CA) was employed together with the array of electrodes.

For the proper verification of the electronic tongue performance, a new set of solutions was used, the test set, which did not intervene in the training process. The test set was formed by 10 synthetic solutions prepared in the same way as the training ones, but with concentrations generated randomly inside the training space, while the latter corresponded to a factorial design.

These 37 prepared solutions, 27 for training and 10 for testing, were measured in three turns; one with all the solutions at room temperature (around 24 ºC), another with half of the solutions at lower temperature (around 10 ºC) and the third with the other half at higher temperature (around 35 ºC). This experimental sequence was designed with the goal of including temperature effect in the response model.

Software

ANNs were built and evaluated using the routines available to the Neural Network Toolbox v.4.0, which are optional add-ons in the Matlab v.6.1 (Maths Works, Inc.) programming environment. Sensor readings were acquired from the radio station by using custom software written in VisualBasic.

On-line application in the greenhouse

The on-line measurements were performed along June of 2006 in a soilless rose crop (*Rosa indica* L. cv. Lovelly Red®) in a climatized greenhouse of 270 m^2 at the IRTA site in Cabrils (41º 25’ N, 2º 23’ E). The growing media were perlite for one half of the greenhouse and coco fiber for the other half. Irrigation was triggered automatically every time that the accumulated radiation reached 200 W m^{-2} h, leaving a leaching fraction of around 20 % of the applied water. The leachates of both types of substrate were collected together and reused for the production of a new nutrient solution. The recomposition of nutrient solution consisted on the dosification of the volumes of leachate, tap water and six concentrated solutions (potassium nitrate and ammonium nitrate; potassium sulphate; monopotassium phosphate; magnesium nitrate; microelements and nitric acid). This dosification was controlled by a programmable logic controller (PLC) MCU "Ferti" (Multi Computer Unit; FEMCO, Damazan, France) based on the pH (measured by a pH sensor from Broadley James Corp., USA), EC and predefined ratios between the volumes of each concentrated solution. The ratios of injection of the diverse concentrated solution were adapted to the case (season, plant phase, etc.), if necessary, according to laboratory analysis of the leachate performed every 2 weeks.

The array of sensors and the temperature probe were installed on-line in a pipe between the tank of recomposed nutrient solution and the irrigation pump. Figure 2 shows a photograph of the installed pipe with the sensor array and the multichannel potentiometer.

Figure 2. Installation of the electronic tongue in the on-line derivation pipe, at the IRTA site in Cabrils, with the sensor array and the multichannel potentiometer.

The monitoring consisted in one measurement per sensor, done every 20 min. These readings, once amplified and filtered, were transmitted to the computer using a radio link. This computer was sited 150 m from the greenhouse with the local road Vilassar-Cabrils passing between the two points as a significant generator of perturbances during operation. The system was left in continuous operation for two weeks which served to validate the different parts of the monitoring system. The block diagram of the proposed manifold is depicted in Figure 3.

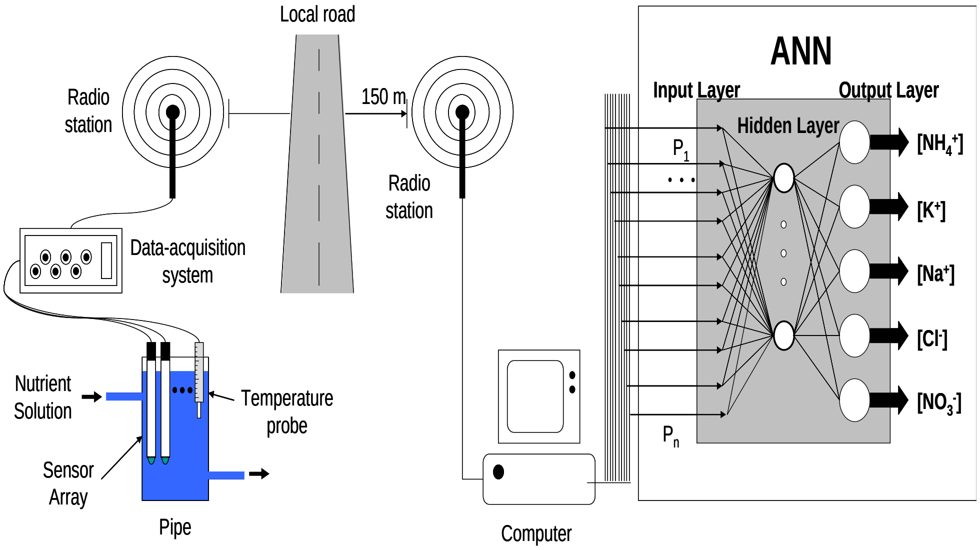

Figure 3. Block diagram of the proposed system, with a schematic representation of the electronic tongue approach.

Results and Discussion

Evaluation of sensor's response

The sensors that formed the array were previously evaluated in doubly distilled water to establish their response characteristics. The results are summarised in Table 3. The obtained sensitivities are close to the corresponding theoretical values (± 59.16 mV/decade at 25ºC for a ion of charge ± 1), in all cases except for the generic response electrodes due to their non-selective nature. Anyhow, these sensors are necessary in order to provide the cross-response terms, the over-determined information needed for the proper operation of the electronic tongue. Concerning their detection limits, the obtained values are around 10^{-4} M in general for each prepared sensor. The regression coefficients obtained for the non-linear fitting of the experimental data to the Nikolskii-Eisenmann equation were ≥ 0.998 for all tested sensors. The initial verification of their behaviour, i.e. the cross-response features, confirmed that these sensors were suitable for constituting the array.

Table 3. Response parameters of each sensor used in the array when calibrated in doubly distilled water

Sensor	Sensitivity (mV/decade)	Detection limit (M)	Regression Coefficient
NH_4^+ (1)	56.7	5.0×10^{-5}	> 0.999
NH_4^+ (2)	56.5	6.2×10^{-5}	> 0.999
K^+ (1)	52.5	3.6×10^{-4}	> 0.999
K^+ (2)	52.6	4.1×10^{-4}	> 0.999
Na^+ (1)	59.9	2.4×10^{-4}	0.992
Na^+ (2)	59.9	2.7×10^{-4}	0.992
Generic 1	28.1	5.6×10^{-4}	> 0.999
Generic 2	40.6	1.3×10^{-4}	> 0.999
Cl^-	-58.9	5.1×10^{-4}	0.993
NO_3^-	-53.7	1.1×10^{-4}	> 0.999
Generic 3	-48.2	2.7×10^{-5}	0.999

Building of the ANN model

ANNs are efficient multicomponent calibration tools for classification and modelling, especially useful for nonlinear systems. Their functioning is inspired on the animal nervous system and its elementary unit is the perceptron or neuron. The properties that characterize a neural network are: the transference function used in the neurons, the network topology and the learning algorithm used. Among the different ANN structures, the multi-layer perceptron is the most widely used. It can be defined as a feed-forward network with one or more layers of neurons between the input and output neurons. These additional layers contain hidden neurons that are connected to both the inputs and outputs by weighted connections.

Many different characteristics define the configuration of an ANN, among others the number of layers, number of neurons in each layer, transfer functions used in each layer, etc. (Cartwright, 1993). Selecting the topology of an ANN is normally done by trial and error, because of the difficulty to predict an optimum configuration in advance (Despagne and Massart, 1998). Normally, the ANN is trained many times with the same experimental data and varying its configuration order to get the best model. The process includes all combinations of the number of neurons of the hidden layer and the transference function used within. The optimization of these characteristics will define the specific configuration leading to the best modelling ability.

Thus, in this optimization we initially fixed the following parameters: the number of input neurons, which is 12 (11 sensors from the array and the temperature); the number of output neurons, which is five (the five modelled analytes); the transference function for the output layer is linear (*purelin*) and a single hidden layer of neurons; these selections are based on previous experience with electronic tongues using potentiometric sensors (Gallardo et al., 2003a). The learning algorithm used was Bayesian Regularization (Demuth and Beale, 1992) and employed as internal parameters, a learning rate of 0.1 and a momentum of 0.4, selected from preliminary tests.

The modelling capacity of the ANN was examined in terms of the root mean squared error (RMSE) in concentration:

$$RMSE = \sqrt{\left(\sum_{i,j}\left(x_{i,j} - \bar{x}_{i,j}\right)^2 / (5n-1)\right)} \tag{2}$$

where *n* is the number of samples (5*n*, as many as 5 species were determined) and $\bar{x}_{i,j}$ and $x_{i,j}$ are the expected concentration value and that provided by the ANN, respectively, for each compound *i*, with *j* denoting samples. Additional desired performance is the correct prediction ability, especially for the external test set, which is checked visualizing the predicted versus expected comparison graphs.

The strategy selected for the learning process, that is, the search of the weighed coefficients best representing experimental data, was Bayesian Regularization. Compared to more classical learning algorithms, it provided better RMSE values, greater consistency between the predicted and obtained values for the training and a higher significance for the external test set (Demuth and Beale, 1992). For example, the algorithm of gradient descent is not a fast training method and it can converge to a local minimum. Also, the more advanced Levenberg-Marquardt algorithm is very efficient, but it provides models with more complex architecture. A further advantage of Bayesian Regularization is that it does not require an internal validation set of samples to avoid overfitting (Demuth and Beale, 1992).

Considering the highly nonlinear behaviour of the sensors, whose response is described by the Nikolskii-Eisenmann equation (1), and from our previous experience in potentiometric electronic tongues (Gallardo et al., 2003a; Gallardo et al., 2003b; Gallardo et al., 2005), only two different nonlinear transference functions were considered for the hidden layer. Specifically, these were a sigma-shaped function named *tansig* function (Freeman and Skapura, 1991) and a logistic function represented by the *logsig* function. After evaluating the different combinations, the best training results were obtained with the *logsig* function and 5 neurons in the hidden layer (tested between three and ten neurons); this configuration provided a RMSE = 3.96 mM for the external test set, those samples not intervening in training. This configuration was the best among the 16 different variants tested.

