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By

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ABSTRACT

Poor availability of nutrients commonly constrains crop production in marginal environments of developing countries. Degraded soils and poor access to fertilizers limit the yields that can be produced by resource-poor farmers. In such circumstances, farmers need to use management techniques that maximize nutrient use efficiency of their crops. There are various techniques available, such as the use of legumes in crop mixtures or rotations, or careful placement and timing of fertilizer applications. In low-input agriculture the direct uptake of dissolved organic N (DON) by plants may be extremely important. DON represents a significant pool of soluble N in most ecosystems. Some plants may possess a greater capacity to take up DON rather than dissolved inorganic N (DIN). DON is composed of many compounds which enter soil from a range of sources (e.g. litterfall, root and microbial exudation, turnover of roots and organisms, urine and faeces, organic fertilizers). My aim was to investigate the impact of plant residues on DON cycling when incorporated into soils and to study the uptake of DON in comparison with DIN by the plant root system. In addition, the secondary aim was to investigate the influence of the rhizosphere on the transformation DON in soil. In the first trial, three experimental treatments were used to alter organic inputs: (1) Soil amended with straw (high C/N ratio), (2) Soil amended with grass residues (low C/N ratio), and (3) Non-amended (control). Results indicated that soil solution NO_3^- and NH_4^+ accumulated in the grass-amended soil in contrast to that amended with straw or in the unamended control soil. Overall, straw immobilized DIN in solution. DON in the grass amended soil increased from day 14 to 21 and sharply decreased thereafter whilst the straw amended soil and control remained relatively constant. Contrary to expectation, the results indicate that addition of organic matter did not cause a large rise in DON relative to that of DIN. This suggests that in this high fertility agricultural soil the microorganisms rapidly break down DON contained in N rich organic

residues to DIN. For N poor residues DON appears to represent a more important source of N, however, its availability to plants remains as yet unknown.

DEDICATION

This thesis is dedicated to my Father, who always inspired me to get higher qualifications and position and to my family. It is gratification for me that I have attained the dreams of my father and my family.

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List of abbreviations

Abbreviation	Description
µM	Micromolar
^{13}C	Carbon 13 isotope
^{15}N	Nitrogen 15 isotope
AA	Amino acids
ANOVA	Analysis of variance
C	Carbon
Cm	Centimeter(s)
CO_2	Carbon dioxide
d	Day(s)
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DON	Dissolved organic nitrogen
EC	Electrical conductivity
LMW	Low molecular weight
MC	Moisture content
mg	Milligram(s)
ml	Millilitre(s)
mm	Millimetre(s)
mM	Millimolar
N	Nitrogen

$NaHCO_3$	Sodium bicarbonate
NaOH	Sodium hydroxide
NH_3	Ammonia
NH_4^+	Ammonium
NO_2^-	Nitrite
NO_3^-	Nitrate
OM	Organic matter
P	Phosphorous
ppm	Parts per million
SEM	Standard error mean
SOC	Soil organic carbon
SOM	Soil organic matter
SR	Soil respiration
TDN	Total dissolved nitrogen
TFAA	Total free amino acids
UV	Ultraviolet
v:v	Volume to volume
w/v	Weight to volume

Thesis plan

This thesis consists of six interlinked sections. The thesis starts with a review of the literature and is followed by two experimental chapters which are designed to test the hypotheses identified earlier. This is followed by a general discussion as detailed below:

Chapter 1: General introduction and objectives of the research.

Chapter 2: Review of the literature on the research themes and related topics.

Chapter 3: Experiment on the effects of plant residues on DON dynamics in soil.

Chapter 4: Experiment on the influence of root N content on N release during root turnover.

Chapter 5: General discussion of the results from all the experimental chapters, highlighting the key conclusions and identifying areas for further work.

Chapter 6: Literature cited.