Figure 4 illustrates the performance of the finally optimized model for the external test set, comparing predicted *vs.* expected values for ammonium, potassium, sodium, chloride and nitrate. Predictions were within tolerable margins for the individual ions and for their mixtures. The accuracy of the obtained response approaches ideality, with unity slopes and zero intercepts for the external test set (all confidence intervals were calculated at the 95% confidence level), that is, predicted values are not distinguishable from the expected ones. The figure also shows a highly significant correlation for the five individual species in the external test set.

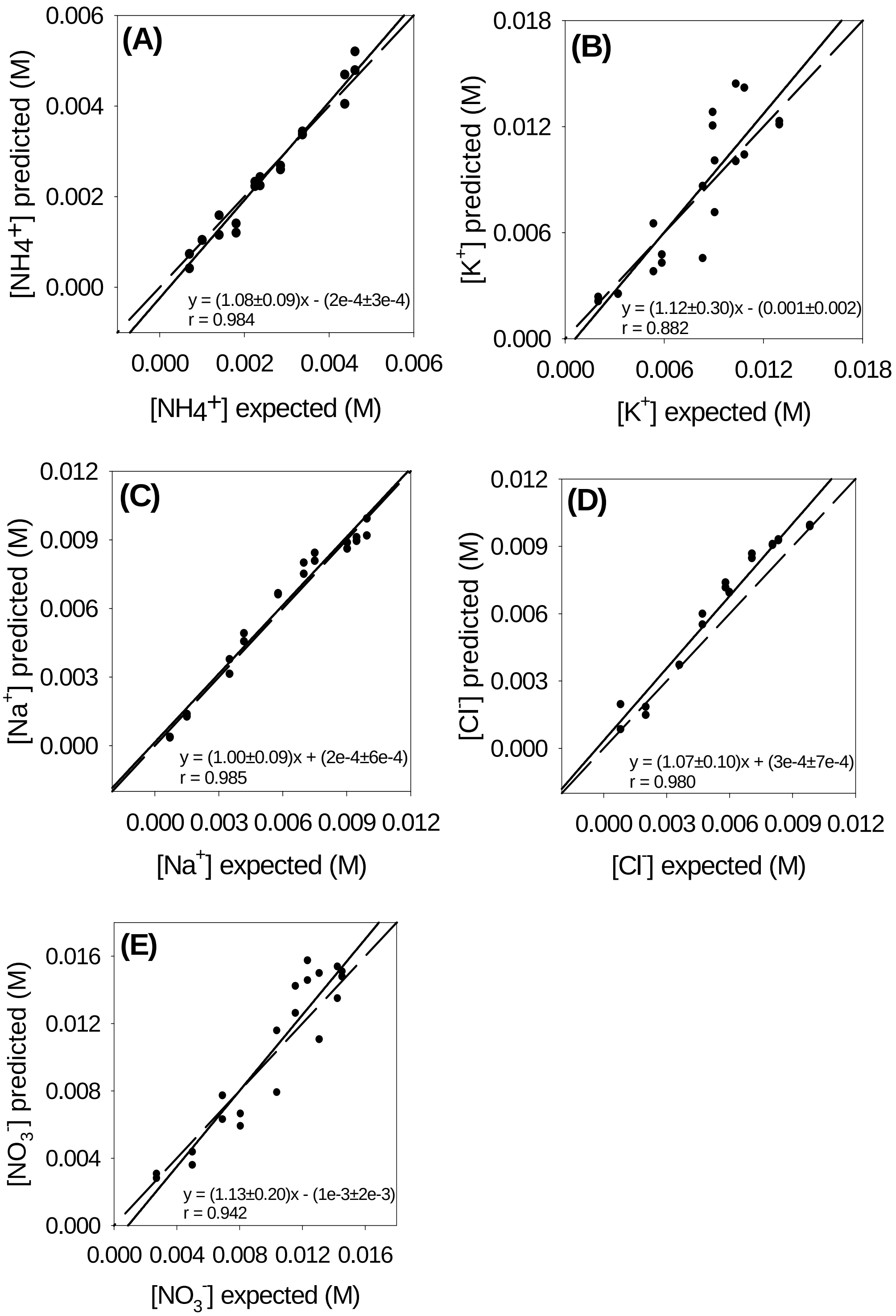

Figure 4. Predicted versus expected comparison graphs for the samples of the external test set: (A) ammonium, (B) potassium, (C) sodium, (D) chloride and (E) nitrate. The dashed line corresponds to ideality and the solid one is the regression of the comparison data. Each sample was processed twice at different temperature.

On-line application in the greenhouse

With the previously optimized ANN, the primary data from the on-line study were turned into analytical information. The concentration of ammonium, potassium, sodium, chloride and nitrate in the nutrient solution was continuously monitored for more than 15 days, from which 5 days were analyzed in more detail. Figure 5 shows the concentration of the considered cations and anions as predicted by the electronic tongue during this period. Additionally, the figure represents the recorded nutrient solution temperature where the day-night cycles are clearly observable. From the observed profiles in the concentrations, one can say that the recirculation system is able to maintain the concentration of the ions in a narrow range. Since no cyclic concentration variations were observed once the electrodes were stabilized, we can consider that the electronic tongue corrected the temperature effect. As read from the graph, the ammonium concentration was between 0.008 and 0.006 M, the potassium concentration, between 0.004 and 0.002 M, the chloride concentration, between 0.01 and 0.011 M, while the nitrate concentration was around 0.016 M. For the sodium ion, there are some perturbing effects, once per day, not clear if related to the sensor or to changes in the feeding water. In any case, this species is just an indicator of accumulated salinity, not a nutrient itself. Then, it can be admitted if its concentration is not maintained at an accurate level.

The predictions of the content in ammonium, potassium, sodium and nitrate ion were satisfactory since they corresponded to dosified values. Although not presented here, the electronic tongue was validated by comparison with the real samples as done before (Gutierrez et al. 2007a). As seen in this chapter, the application showed that the ANN succeeded in compensating the temperature effect and the cross-interference effects among the different ions considered.

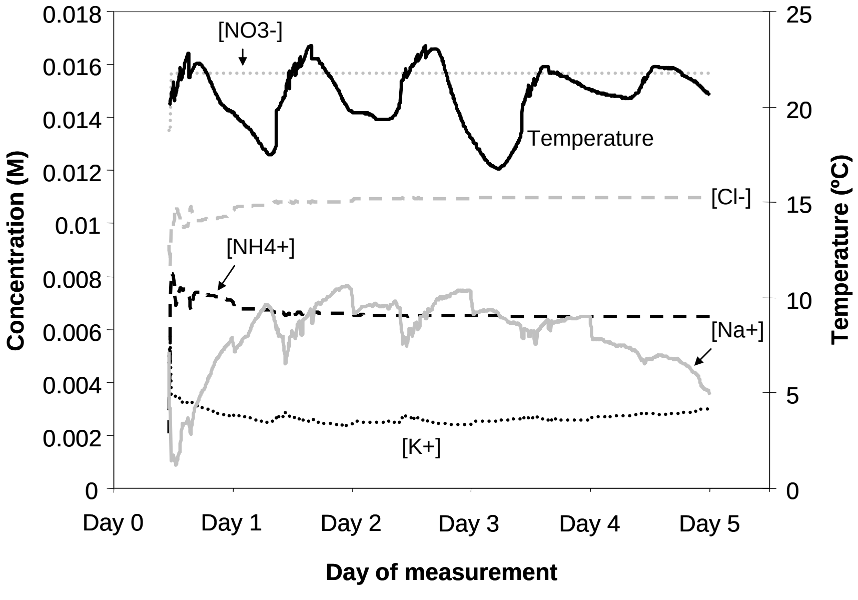

Figure 5. Representation of the concentration values predicted by the electronic tongue for the five considered ions in the nutrient solution during 5 days of continuous monitoring. The variation of temperature is also shown.

Conclusion

A potentiometric electronic tongue for the simultaneous determination of ammonium, potassium, sodium, chloride and nitrate in greenhouse samples with fertigation strategy was developed and optimized. The on-line application showed that the ANN succeeded in compensating the temperature effect while concentrations of the five studied ions could be correctly predicted. These results are very promising, given the intrinsic difficulty of the studied case, where different nutrient ions with strong interfering effects are present at high concentrations. Ongoing experiments are focused to study the effect of response drifts of the sensor array, and how to correct its possible distortions. One alternative to correct this effect would be to increase the number of sensors used in the array, incorporating new species not considered now such as calcium ion. In any case, the system was able to compensate natural temperature variations by incorporating the solution temperature as input in the ANN model. The used radio link demonstrated a robust operation in occurrence of perturbances, so we can conclude that systems of the studied type can be a promising tool for automatic wireless chemical monitoring in horticultural applications. With a tool like this a very precise control of the dosing of nutrient species may be attained, a goal in advanced use of fertilizers.

Acknowledgment

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Chapter X

Organo-Zeolitic-Soil Systems: A New Approach to Plant Nutrition

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Abstract

A collection of papers, selected from a natural zeolite conference in 1982, was published in 1984 under the heading "Zeo-Agriculture: Use of Natural Zeolites in Agriculture and Aquaculture" edited by Wilson G. Pond and Frederick A.Mumpton. This publication, for the first time, focused attention on the agricultural potential of zeolitic rocks and demonstrated the application of these materials over a broad area of plant and animal sciences. This work by associating mineralogical and biological research was a precursor to what is now become known as "Geomicrobiology" and as such has lead to new discoveries that will be of great benefit to agronomy. It is shown, in this chapter, that the interaction of zeolite mineral surfaces with the microbial activity of waste organic material results in a soil amendment that introduces both available carbon and nitrogen into damaged and degraded soils that are lacking in biodiversity. During the decomposition of the organic phase ammonia is released and quickly adsorbed by the zeolite mineral. This reaction promotes the formation of a large population of nitrifying bacteria which oxidize ammonium ions from the surface of the zeolite crystals to produce first nitrite and finally nitrate ions, which enter the soil pore-water. This process is continuous in that adsorbed ammonium ions are oxidized and replaced by further ammonium ions until their source is exhausted. In this respect the presence of the zeolite phase acts to buffer the system against the loss of ammonia by volatilization and aqueous leaching. Further more the enzyme reactions involved in the nitrification produce protons

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which react with the soil to liberate cations and provide plant nutrients in a form that can be taken up from the soil solution. Non-metals such as phosphorous and sulphur as well as trace metal elements, that are essential to plants, are present in adequate concentrations from the decomposing organic waste and reactions with inorganic soil matter. Plant growth experiments clearly demonstrate the efficacy of the organo-zeolitic-soil system in both clean and contaminated cases. By introducing organic waste the explosion of the microbial landscape, that occurs as a result of the application of the biological fertilizer, helps satisfy the carbon demand imposed by a greatly expanded population of heterotrophic microorganisms. This can overcome the problem imposed by the heavy use of inorganic fertilizers as the loss of carbon from the soil is limited by the incorporation of organic material. It therefore appears that the loss of soil biodiversity, and consequent degeneration of soil structure that results from the over use of synthetic chemical fertilizers can be to a greater or lesser extent, depending on climatic conditions and access to suitable materials, reversed by this geomicrobial approach to soil fertility.

Introduction

The concept of using natural zeolite minerals together with animal waste to provide a source of plant nutrient has been made feasible by the discovery of large deposits of readily available zeolitized volcanic ash in many parts of the World. The discovery of these rocks in the 1950’s and 60’ [Coombs, 1954; Sheppard and Gude, 1965; Hay, 1966.] has lead to its recognition on all continents. Glass particles, falling as ash, occurs in all eruptions but in the explosive “Plinian” type, characteristic of acid and intermediate magma, the fallout often occurs on a catastrophic scale. Enormous volumes of ash, deposited from ash flows, form thick beds of volcanoclastic sediment, which in many cases have accumulated below water in either lacustrine or marine environments, Fig 1.

Figure 1. Pyroclastic ashflow entering the sea from the Soufriere Hills volcano; an active stratavolcano on the Caribbean island of Monserrat.

Uplift of land during the Cenozoic Era, 0 – 65 million years ago, has revealed large numbers of such deposits which can be open cast mined in a manner which, relative to metal or coal mining, causes a minimum of environmental damage, Fig 2.

Figure 2. Typical outcrop of zeolitized tuff occurring as a cliff along the Iza River at Calinesti Bridge, ca.23 km south west of Sighetu, Northern Romania.

The volcanic glass, found in such rocks, is mostly altered to zeolite, the type of which is dependant on the original composition of the glass and the physical chemistry of the geological environment in which alteration takes place. These rocks commonly contain high abundances, 80 – 90 volume percent, of zeolite minerals. Generally, the zeolites found in any one volcanoclastic sedimentary rock comprise one or two types of the following minerals; clinoptilolite, heulandite, mordenite, chabazite, phillipsite, erionite. These minerals are commonly found among the forty plus zeolites known to occur in nature. Zeolites of the clinoptilolite-heulandite family are most frequently used, crushed and mixed with animal waste, to produce the biofertilizer.

Early work in the 1960's found that ammonia could be easily ion-exchanged from agricultural waste water [Ames, 1967] and since then zeolitic tuffs have been used for this purpose. Several authors have drawn attention to the application of zeolitic tuffs in various areas including water and waste-water treatment, agronomy and horticulture but none have been as prolific as F.A.Mumpton who for the last thirty years organized international conferences and wrote many papers on the applications of natural zeolites. Among his many articles and papers is a collection edited by W.G Pond and F.A Mumpton in 1984 entitled "Zeo Agriculture" which has formed the basis for much of the later work on the subject. Hideo Minato was the first to report on the use of natural zeolite to enhance plant growth [Minato, 1968] and this work was followed by other scientists [Barbarick and Pirela, 1984; Tsitshishvili, 1988; Huang and Petrovic, 1994; Ming and Allen, 2000]. These authors focused on the zeolite ion-exchange properties of ammonia as the cause of plant growth enhancement in substrates amended with zeolitic tuff.

Further investigations revealed the essential role that soil micro-organisms play in organo-zeolitic-soil systems and in particular the function of amonium oxidisers. This was first brought to light in a study in which organo-zeolitic fertilizers were used to enhance plant growth [Andronikashvili et al., 1999]. It has now been found, [Leggo and Ledésert, in prep) that the zeolite–microbial interactions act to buffer ammonia against loss by volatilization to the atmosphere and to soil via aqueous leaching. Other work has demonstrated that an organo-zeolitic amendment to metal polluted soils can sustain plant growth in extreme phytotoxic conditions. This work has been conducted over several years [Leggo and Ledésert, 2001; Leggo et al., 2006; Ledésert et al., in prep] and early field trials in Canada have revealed the long sustaining effects of this treatment; see section on polluted soils.

Zeolite Mineral Properties

Natural zeolites are open framework aluminosilicates and differ from most other rock forming silicates in having a porous structure. As regards size the zeolites found in volcanoclastic sediments have very small dimensions with crystal lengths being in the order of 10 – 100 microns (10^{-6}m). In this respect these crystals approach clay mineral size but unlike clays zeolites have rigid structures with well defined void architectures. Chemically these minerals are composed of silicon, aluminium and oxygen with water molecules and cations in extra framework positions. The basic building unit is a tetrahedral arrangement having a central silicon atom bounded by four oxygen atoms at the apices of the tetrahedron. All oxygen atoms are shared between adjacent tetrahedra producing a continuous lattice structure in which a complex system of pore channels and polygonal voids are formed. The substitution of aluminium for silicon produces a negative charge on the framework which is compensated by the water molecules and cations occupying the void space by entering the mineral pores. In this respect the extra-framework water molecules and cations are weakly bound within the water filled pore space and can often be readily removed or exchanged between the solid zeolite phase and an aqueous phase in contact with the mineral. The charge, size and abundance of the cation in the external solution, determines its selectivity in any particular zeolite structure. In the case of clinoptilolite-heulandite, mordenite and phillipsite the NH_4^+ cation is highly selective and the fact that these minerals are commonly found in zeolitized tuffs makes them highly desirable components of organo-zeolitic fertilizers.

Occurrence and Formation of Zeolitic Tuff

Zeolitic tuffs are found in many parts of the World as surface outcrops that can be extracted by open-cast mining operations [Hay, 1995] and it is to be expected that more will be discovered as the use of these unique materials becomes generally acknowledged. Such quarrying activities are no different to those used for the extraction of building stone and in this respect do not present an environmental problem. The only question being the possible lack of this natural resource in future times.

Zeolitic tuff is a hard, fine grained sedimentary rock composed of altered volcanic glass shards commonly occurring together with pumice fragments, pyrogenic and clay minerals. Sometimes marine micro fossils and rarely fossilized remains of land plants, occurring in sedimentary concretions, are found as additional components which testify to its origin as sediment deposited under aqueous conditions. Due to the fine grained texture of zeolitic tuffs optical methods of investigation and mineral identification are limited, thus Scanning Electron Microscopy (SEM) and X-ray diffraction methods are used to characterise this rock type.

Under the microscope the outlines of glass shards, that have opaque interiors, can be observed. At higher resolution, Fig 3, monoclinic plates of, in this case, clinoptilolite are clearly seen growing on clay minerals which form an outer coating to the shard.

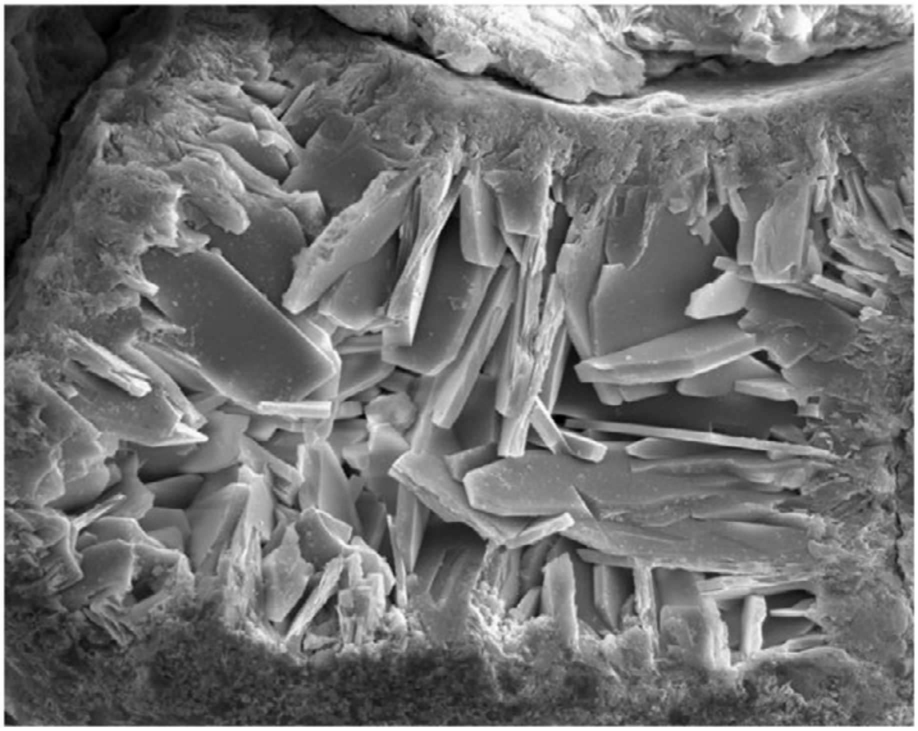

Figure 3. Highly resolved SEM image of a glass shard altered to clinoptilolite. The zeolite crystals are nucleated by a clay coating covering the outer surface of the shard.