Chapter 1

1.1. General introduction

The rapid expansion of the human population compared to the reducing capacity for increasing food production represents a major global challenge. An intensification of crop production is therefore immediately required to address this imbalance. Globally, most of the lands suitable for agriculture have already been cultivated and there is little possibility of expansion into new areas to increase food production. Hence, modern farming systems aim to increase the yield per unit area. Crop yields can only be maintained or increased by employing sustainable agricultural management practices, particularly in relation to the maintenance or enhancement of soil fertility. The continual addition of organic wastes or mineral fertilizers is therefore required worldwide to improve soil fertility and consequently enhance crop production. Soil fertility depends on a multitude of factors: physical and chemical properties of the soil, microbial activity, and the intrinsic concentration of soil nutrients. The fertility of productive soils may be progressively degraded over time due to one or a combination of the following factors: soil erosion, water shortage, and irrigation using wastewater or over-fertilization. These factors may result in the salinization and degradation of soils, which leads to further decreases in soil fertility and simultaneously may cause environmental pollution threatening the health of plants, animals and humans.

Soil fertility depends primarily on the reserves and availability of plant nutrients in soil and complex interactions can occur between them. For example, the releases of N and P from soil organic matter often occur at the same time while K is not (Martinez and Cerda, 1989). Often the price of inorganic fertilisers precludes their use by farmers in which case N and P can be supplied from organic sources such as animal manures or plant residues (if available). The incorporation of

organic materials is also necessary to maintain soil fertility and productivity irrespective of cheaper sources. Such practices may also reduce the environmental pollution associated with the excessive application of inorganic N and P fertilisers.

Plant residues represent the primary source of organic matter entering soil and their input is known to be crucially important for the maintenance of soil organic matter (SOM) reserves (Varvel, 1994) and for sustaining soil biological activity (Tian *et al*., 1992). Plant residues are also a major source of N and P (Bhatti *et al*., 2005; Masri and Rayan, 2006) and consequently their addition always replenishes soil nutrient reserves to some extent (Richardson, 2004). The addition of plant residues also improves soil structure by promoting soil aggregation (Oades, 1993), which in turn also improves other soil properties such as soil porosity (Halkiah *et al*., 1981), water infiltration, aeration, resistance to compaction and the soil's water-holding capacity (Walker and Bernal, 2008). The effect of plant residues on soil nutrient bioavailability, microbial activity and crops growth, however, differs greatly depending on the chemical composition of the residues and their subsequent rate of decomposition. Plant residues with high carbon-nitrogen ratios (C/N) and carbon-phosphorus ratios (C/P) and high lignin and polyphenol levels decompose slowly, releasing nutrients over long time periods (Tisdale *et al*., 1993; Green and Blackmer, 1995). These types of residues may have a slow effect on soil nutrient levels but may indirectly affect crops when used as mulching components (e.g. by reducing water evaporation from the soil surface). In contrast, residues with low C/N and C/P ratios and lignin and polyphenol levels decompose rapidly, which has an immediate effect on soil nutrient levels and microbial activity. Notably, the combined application of organic and inorganic fertilisers can also increase the rate of residue decomposition (Chen *et al*., 2007).

Soil degradation, a present concern worldwide, results from inappropriate land-use and cultivation, resulted in soil nutrient deficiency, low plant productivity and eventually low

standards of living for farmers. Presently, agriculture often results in manipulation of soil productivity without sufficient attention being paid to enhancing the fertility of degraded soils. Furthermore, as the world population is projected to increase by nearly 35% in 2020 compared to the 1995 population, soil degradation is now observed as a severe constraint to agricultural crop production (UN, 1996). In order to fulfill future world food requirements it is possible to a large extent if the benefits of SOM preservation are recognized by farmers. For long-term sustainable agricultural production, management of natural resources, particularly SOM is considered a key measure for ameliorating deteriorated soils (FAO, 2003). Promoting the accumulation of plant residues has therefore now become a fundamental approach for soil fertility improvement (FAO, 2003). Awareness of the processes involved in plant residue decomposition and the factors regulating SOM stability are therefore critical for realizing sustainable agricultural management (Angers and Caron, 1998). Understanding the dynamics of plant residue breakdown and nutrient release represents one of the first steps towards a better understanding of SOM management.