Textures of this type are common and are characteristic of zeolite alteration [Leggo et al., 2001; Cochemé et al., 2003]. It is on exposed zeolite crystal surfaces such as these that interaction with soil micro-organisms take place.

Natural zeolite as a plant fertilizer

Much attention has been focused on the ion-exchange behaviour of natural zeolites and use has been made of this mineral property to store essential plant nutrients such as K^+ and NH_4^+ions, exchanged from solutions of potassium and ammonium salts. On application these ions are reversibly exchanged from the charged zeolite into the substrate pore water [Hershey et al., 1980; Perrin et al., 1988; Ming et al., 1995] and from there taken up by the plant. This however was found to be a slow process and which lead to the concept of the “slow release fertilizer”. Taking this one stage further the nutrient exchanged zeolite was used in artificial soil-less substrates consisting of zeolites, peat, and vermiculite and the term “Zeoponics” was introduced to name such systems [Parham, 1984]. To provide a source of phosphorus the mineral apatite, a major component of natural phosphate rock, was introduced [Lai and Eberl, 1986; Allen and Ming, 1995] and an improvement was made by the substitution of synthetic hydroxyapatite for phosphate rock [Ming et al., 1995]. It has since been reported that when a zeoponic substrate is inoculated with a commercial inoculum or soil enrichment culture of nitrifying bacteria, nitrification is greatly increased [McGilloway et al., 2003]. However this study showed that in synthetic systems it is difficult to control both nitrite and nitrate concentrations in plant tissues.

Among the first to report on enhanced microbial activity in organo-zeolitic-soil substrates was T.Andronikashvili who examined the effect of organo-zeolite fertilizers on the microbial landscape of soil [Andronikashvili et al., 1999]. As pointed out in this work zeolitic tuff contains very little, if any, ammonium ions but its freely exchangeable water content does play a role in maintaining moist soil conditions [Huang and Petrovic, 1996; Leggo et al., 2006]. Such conditions are essential to avoid stress in both soil bacteria and plants. However, in organo-zeolitic fertilizers made with animal wastes ammonium ions are abundant, due to the microbial decomposition of organic nitrogenous compounds such as urea, uric acid, amino acids etc., present in the waste and it is this source of ammonia, in cationic form, that is adsorbed on the zeolite surface. In an aerobic soil environment ammonium oxidising micro-organisms (AOBs) convert ammonia, via hydroxylamine, to nitrite which is then further oxidised by nitrite oxidising micro-organisms (NOBs) to nitrate. As a result of these enzyme catalysed reactions protons (H^+) are produced that dissociate cations from the substrate and in so doing greatly increase the ionic mobility of the soil pore-water. It has been possible, by studying the chemistry of aqueous leachates from organic-soil and organo-zeolitic-soil substrates, to confirm that these reactions take place in such substrates [Leggo et al.,in prep]. In addition it has been found that a linear relationship exists between the NO_3^- concentration and the electrical conductivity of the aqueous leachate solutions, Fig 4. This empirical relationship implies that ionic mobilization of the soil pore water increases with the degree of nitrification and results in the availability of elements essential for plant growth.

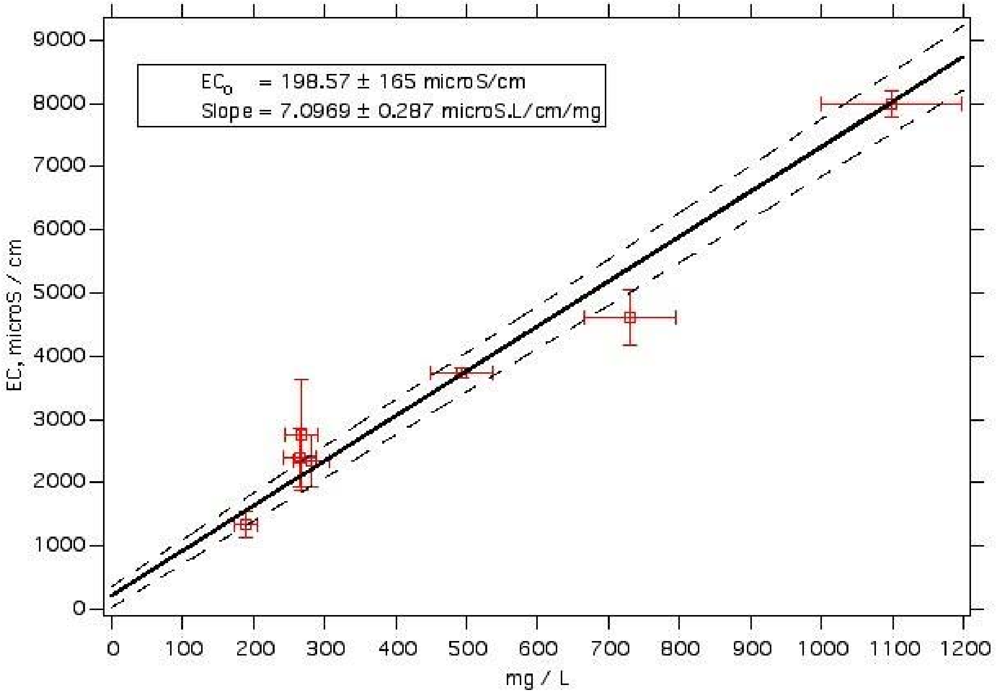

Figure 4. Plot showing the linear relationship between electrical conductivity and nitrate of aqueous leachates. Error bars are given together with the estimate of error on the slope shown as dotted lines.

Earlier studies [Leggo 2000 ; Leggo and Ledésert, 2001] on the chemistry of leachates from substrates with and without an organo-zeolitic amendment, had also indicated this relationship as concentrations of K^+, Na^+, $Ca^{2+}$and Mg^{2+} all increased by an order of magnitude in the leachates from the organo-zeolitic amended substrates. A similar effect was seen in another study [Buondonno et al., 2000] in that the availability of K, Na, Ca, Mg, Mn, Al and Fe increased in soils as a result of an organo-zeolitic addition. It therefore appears that the relative increase in nitrification, that is characteristic of these substrates, produces an abundant supply of nutrients that explains the large response in plant growth that is found when soils are amended with organo-zeolitic fertilizer, Fig 5.

With the exception of phosphorus, nutrient elements are available from the mobilized soil pore-water as a result of nitrification and for most are found to be in concentrations that are in the adequate range of many higher plants. Phosphorous, on the other hand, is directly available in an organic form from the decomposing manure and appears in sufficient quantity, as defined for Spring Wheat at the whole shoot, booting stage [Marschner, 1995].

Andronikashvili et al., [1999] found evidence for a 30-40 % increase in the diazotrophic bacteria *Azotobacter*, in organo-zeolitic amended soils. This is an important observation as these bacteria fix atmospheric nitrogen and although diazotrophs require extensive energy, released from the hydrolysis of adenosine triphosphate (ATP), to reduce nitrogen to ammonia the increased presence of *Azotobacter* in organo-zeolitic-soil substrates infers another, although more limited, source of ammonia that is available for oxidation by nitrifyers.

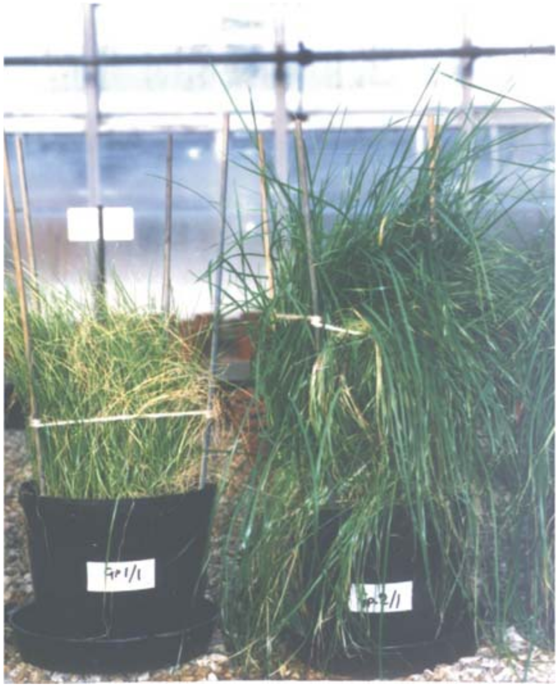

Figure 5. Ryegrass (*Lolium perenne*). Comparison of plant growth in untreated soil (Gp 1/1) with the same grass growing in organo-zeolitic amended soil (Gp 2/1). In each case 1.25g of seed was sown contemporaneously into 2 kg substrates, germinated and grown under identical conditions over the same time period. The pots were watered with de-ionized water, without the addition of any chemical fertilizer.

Our current time-course leachate experiments show that the NH_4^+ concentration, in leachates from an amended substrate, was reduced to a low level, of between 0.10 – 1.10 mg/L, by thirty days but after one hundred and forty days the concentration had increased by an 1.40 mg/L. Although not clearly understood this behaviour suggests that diazotrophs do play a role in maintaining a low level of NH_4^+ that would subsequently undergo oxidation by nitrifying micro-organisms and supply a low level of nitrate at the mature stage of plant growth.