In soils, 90% of N is present in organic forms, which are dominated by solid N compounds (Stevenson, 1982). This solid organic N is not directly available to plants and microbes (Jones *et al.*, 2005a). Nutrients stored in solid soil organic matter (SOM) become available to the soil microbial community and plants as they are processed into smaller units (dissolved organic forms) by the soil microbial community (Schimel and Bennett, 2004; Jones *et al.*, 2005a; van Hees *et al.*, 2005). Dissolved organic nitrogen (DON) is thought to be a major constituent of terrestrial and marine N cycles (Näsholm *et al.*, 2000; Neff and Hooper, 2002) and needs to be included in ecosystem budgets and N cycling studies, as many studies have confirmed that DON is the major N form in stream water and/or forest floor leachates (Campbell *et al.*, 2000; Perakis and Hedin, 2002).

However, the importance of DON in the soil N cycle has not always been recognized.

Especially in agriculture, information on mineral N remains critical due to its importance in crop nutrition, the environmental impact of NO_3^- leaching and N_2O emissions (Bhogal *et al.,* 2000). Moreover, in the last decades, global N cycles have been dramatically altered by anthropogenic activities such as industrial combustion processes and fertilizer application (Gruber and Galloway, 2008). Increasing atmospheric N deposition caused higher nitrification rates and higher leaching losses of NO_3^-, therefore, the traditional N cycling model, which is inorganic N-centered, has been reinforced (Aber *et al.,* 1998; Fenn *et al.,* 1998; Gundersen *et al.,* 1998). As a consequence, biogeochemical studies have focused on the dynamics of dissolved inorganic N (DIN, especially NH_4^+ and NO_3^-) and processes such as soil N mineralization, nitrification, plant uptake of DIN and NO_3^- losses (e.g. Aber, *et al.,* 1998; van Breemen *et al.,* 2002).

In particular, sub-tropical regions are expected to receive increasing inputs of N (e.g. from fertilizer inputs or fossil fuel consumption) over the next few decades (Galloway *et al.,* 1994, 2008), but until now studies on DON dynamics from these regions in particular and the influence of changing ecosystem N status on key processes in the soil N cycle (e.g. biodegradation) in general are scarce.

Recently, more studies have concentrated on DON in soils, probably due to various reasons. First, the development of new analytical techniques made the determination of DON in soils faster and easier (Campbell *et al.,* 2000). Second, various plants take up DON in N-limited ecosystems (e.g. Kielland 1994; Näsholm *et al.,* 1998), challenging the inorganic-N centered model of the N cycle. The third reason for the increasing interest in DON lies within new results regarding the composition of total dissolved N (TDN) export from forest ecosystems. While the dominance of DON over DIN in N losses from forest ecosystems has been reported from unpolluted forest (Perakis and Hedin, 2002), a number of studies recently confirmed, that not only NO_3^- but also DON can account for the majority of TDN losses in ecosystems receiving high

atmospheric inputs of anthropogenically derived N (McDowell *et al.,* 2004; Pellerin *et al.,* 2006; Brookshire *et al.,* 2007).