Advantages of organo-zeolitic fertilizers

In discussing the merits of organo-zeolitic fertilizers it should be realised that these fertilizers are subtly different to what is generally understood to be an organic fertilizer. The presence of the zeolite bearing rock enables the retention of ammonia that would otherwise be largely lost to the atmosphere by volatilization resulting in the limitation of nitrification. In the case of organo-zeolitic-soil substrates ammonia released by the microbial degradation of proteins, amino sugars, nucleic acids, urea and uric acid etc., is adsorbed to the surface of the zeolite minerals and then oxidized by nitrifying bacteria within a few days, [Leggo et al.,in prep]. This is a process that is continuously repeated while the decomposition of the organic material is supplying ammonium ions to the substrate; the nitrate, residing in the soil pore water, being consumed at an increasing rate during plant growth. It therefore appears that this slow, natural process is not so vulnerable to the loss of nitrate that can occur from the use of inorganic fertilizer. It is well known that the use of inorganic fertilizers results in an enormous population expansion of nutrient limited soil microbes and as microbial life is dependant on a source of carbon the soil organic matter (SOM) becomes deprived of this element [Ball, 2006]. The effect of long term use of inorganic fertilizer leads to the loss of soil structure and eventual erosion of the land surface. It is now recognized that to sustain a healthy soil, organic matter must be re-introduced either by primary tillage, the application of animal manure or other organic waste. In this way the stress imposed on soil ecology and structure, by the application of synthetic fertilizers, can be avoided.

The new class of fertilizer has all the advantages of the strictly organic fertilizer in being a natural product that improves soil structure and supports the microbial and invertebrate life forms that exist in a healthy soil environment. The added attraction of using organo-zeolitic fertilizers is that, unlike inorganic chemical fertilizers, they appear not to be subject to the high degree of leaching experienced in wet conditions. Within the constraints of our present knowledge it is clear that plant growth is greatly increased in an organo-zeolitic substrate and from empirical observations it appears that the rate of formation of nitrate in the soil pore-water is such that plant uptake accounts for a large proportion, and in so doing limits the amount that is available for leaching. In this respect the organo-zeolitic-soil system can be considered as a slow release mechanism but one that is controlled by biological activity rather than a process of physical diffusion.

However, the application of an inorganic fertilizer, is less labour intensive with, in the short term, a cost saving benefit. In the past the use of these fertilizers were of enormous benefit and averted a worldwide food shortage to the great advantage of the world’s human population but this comes at the risk of concentrating salts in the soil and groundwater that are detrimental to both plant and soil microbes. It is now well established that a relationship exists between the continued increase in the use of these fertilizers and the loss of biodiversity [Mozumder and Berrens, 2007]. As pointed out in this paper i.e., “Ecosystem processes are controlled by the diversity of living communities in the ecosystem ------ and modifications to these processes can alter the ecological functions that are vital to human well-being”. This is clearly shown by the impact inorganic fertilizers are having on the increasing nutrient pollution, in the hydrosphere. The resulting irreversible loss of biodiversity is fast becoming critical and is a situation that, unless modified by better agronomic practices, could have disastrous consequences by 2050 [MEA, 2005].

The organic material used in the preparation of the organo-zeolitic fertilizer includes any form of animal waste, food waste or green waste that, which on decomposition, provides a source of ammonia. It has been found that animal manure is very effective due to the presence of the three major plant nutrients namely nitrogen, potassium and phosphorous together with trace elements that are essential for healthy growth. An initial mixture is made in which the volume of the crushed zeolitic tuff varies according to the requirement, as clean soil needs less of the amendment than that of a polluted environment to sustain plant growth. In the case of the phytoremediation of polluted soils it is important to know the soil chemistry so that plants suited to the particular soil conditions are chosen. Soil water pH is an important parameter as nitrification is one of the most pH-sensitive natural soil processes. Apart from forest soils in which nitrification can occur at values below 4.0, [Killham.1990], agricultural soils require a pH value of above 5.5 before nitrification can be fully effective [Paul and Clark., 1996]. However, soil pH can be adjusted to a suitable level by the addition of lime, as in arable practices; a detailed explanation of which can be found in many texts [Rowell 1994]. Little composting is required since it has been found desirable to rotovate the freshly made mixture into the top soil as an active biological system in order to maximise efficiency. This approach offers the best opportunity to control nitrate leaching as ammonium ions released from the decomposing manure are immediately adsorbed to the zeolite mineral surface and then oxidized by nitrifying micro-organisms. As a result nitrate accumulates slowly in the soil pore-water from which it is readily taken up by the growing plant and it appears that residual nitrate is low by the end of the growing season.

In the case of countries that have large economic deposits of zeolitized tuff the low cost of production of organo-zeolitic bio-fertilizer is a considerable economic factor in its use. In many cases countries in Africa and South East Asia will benefit greatly from the use of their own resources as the cost of chemical fertilizers, due to their manufacture being linked to the rising costs of oil and gas, will inevitably increase in the future.

Agronomic achievements in the use of natural zeolite

Although the beneficial use of zeolitic tuffs to soil health has been known for many years [Mumpton, 1999] studies of the use of organo-zeolitic soil amendments, without additions of chemical fertilizers, have been conducted in few institutions. Plant growth in potted substrates and trials in plant beds have been used to compare performance in organo-zeolitic-soil substrates with that of soils fertilized with commercial chemical fertilizers. Studies have been made with a variety of edible plants which include arable farm crops, vegetables and fruits. In several cases it has been found that the addition of zeolitic tuff together with chemical fertilizer has the effect of increasing the yield of arable crops, potatoes, oil bearing geraniums and radishes [Tsitsishvili et al., 1984]. It was later discovered that a 16.7 volume % organo-zeolitic amendment made of poultry manure and zeolitic tuff containing abundant clinoptilolite produced a higher wheat dry grain yield without the addition of chemical fertilizer [Leggo, 2000]. This study was later substantiated by controlled experiments using similar organo-zeolitic amendments for the growth of a large variety of food plants. Wheat, maize, cabbage, carrot, beetroot, cucumber, onion, and garlic all showed large increases in yield relative to amendment with chemical fortified organo-zeolitic fertilizers or chemical fertilizers alone [Adronikashvili, T.G, 2003]. Again, it has also been shown that soybean (*Clicine hispidee Maxum*) grown in an organo-zeolitic-soil substrate, without the inoculation of nitragin, a biological fertilizer, has about a 40% increased yield compared with the same plant grown with chemical fertilizers [Mgeladze, et al., 2005].

It would appear that the introduction of chemical fertilizers together with an organo-zeolitic amendment could, apart from inhibiting the soil nitrifying bacteria, produce an elevated supply of NH_4^+ and NO_3^- ions that is known to cause increasing nitrite concentrations in vegetables; which is undesirable as a high nitrite concentration increases the risk of the formation of nitrosamine, a known carcinogenic compound [Tannenbaum et al, 1978].

An un-reported experiment with tomato (*Solanum* Ailsa Craig) was made in the Cambridge laboratory to investigate any differences in yield and quality that might occur between plants grown in an organo-zeolitic amended soil and a chemically fertilized soil. Three substrates were made using soil from the Botanic Garden, University of Cambridge; as used in earlier work [Leggo, 2000]. The control substrate, substrate 1, was garden soil. Substrate 2 was garden soil amended with an organo-zeolitic fertilizer using 16.7 volume % of the standard mixture, with 157 wt % excess zeolitic tuff. In substrate 3 commercially available Miracle-Grow was used as the fertilizer, applied at the recommended concentration once every seven days. All substrates were watered regularly with de-ionized water to keep the substrates moist. The plants were grown for a total of 84 days under controlled laboratory greenhouse conditions. At harvest the organo-zeolitic-soil substrate produced 13.4 wt% more fruit than the chemically fertilized substrate. Furthermore the plants grown in this substrate had scarlet red fruit whereas those grown in the chemically fertilized soil had larger pink fruit having an apparent increase in water content. The scarlet fruit had a greatly enhanced flavour and the intense red colour indicated an increase in the lycopene content which is considered to be a desirable antioxidant.

Other studies on the yield and quality of fruit and vegetables have found similar results. In general plants grown in organo-zeolitic amended soils have greater yields than those grown in soil with chemical fertilizers. In a study on the growth of garlic (*Allium sativum*) it was found that plants growing in an organo-zeolitic-soil system increased the productivity by 19.7 % relative to plants growing on plots amended with chemical fertilizers [Janjgava et al., 2002]. It should be mentioned that in this case zeolitized tuffs containing phillipsite and clinoptilolite were used with poultry manure as the organic component. The organo-zeolitic mixture containing phillipsite was found to produce approximately a 3 wt % greater yield than that using clinoptilolite; the application rate of the amendment being 20 tonnes per hectare. This illustrates that the type of zeolite mineral is important as ion exchange properties differ between mineral species due to differences in pore architecture and cation selectivity. As clinoptilolite is more commonly found in zeolitized tuffs much of the work on agricultural applications has been made with this mineral but future studies could well reveal the importance, in this area, of other natural zeolites. In some studies zeolitized tuff, ammoniated with ammonium chloride, has been used as a soil amendment. These have also shown improved plant yield and quality. Although not strictly organo-zeolitic-soil systems, as these substrates lack the organic component, the analogy exists in that ammonium ions are exchanged into the zeolite pore structure and are available at the mineral surface for oxidation by nitrifying bacteria. An experiment with grapes showed that when the modified zeolitic tuff (clinoptilolite) was used to amend soil the average yield per vine in comparison with a soil control doubled, [Andronikashvili et al., 2007].

These results are in agreement with our concept that plant growth in an organo-zeolitic-soil system occurs at a rate that is compatible with the uptake of nutrients from nitrification as when chemical fertilizers are used it is more difficult to control the supply of nutrients to satisfy changes in uptake during plant growth.