Until now, the connection between DON export from forested watersheds and N loadings remains unclear. DON losses have been found to be independent of N loading rates (Lovett *et al.,* 2000; Perakis and Hedin, 2002; Pellerin *et al.,* 2006), while other studies reported a positive relationship between DON fluxes in soil solution and N loading rates in long-term forest fertilization experiments (McDowell *et al.,* 2004; Pregitzer *et al.,* 2004). Fang *et al.,* (2009) listed several mechanisms that could lead to DON losses under N-saturated conditions. Firstly, increasing dissolved organic carbon (DOC) losses have some-times been reported from N fertilized forests (Pregitzer *et al.,* 2004). Therefore, a concurrent increase of DON is likely, as DON export has been shown to follow DOC losses in a strict stoichiometric relationship (Rastetter *et al.,* 2005; Brookshire *et al.,* 2007). If organic matter decomposition is low, DOC losses will increase as compared to C losses in the form of CO_2; therefore, DON export is directly controlled by SOM dynamics rather than by biological N demand (Brookshire *et al.,* 2007). Secondly, increasing N mineralization could trigger DON production in the critical depolymerization step before ammonification (Schimel and Bennett, 2004). Third, the role of abiotic DIN incorporation might play an important role under high atmospheric N deposition (Berntson and Aber, 2000; Dail *et al.,* 2001). Whether DON is dominant over DIN due to a potentially large DON source in soils (Jones and Kielland, 2002) or due to the lacking utilization of DON by plants and microorganisms (Jones *et al.,* 2005a), remains an open question. In contrast, factors, that could explain the dominance of DIN over DON in eco systems are soil C/N ratios (Hood *et al.,* 2003) and N supply from biological N_2 fixation by plants (Schwendenmann and Veldkamp, 2005). Despite the growing interest in DON in both temperate and (sub)tropical ecosystems, information on DON dynamics in the soil of forest ecosystems under high and low anthropogenic N deposition is still scarce.

It is possible to increase SOM levels, as well as increase soil fertility, in several ways. Among them, adding different types of plant materials is currently one of the most cost-effective means. However, in order to optimize the benefits of plant residues on soil quality improvement, it is critical to synchronize the release of nutrients from residue decomposition with patterns of plant nutrient uptake, which may minimize the loss of available nutrients via leaching, runoff and erosion (Sylvia *et al.,* 2005). There are many factors which regulate the rate of plant residue decomposition and numerous studies have been carried out on the dynamics of litter decay in soil in order to obtain an in-depth understanding of decomposition mechanisms (Chintu *et al.*, 2004; Potthoff *et al.,* 2005; Kuzyakov *et al.,* 2007). In both agricultural and natural systems, plant residues are added to soil continuously as root and shoot litter as well as in pulse additions (e.g. animal manure). To date most studies have focused on the turnover of leaf litter, however, more work is required on the role of root and mycorrhizal turnover on soil nutrient cycling. Presently, the methods for examining the decomposability of plant residues and the release of different elements have not been comprehensively studied. This thesis aims to directly address this issue.

1.1.1. Study objectives

The main objectives/aim of the thesis are as follows:

- To study the effects of plant residue quality on DON cycling after residue incorporation into soils.
- To investigate the production and cycling of DON after the incorporation of root residues of different N contents into soil.

1.1.2. Hypotheses to be tested

1. DON availability in cropping soils is enhanced by shoot residue addition and root turnover.

2. That the labile low molecular weight DON pool in soil increases after organic residue addition and that this constitutes an important source of N to plants in comparison to inorganic N.

3. The production of dissolved inorganic N (DIN) is limited by the transformation of complex DON to labile DON and not by the transformation of labile DON to DIN.

1.2. References

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Chapter 2

2. Literature review

2.1. Soil fertility and plant nutrients

Soil fertility can be defined as the capability or quality of a soil that allows it to provide chemical elements in quantities and proportions that are conducive to plant growth. Soil fertility and plant nutrition, therefore, cannot be separated. Plant nutrition involves the study of the chemical elements necessary for plant growth. Plants take up essential elements from the soil through their roots and air through their leaves. Notably, there are about 17 micro- and macro-elements required for crop growth.

Plant residues provide organic matter and nutrients, which are essential for soil productivity (Kumar and Wagenet, 1985; Varvel, 1994). The plant residues added to the soil are transformed into CO_2, inorganic nutrients, microbial biomass and relatively stable humus (Berg *et al*., 1993). SOM sustains favourable physical, chemical and biological soil properties and allows for the release of nutrients through the decomposition of plant and microbial residues (Tutua *et al*., 2002; Sahrawat, 2004).

2.1.1. Nitrogen

In the context of crop production, N is one of the most frequently growth-limiting nutrients (Lebauer and Treseder, 2008) and is one of the main causes constraining the production of crops such as wheat and other cereals (Glass, 2003). N is a major nutrient element controlling the cycling of organic matter in the biosphere (Knicker, 2011) and N has great impact on the global C and N cycling (Gardenas *et al.,* 2011).Consequently, the majority of non-legume cropping systems require additional N inputs to produce good yields (Graham and Vance, 2000). Over the last 50 years it is therefore not surprising that there has been a progressive increase in the use of N

fertilisers to enhance agricultural production worldwide (Hirel *et al.*, 2007).

For environmental and economic reasons, N fertilisers should be used as efficiently as possible. This is exemplified by the deterioration in the diversity of bacterial, animal, and plant ecosystems adjacent to farmland due to the intensive use of N fertilisers (Hirel *et al.*, 2007). Despite these destructive effects on the biosphere, the use of fertilisers (N in particular) in agriculture, which generally improved the efficacy of the available cropping methods, primarily in advanced countries, has undoubtedly helped in providing food, feed and fibre to the global population (Sutton *et al.*, 2012). The main feature of the terrestrial N cycle is shown in Fig 2.1.

2.1.1.1. Soil N and its organic forms

Soil N appears in one of three general forms:

(i) organic nitrogen compounds, (ii) ammonium (NH_4^+) ions and (iii) nitrate (NO_3^-) ions.

About 95-99% of the N available in soil is present in organic forms: in plant, animal and microbial residues. Generally, it is believed that plants cannot directly utilise N in these macromolecular organic forms with microorganisms required to convert organic N to an inorganic form that can be subsequently used by plants. A very minor amount of organic N may be available to plants as soluble organic compounds (e.g. urea, amino acids, and peptides).

The majority of plant-available N in soil is present in an inorganic form as NH_4^+ and NO_3^- with very small amounts of NO_2^- also present. NH_4^+ and NO_3^- ions may exist in the crystal structures of certain soil minerals and may be relatively bioavailable; however, this form of N is abundant in only a few soil types worldwide. The available N in the majority of soils is supplied by the release of N from SOM or organic materials added to soils (e.g., manure, forage legume residues). The magnitude of N released from these materials depends on the composition of the

material, predominantly its C/N ratio, and the prevailing climate. The availability of inorganic N represents the primary constraint to vegetation productivity in most ecosystems (Schalze, 2006).

Inorganic fertilisers are typically applied as NH_4NO_3 or $(NH_4)_2HPO_4$ within many blends of liquid and dry fertilisers as well as being applied directly. Urea is an industrialised, organic form of N ($CO(NH_2)_2$, which is generally used in solid and liquid fertilisers. N fertiliser rates are determined by the crop to be grown and the quantity of N delivered by the soil. The standard protocol for fertilization rates are generally built on local recommendations and experience.

2.1.1.2. Nitrogen leaching

For several decades, research has focused on the efficient use of nutrients within agricultural production systems as poorly managed agricultural land is recognised to be a major source of contamination to both surface and ground water (Randall and Mulla, 2001). In Asia, Europe and Northern America, intensive agricultural practices have led to both higher production costs and a greater risk of environmental hazards such as ground and surface water pollution by NO_3^- leaching (Randall and Mulla, 2001).

2.1.2. Decomposition and mineralization

Decomposition and mineralization processes are important for sustaining life on Earth, facilitating the massive recycling of chemical elements in the biosphere. Decomposition is the process of metabolic degradation of organic matter (e.g. plant residues, animal tissues and microbial material) into simple organic and inorganic compounds. Decomposition is basically a process of breakdown of the organic matter carbon structure with the subsequent release of energy. Three major procedures are involved in decomposition: leaching, disintegration and chemical alteration.

Decomposition and mineralization are strongly related processes, where mineralization is

generally considered as a subset of decomposition, whereas decomposition does not always lead to mineralization. Generally, decomposition is associated with the C cycle whereas mineralization contributes to nutrient cycling. Basically, mineralization refers to the process where matter is converted from an organic to an inorganic substance, as a result becoming mineralized. In N mineralization, organic N from decomposing plant and animal residues (proteins, nucleic acids, amino sugars and urea) is converted to NH_3 and NH_4^+. This process is also called ammonification. The resulting NH_4^+ can be converted back to organic N (immobilization) where it is taken up by microbes and plants or nitrified to NO_3^- by nitrifying bacteria. The mineralization of soil organic soil N during the growing season is vital for the provision of N for crop growth (Appel and Mengel, 1992). N mineralization is regulated by a multitude of factors including the composition or quality of the organic material, agricultural management practices, temperature, humidity, soil pH, aeration and soil structure and texture (Jarvis *et al.*, 1996).