Organo-zeolitic fertilizers applied to metal polluted soils

In his comprehensive review on the removal of heavy metals from wastewater C.Colella has shown the versatility of natural zeolites in the removal of heavy metals from aqueous solution and has referred to many important papers on this subject [Colella,C; 1995]. The use of zeolite cation-exchange to improve the quality of wastewater has met with considerable success but the same approach with soils present insuperable difficulties. The problem of getting heavy metal cations into solution on a large scale to successfully treat a contaminated site is clearly not feasible. Therefore an alternative approach has to be made. The immediate problem with mine and metallurgical waste sites is to prevent the spread of toxic material by leaching of the top soil, by rainfall and consequent run-off , together with erosion by the force of the wind. In this respect it is clear that phytoremediation affords an opportunity to limit or prevent the transport of metals in dust clouds and water bourn particles that inevitably enter the food chain. In the past the problem has been in sustaining plant growth on sites contaminated with metal residues. This problem has now been greatly reduced by the use of organo-zeolitic materials to sustain perennial plants and stabilize the surface of such sites. In a recent study [Ledésert et al., in prep] it was found that the metal accumulator plant *Arabidopsis halleri* could be sustained on a site covered by metal concentrate rejected from a metal refinery. Although several other case studies have been made in which base metal concentrations were in the order of 1000 mg/kg this study involved metal concentrations in weight percent. It therefore came as a surprise to find that by amending the waste material with the biofertilizer it was possible to grow and sustain plants in such a highly phytotoxic environment, Fig 6.

As it was thought that nitrifying micro-organisms would not be able to function and provide nutrients in such extreme concentrations of metal ions the result was, at first, not understood. An apparent solution to this problem comes from the observation of biofilm formation occurring on zeolite mineral surfaces in aqueous and moist soil environments [Garcia et al, 1992., Kalló.,2001., Leggo et al, 2006] as other work had shown that bacteria when encapsulated in biofilm function normally. It is suggested that the biofilm, being an aqueous polysaccharide containing sugar end members, reduces and precipitates metal ions in an external environment outside the cell, thus preventing oxidization of the enclosed bacteria [Kemner, et al., 2005].

A site trial at Lynn Lake, Manitoba, Canada in 2002 again demonstrates the efficacy of amending metal polluted land with an organo-zeolitic mixture. At this locality a sulphide deposit had been mined for its nickel content from 1953 to 1976 and an area of 213 hectares is covered in mine tailings from the operation. The site has remained barren of vegetation since the deposition of the waste which has resulted in wind blown migration of fine dust containing many toxic metal elements. In order to try and control this situation it was decided to use a phytoremedial approach using a reclamation mix consisting of creeping red festue (*Festuca rubra*), timothy (*Phleum pratense*) and red clover (*Trifolium pratense*) in amended mine waste. The test involved the addition of organo-zeolitic fertilizer together with lime to reduce the acidic nature of the site. Seven treatments were randomly assigned to 6 m x 3 m plots in which the amendment was tilled directly into the top 15 cm of the waste just prior to

Figure 6. Plant growth experiment (*Arabidopsis haleri*). Plants growing in untreated, clean soil, used as the control are shown above the experimental pots, in each case. The upper image shows plants in untreated waste ore concentrate and the lower image shows plants in treated waste ore concentrate (Ledésert et al., submitted for publication).

seeding. The treatments involved the application of the fertilizer at two concentrations (162 and 45 tonnes / hectare) and lime applications of 11 tonnes / hectare. Chemical fertilizer, to match the nutrients of the organo-zeolitic fertilizer, plus lime at 11 tonnes / hectare and a control containing no amendment were also included. It was found that plant growth could not be sustained on either the chemical fertilized waste or the un-treated waste. However, in two seasons the plots amended with the organo-zeolitic mixture had sustained plant growth without further treatment and being left entirely under natural weather conditions, the plants have now survived six growing seasons, Fig 7.

Figure 7. Organo-zeolitic treated test strip supporting grass and clover at Lynn Lake, Manitoba, Canada. This area of more than 200 hectares was covered in mine waste from sulphide mining. This site had not supported any vegetation before the test carried out in 2001. The plants have been sustained over six seasons and are still producing growth in the summer months.

Conclusion

The research conducted to date has clearly demonstrated the efficasy of organo-zeolitic soil amendment. At the moment although there is a lack of geological evidence on which to base an estimate of world resources those available in Eastern Europe, Turkey, Iran, USA, Russia, South East Asia, Japan and China are very large and will not be exhausted in the near future. In the current situation regions that lack economic deposits, such as Western Europe and Scandinavia the cost of importation restricts its use to the remediation of contaminated land and horticulture. This condition may not be an over riding factor as natural zeolites are relatively strong, rigid structures that are stable in normal soils. Clinoptilolite, for example, is stable to a rise in temperatures of 450^0 C and can resist acid conditions to a level of pH 2. It is therefore realistic to think of an organo-zeolitic amendment as being re-chargeable when the organic component looses its ability to provide a supply of ammonium ions. The addition of more manure would sustain the system although the concentration of zeolitic tuff would be diluted. In this case it might be necessary to increase the quantity of tuff above the minimum concentration. Early unpublished experiments have shown that successive crops can be obtained without increasing the amount much above its minimum level but the efficiency of such a procedure will only become made apparent by the results of further research.

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Chapter XI

Radio-Environmental Impacts of Phosphogypsum

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Abstract

Phosphogypsum is a waste by-product of the phosphate fertilizer industry, which is usually disposed in the environment because of its restricted use in industrial applications. The main environmental concerns associated with phosphogypsum are those related to the presence of natural radionuclides from the U-238 decay series e.g. increased radiation dose rates close to the stack, generation of radioactive dust particles, radon exhalation, migration of radionuclides into neighbouring water reservoirs and soil contamination. Environmental impact assessment studies related to phospogypsum disposal, indicate that usually direct gamma radiation originating from the stacks and inhalation of radioactive phosphogypsum dust don't pose any serious health risk to people working on phosphogypsum stacks. On the other hand, radon emanation is one of the greatest health concerns related to phosphogypsum, because inhalation of increased levels of radon and its progeny may pose a health risk to people working on or living close to stacks. Physico-chemical conditions existing in stack fluids and leachates are of major importance and determine radionuclide migration in the environment. Among the radionuclides present in phoshogypsum U, Po-210 and Pb-210 show increased mobility and enrichment in the aquatic and terrestrial environment. Regarding Ra-226, the largest source of radioactivity in phosphogypsum, newer investigations show that the Ra-226 concentration in stack fluids is moderate and its release form stacks to terrestrial environments insignificant. Nevertheless, further research is required to improve the understanding of the radionuclide geochemistry occurring within, and beneath, phosphogypsum stacks.

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Introduction

Phosphogypsum is a waste by-product of the phosphate fertilizer industry produced during the wet process production of phosphoric acid from phosphate rock (Figure 1). Approximately 5 tonnes of phosphogypsum are produced per tonne of phosphoric acid (P_2O_5). According to published data, several hundred million metric tonnes of phosphogypsum are produced yearly and out of that, 14% is reprocessed, 58% is stored and 28% is dumped into water bodies (Rutherford et al., 1994; Hull and Burnett, 1996).

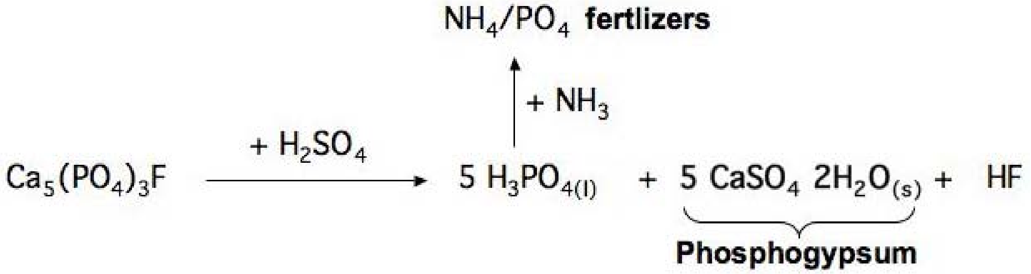

Figure 1. Chemical equations of reactions occurring during the production of ammonium-phosphate fertilizers from phosphate rock

Phosphogypsum is composed mainly of gypsum ($CaSO_4\ 2H_2O$), but contains relatively high levels of impurities such as fluoride, certain natural-occurring radionuclides and toxic trace elements, which originate primarily from the source phosphate rock. The main problem associated with this material concerns is the elevated levels of impurities and in particular natural, uranium-series radionuclides, which could have an impact on the environment. Because of these concerns commercial use of phosphogypsum and dumping into water bodies is restricted and therefore phosphogypsum storage on stacks is environmentally and economically the most viable waste disposal practice. Nevertheless, phosphogypsum stacks may represent sources of radiological contamination associated with (i) gamma radiation which may increase the average dose received by people working on to the stack; (ii) wind erosion and radionuclide-contaminated dust dispersion; (iii) radon (Rn-222) emanation which may pose a health risk to workers on the site or people living close to stacks; and (iv) migration of radionuclides from the U-238 decay series below phosphogypsum stacks into adjacent soil and water systems (Van der Heijde et al., 1990; Rutherford et al., 1994; Hull and Burnett, 1996; Perez-Lopez et al., 2007).

The present paper, which is mainly based on previous articles related to the subject, summarizes the environmental impacts and gives a short overview of the radiological aspects of phosphogypsum dumping. The main environmental concerns related to the storage of phosphogypsum and the direct impact of environmental compartments (e.g. atmosphere, hydrosphere, soil) is schematically presented in Figure 2.

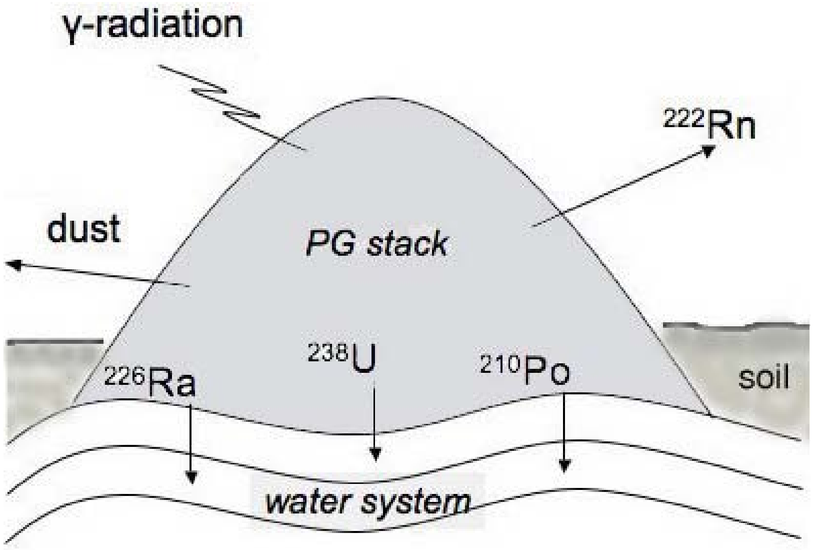

Figure 2. Schematic illustration of the radio-environmental impacts of phosphogypsum

Gamma Radiation emmision

Gamma radiation may affect living organisms by damaging their cells. In general, the risk of harmful health effects increases generally with the amount of radiation absorbed. Health effects of radiation are divided into two categories: threshold effects and non-threshold effects. Threshold effects appear after a certain level of radiation exposure is reached and enough cells have been damaged to make the effect apparent. Non-threshold effects can occur at lower levels of radiation exposure (UNSCEAR, 2000). Because of the harmful effects, human activities, which lead to increased radiation exposure (above the background radiation) have to be under continuous control to keep radiation exposure as low as possible and avoid any health hazards particularly to people residing or working close to radiation sources.

Phosphogypsum stacks are a direct source of gamma radiation because phosphogypsum is enriched in naturally-occurring radionuclides which originate from phosphate rock, that contains elevated levels of radionuclides belonging to the U-238, U-235 and Th-232 decay series. U-238 and its daughter radionuclides are the principal contributors regarding the radioactivity of phosphogypsum because of their relative high concentration in the parent material (Manyama Makweba and Holm, 1993). The U-235 decay series is of minor importance because of the low natural abundance of this uranium isotope (0.72 % of total uranium). Thorium (Th-232) is also not considered an environmental concern, because its content in sedimentary phosphate ores, which represent about 85% of the known and easy mined phosphate deposits, is usually low (Metzger et al., 1980). In undisturbed phosphate rock deposits the members of the U-238 decay series are in approximate radioactive equilibrium and the phosphate ore beneficiation process does not disrupt this equilibrium but increases the radioactivity of the concentrated mineral by up to 400% compared to the non-processed material. However, during the wet phosphoric acid process, the radioactive equilibrium is disrupted and the radionuclides are partitioned between two phases according to their solubility. Uranium, Th and Pb-210 distribute primarily into the phosphoric acid while most of the Ra-226 and Po-210 are fractionated to the phosphogypsum. Besides radium-226, which is usually the largest source of radioactivity, U-234, U-238 and Po- 210 may also be present in phosphogypsum (Rutherford et al., 1994; Hull and Burnett, 1996). Figure 3 summarizes in the form of a bulk diagram the concentration of different natural radionuclides measured in various phosphate rocks (PRock) and phosphogypsum (PG) samples and demonstrates clearly the process related radionuclide separation.

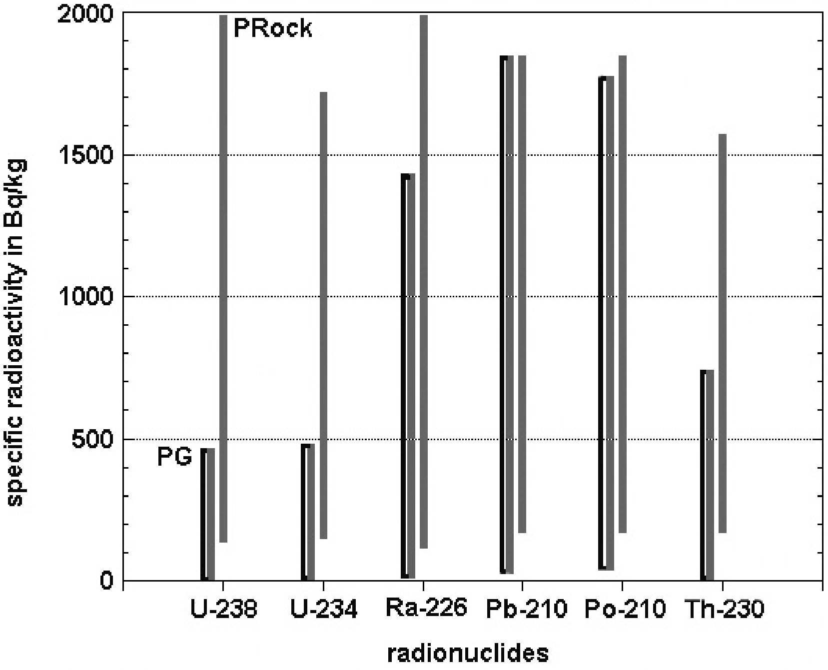

Figure 3. Concentration of different natural radionuclides measured in various phosphate rock (PRock) and phosphogypsum (PG) samples. The concentrations used in the above figure are a compilation of literature data (Van der Heijde et al., 1990; Makweba and Holm, 1993; Rutherford et al., 1994; Hull and Burnett, 1996).

Because phosphogypsum stacks are direct sources of gamma radiation, they may cause increased ionizing radiation exposure to people spending long periods of time working on a stack. In order to protect workers, radiations doses have to be monitored and if needed protective measures must be taken. In radiation protection and dosimetry the concepts of radiation absorbed dose and dose equivalent are of particular importance. The radiation exposure is defined as the energy absorbed from any type of radiation per unit mass of the absorber. In the SI system the unit for absorbed dose is the Gray (1 Gy = 1 J kg^{-1}), and is equivalent to 100 rad, which is conventional unit for radiation absorbed dose. The dose equivalent, H measured in Sieverts (Sv), quantifies more adequately the probable biological effect of the absorbed energy, since the absorption of equal amounts of energy per unit mass under different irradiation conditions does not have the same biological impact (UNSCEAR, 2000).

Gross gamma dose rates measured on the surface of a phosphogypsum stack in Cyprus reached values, which exceeded the corresponding background values up to 400 nSv h^{-1}. However, these values decreased to about 300 nSv h^{-1} at 1 m above the stack surface and almost to the background levels (80 nSv h^{-1}) in areas where the surface was covered by soil. Similar dose rates measured above phosphogypsum stacks have been reported also by other investigators (Laiche and Scott, 1991; Hussein, 1994; Mas et al., 2001). Generally, the dose rate levels measured correspond to a working year exposure of 0.85-0.45 mSv above background but are far below the occupational exposure limit (50 mSv y^{-1}) and the NCRP (1984) recommendation for general public continuous exposure to gamma radiation above background (10 mSv y^{-l}). The average worldwide exposure due to natural radiation sources reported by UNSCEAR is approximately 2.4 mSv y^{-1} (UNSCEAR, 2000). Even the highest gamma dose rates measured on the surface of phosphogypsum stacks are relatively low because it represents less than 35% of the average worldwide exposure due to natural radiation sources. Nevertheless, the dose rate levels measured are above background values and hence unnecessary time spending or even residing on phosphogypsum stacks should be avoided.

Radioactive Dust

Airborne particulate matter originating from phosphogypsum piles by wind erosion or operations on the stack is also a potential source of radioactivity. However, wind erosion is usually not a serious problem on stacks because inactive stacks develop a crust while active stacks generally have a high moisture content, which reduces dust generation. Analysis of air samples for U-238, Th-230, Ra-226, Pb- 210 and Po-210 close to a phosphogypsum pond show that the concentration of all radionuclides except Ra-226 are within background levels. The annual dose corresponding to the Ra-226 intake is estimated to be 2.5 µSv, which is significantly lower than the typical annual exposure because of the natural background radioactivity (Rutherford et al., 1994). Generally, the annual radionuclide transport from a phosphogypsum stack is very low and the associated radiation exposure insignificant. However, excavation or drilling works causing increased dust production, may (after inhalation) affect people working on the stack and therefore protective measures have to be taken when phosphogypsum dust generating operations are carried out on the stack.

Radon Emanation

Exposure to radon gas is one of the greatest health concerns related to phosphogypsum. Radon (here referring exclusively to Rn-222) is an ubiquitous, naturally occurring, radioactive gaseous element and member of the U-238 decay series (alpha decay product of Ra-226). Rn-222 is an inert, noble gas with a relatively short half-life, 3.82 days, which is an environmental concern because it can be highly mobile and eventually decays to form two relatively long-lived radioactive daughters, Pb-210 and Po-210. Inhalation of radon along with its daughter nuclides attached to microscopic particulate matter delivers highly ionising radiation to lung cells, causing biological damages and ultimately leading to lung cancer. From epidemiological and experimental studies, the carcinogen effects of radon, such as the increased risk of lung cancer for exposed miners compared to the non exposed population, have demonstrated potential negative impact of this radionuclide on human health. Radon accounts for about 40% of the average annual dose equivalent to which a person is exposed in the industrial world (Berger, 1990).