SOM decomposition represents a considerable contributor to nutrient availability in soil. Incorporating animal dung and other organic materials into the soil is well documented to have positive effects on soil physical, chemical as well as biological properties. SOM consists of various pools, humus, fresh plant residues and root exudates (NRCS, 2000). These pools differ in their rate of decomposition, decreasing in the order: root exudates > fresh residues > humus. These components are the food source for the community of heterotrophic organisms. Decomposition processes are controlled by a range of factors of which nutrient availability, soil microorganisms, physical environment, crop residue quality, root exudation and rhizosphere priming effects are particularly important (Singh *et al.,* 2004). Natural factors such as soil texture, moisture and temperature are very important since they can affect decomposition rates due to their effects on microbial activity. The chemical qualities of plant residues (e.g. C/N and C/P ratio) as well as the physical structure (e.g. protection of protein within plant tissues) are usually considered important

for regulating mineralization rates. According to NRCS (2000), a typical green plant residue contains: cellulose (45%), hemicellulose (20%), lignin (20%), proteins (8%), sugars and starches (5%) and fats and waxes (2%). When plant residues enter soil in some cases the organic compounds undergo rapid microbial decomposition (e.g. low molecular weight (MW) sugars and amino acids) whilst other components degrade more slowly (e.g. lignin, cellulose). Plant residue decomposition is a biologically complex process and requires the concerted action of numerous soil organisms. The end products of plant residue decomposition include CO_2, water, energy, microbial biomass, inorganic nutrients and re-synthesized organic C compounds such as humus, phenolics, celluloses, hemicelluloses and lignin (Baldock, 2007). Humus has a very significant effect on soil properties, becoming darker, promoting soil aggregation in addition to serving as a slow-release storage pool of N, P and other nutrients. Under aerobic conditions plant residue decomposition leads to the production of CO_2, however, under anaerobic conditions, organic acids or CH_4 may be produced instead of CO_2 (Wood, 1989).

The decomposition of plant residues has been studied by evaluating by several parameters, such as CO_2 evolution, residue mass loss, nutrient release and residual C. Since plant residues are decomposed, microbial populations increase rapidly, evidenced by the increased release of CO_2. By measuring CO_2 evolution, the rate of mineralization of C in residues can be determined under aerobic conditions, however, the interpretation of CO_2 data becomes more difficult under anaerobic conditions when C may leave the system as CH_4. Nutrient release during decomposition is critical in terms of nutrient recycling to plants; however, nutrients may also become immobilized in the microbial biomass leading to reduced availability in soil (Trinsoutrot *et al.*, 2000, Corbels *et al*., 2003, Baggie *et al*., 2005).

Soil respiration represents the best marker for assessing the decomposition rate of plant residues and respiratory activities of plant roots (Hu *et al*., 2006). Various different research studies

have revealed that temperature, water availability and pH are the major factors affecting the rate of soil respiration (Osono *et al*., 2003; Marschner *et al*., 2005). The results of Nikliska and Klimer (2007) revealed that temperature has a considerable effect on soil respiration and on the loss of plant residues both directly and indirectly by means of its effect on water loss. Respiration losses of C from plant residues with a high C/N ratio are typically much lower than from those with a low C/N ratio due to the plant residues not containing sufficient N to satisfy the requirements of the soil organisms. Additionally, they often contain large amounts of poorly decomposable C compounds such as cellulose or lignin. The rate of respiration of diverse plant residues (high and low C/N residues) is generally higher than that of high C/N residues, whereas N release is higher. The dynamics of decomposition and N release from cover crop residues applied as mixed and single applications has also been investigated (Aita and Giacomini, 2003). Their findings were that mixed residues of common vetch-oat possessed lower decomposition rates than the vetch alone but released higher amounts of N. In addition, Jensen (1997) established that mixing plant residues in soil caused a spatial redistribution of the soil microbial biomass within the soil profile. Nevertheless, the mechanisms of nutrient release after plant residues are added to soil and their effect on living plants are still not entirely clear.