Emanation is the process whereby radon is released from the crystal structure of the hosting mineral. The emanation coefficient is the number of Rn-222 atoms that escape from the solid phase divided by the total number of Rn-222 atoms that are formed from the decay of Ra-226. When Ra-226 decays a high energetic alpha particle is ejected and the newly formed Rn-222 atom recoils in the opposite direction with a kinetic energy which is 104-105 times greater than typical bond energies. As a result, the recoiled Rn-222 atom can move within mineral structure up to a distance of 70 nm. If the mineral surface is present within this distance the Rn-222 atom may escape the crystal and be free to enter pore air or pore water. On the other hand, the energy of recoiling atoms is sufficiently high that some atoms cross intraparticle pore spaces and become embedded in adjacent particles. Hence, only a fraction of the formed radon can escape from the solid phase. Water in intraparticle pores increases the emanation coefficient by reducing the energy of the ejected atoms (Rutherford et al., 1994; Duenas et al., 2007). Phosphogypsum typically has an emanation coefficient of approximately 15%. However, the emanation coefficient can reach values up to 30% when the material is present as powder, because the amount of Rn-222 that is potentially available for release from a mineral is also related to the surface area to mass ratio (Lysandrou et al, 2007). The emanation factor is one of the main factors (e.g. Ra-226 content, atmospheric pressure, and diffusion coefficient for radon) that determine the exhalation rate of Rn-222 from phosphogypsum. Typical exhalation rates from soil, which is the largest source of background radon in the environment, is approximately 0.5 Bq m^{-2} s^{-1} per Bq Ra-226 g^{-1} soil and Rn-222 levels near open ground are about 5 Bq m^{-3} and the average concentration of Rn-222 in the global atmosphere is about 3.7 Bq m^{-3}, however concentrations may be 10-times higher over some land areas. The mean exhalation rate of Rn-222 from active phosphogypsum stacks is about 1 Bq m^{-2} s^{-1} although measured rates vary by almost two orders of magnitude (<0.004 to >3 Bq m^{-2} s^{-1}). Based on reports regarding estimated total Rn-222 fluxes from many stacks throughout Florida and estimated maximum exposed individual and collective risks, U.S.E.P.A. adopted an average 0.74 Bq m^{-2} s^{-1} limit on Rn-222 emissions from phosphogypsum stacks (Rutherford et al., 1994; Duenas et al., 2007).

Despite the increased emanation, populations living close to phosphogypsum stacks are usually not subjected to a significant health risk because air movement readily dilutes Rn-222 concentrations (Rutherford et al., 1994). However, it is still not clear whether employees who spend numerous hours on active phosphogypsum stacks are subjected to a significant health risk and what will be the radiation exposure of the population if an inactive stack is converted to residential usage and/or phosphogypsum is used a building material. Preliminary estimations indicate that in extreme cases the excessive radiation exposure due to radon and its progeny may increase up to 17 mSv y^{-1} (Lysandrou et al., 2007). Soil caps and vegetation covers reduce radiation exposure and release of Rn-222 from phosphogypsum surfaces (Duenas et al., 2007). However, these covers may induce sulfate reduction (Carbonell-Barrachina et al., 2002) that may lead to increased release of radium due to reductive decomposition of the highly insoluble $RaSO_4$ ($\log K_{sp}$= -10.2).

Radionuclide Leaching

One of the prime concerns regarding phosphogypsum storage is the potential for contamination of water aquifer in the vicinity of the stacks. Process water seepage during operation that leads to formation of acidic fluids underneath the stack and long-term downward leaching which occurs when rainwater or seawater (in the case of coastal stacks) infiltrates through an inactive stack may result in groundwater contamination. There are several laboratory and field studies with respect to potential leachability of phosphogypsum constituents and groundwater pollution (Rutherford et al., 1994; Bolivar et al., 2000; Burnett and Elzerman, 2001; Alcaraz Pelegrina and Martínez-Aguirre, 2001; Haridasan et al., 2001; Lysandrou and Pashalidis, 2007).

Laboratory studies based on standard extraction procedures indicated that very little Ra would be leached, because of the relatively increased inclusion of Ra into the phosphogypsum crystal structure (about 90% of the Ra amount originally present). On the other hand leachate studies on two composite phosphogypsum samples taken from an inactive and an active stack showed that among the radionuclides present in phosphogypsum, only Pb-210 was 100-fold greater in the phosphogypsum leachate obtained from the active stack and exceeded the World Health Organization Guideline for beta particle activity (1.0 Bq 1^{-1}) by three times (Rutherford et al., 1994). Recent leaching experiments performed with phosphogypsum samples taken from an inactive stack showed that leaching with seawater is far more effective (about three orders of magnitude) than leaching with deionized water (Lysandrou and Pashalidis, 2007).

Field studies showed that selected members from the U-238 decay series are present in the groundwater (below the phosphogypsum site investigated) at levels within drinking water standards. Literature data for Ra-226 are less clear, because there are investigators concluding Ra-226 migration through groundwater systems and others who deduce that Ra-226 is not leached from the phosphogypsum stockpiles (Rutherford et al., 1994). Recent investigations performed by Burnett and Elzerman (2001) show that stack fluids were very high in dissolved uranium and Pb-210 with only moderate concentrations of Ra-226. The investigators state that the slightly elevated Ra-226 concentrations, which are not surprising because of the radium-rich nature of the surrounding phosphogypsum, argue against the stack as a source of radium to the aquifer. Modeling of the data obtained by the previous investigation shows predominance of radionuclide complexes with sulfate and phosphate, resulting in relatively mobile uncharged or negatively charged solution species within the stacks. However, enrichment of underlying soils in U and Pb-210 indicates precipitation/sorption when acidic stack fluids enter the buffered environment and implies that these removal mechanisms will prevent large-scale migration of radionuclides to the underlying aquifer. Measurements of stack fluids collected form an inactive stack at a coastal area in Cyprus indicate that the conditions regarding radionuclide leaching and migration differ if a stack is in contact with seawater. The efficient dissolution of phosphogypsum and leaching of its contaminants as well as the stabilization of the dissolved radionuclides (e.g. uranium, Figure 4) in seawater suggests that in long-term the sea will the final receptor for both phosphogypsum and its contaminants. Species distribution modeling indicates that uranium is leached out of the phosphogypsum stack in the form of neutral and negatively charged uranium(VI) fluoro- and phosphato- complexes and is stabilized in the marine environment (pH 8) in the form of the very stable uranium(VI) tricarbonato species (Lysandrou and Pashalidis, 2007).

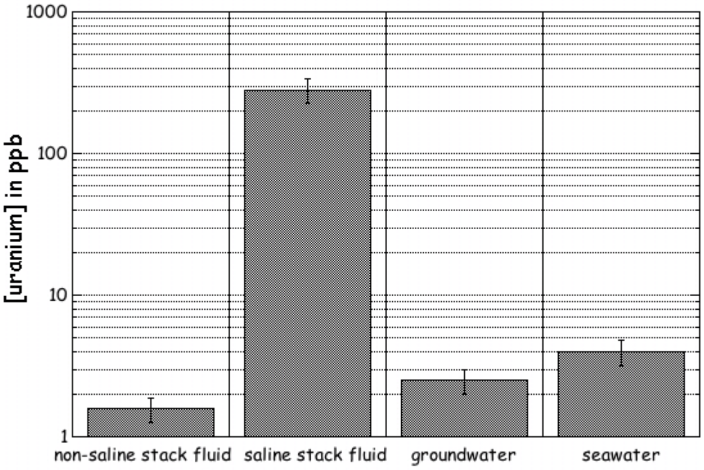

Figure 4. Average uranium concentration in phosphogypsum non-saline and saline stack fluids, seawater and non-contaminated groundwater samples.

Conclusion

Usually, direct gamma radiation originating from the stacks and inhalation of phosphogypsum dust does not present any significant health risk.

The exhalation of Rn-222 from phosphogypsum stacks may vary by several orders of magnitude, ranging from < 0.004 to >3 Bq m^{-2} s^{-1} and is one of the greatest health concerns related to phosphogypsum. According to several studies, populations living close to phosphogypsum stacks are not subjected to a significant health risk because air movement readily dilutes Rn-222 concentrations. However, it is still not clear whether employees who spend numerous hours on active phosphogypsum stacks are subjected to a significant health risk and what will be the health risk if a stack is converted to a build up area.

Acidic process water and the phosphogypsum itself are potential sources of contamination of groundwater and the terrestrial environment beneath phosphogypsum stacks. Leachate chemistry of the radionuclides is of major importance in determining the environmental impact of phosphogypsum. Studies dedicated to radionuclide mobility indicate that uranium, Po-210 and Pb-210 are relatively mobile. Regarding the contamination of aquifers by Ra-226 originating from phosphogypsum stacks there are some inconsistencies in the literature, although newer investigations indicate on moderate concentration of radium in stack fluids and no contamination of underlying aquifers and soils.

The application of soil caps and vegetation covers may reduce the release of Rn-222 from phosphogypsum surfaces and induce sulfate reduction and subsequently the formation of toxic metal sulfides, that are highly insoluble and less mobile. However, progressive sulfate reduction may lead to increased release of radium due to reductive decomposition of the highly insoluble $RaSO_4$.

Further research is required to deeper understanding of the geochemistry occurring within, and beneath, phosphogypsum stacks. In order to accomplish this it is necessary (i) to determine the physicochemical conditions (e.g. redox, pH and ionic strength, complexing agents) that exist within, and beneath, phosphogypsum stacks and (ii) to identify the solid phases and reactions that control the solubility and mobility of the (radio)toxic elements contained in phosphogypsum.

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Index