2.2. Factors affecting the decomposition of plant residues

2.2.1. Plant residue properties

The main chemical and physical properties of the residues that determine the rate of decomposition and nutrient release are discussed in the following sections.

2.2.1.1. Chemical properties

Several different chemical properties of plant residues provide good predictors of the rate and

course of their subsequent degradation when introduced into soil. Residues typically consist of three main chemical fractions which differ in their respective decomposition rate: 1) easily decomposable sugars, organic acids and amino acids; 2) slowly decomposable compounds comprising cellulose, pectin and hemicellulose; and 3) recalcitrant materials such as lignin (van Veen *et al*., 1984). Numerous studies have found that the primary concentration of N (Jensen, 1997), P (Soon and Arshad, 2002), lignin (Muller *et al*., 1988), polyphenols (Constantinides and Fownes, 1994) and soluble C (Kachaka *et al.*, 1993) can all provide good indicators for assessing plant residue quality and residue decomposition rates.

The initial N content of plant residues and the soil microbial biomass responsible for their decomposition are two crucial factors which can both accelerate or constrain residue decomposition (Heal *et al.*, 1997). The N content of plant residues is usually positively correlated with the amount of N mineralization. The soil C/N ratio of agricultural soils is typically around 12 whilst that of microorganisms is typically around 8. The optimum C/N ratio of plant residues to support microbial growth is around 25. In context, the C/N ratio of crop residues usually ranges from 20 to 500 and depends greatly on plant maturity and species. According to Baldock (2007), plant residues with a high C/N ratio (>40) are mineralized far more slowly than residues with a C/N ratio <40. Plant materials with low C/N ratios will meet the N requirements of the soil microbial population and extra N will be mineralized and become available for plant uptake (as NH_4^+ and NO_3^-). Usually, plant residues of the Poaceae family (e.g. wheat, oats, and barley) have a high C/N ratio and the Fabaceae or leguminous family (e.g. vetches, lupin and beans) have a low C/N ratio. Soon and Arshad (2002) found that the decomposition rate of straw from three crops was in the order: pea > canola > wheat, whose residue N contents were 7.1, 7.0 and 5.1 mg N g^{-1} straw and C/N ratios of 66, 71 and 97, respectively.

The lignin/carbohydrate ratio also influences the rate of plant residue decomposition (Heal

et al., 1997). Lignin plays an important role in plant cell wall structure and makes the cell walls resistant to microbial breakdown (via blocking access to cellulose and protein inside the tissues). The decomposition of plant cell walls is therefore vital in the breakdown of plant residues because it permits microbial access. According to Herman *et al*. (1977), the decomposition of organic matter and N mineralization will decline as the concentration of lignin and the C/N ratio increase or the N content decreases. In the later stage of plant residue decomposition when simple decomposable compounds are exhausted, lignin decomposers will predominate (i.e. lignolytic fungi) regulating the course of degradation (Berg and McClaugherty, 2003).

Plant components with different biochemical compositions show different C mineralization kinetics. Generally, the organic C content of most plant materials is about 40%, and, while most of this will be returned to the atmosphere as CO_2, about 20-32% remains in the soil at least in the short-to-medium term as SOM (NRCS, 2000). Reinertsen *et al*. (1984) showed that the decomposition rate of wheat straw and the amount of N immobilized in the microbial biomass in the early phases of decomposition was largely dependent on the soluble and available C pools decomposed within the first few days.

The influence of the initial polyphenol concentration and the polyphenol: N ratio of plant residues on mass loss and N release have also been extensively studied (Palm and Sanchez, 1991; Oglesby and Fownes, 1992). According to Sivapalan *et al.* (1985), plant residue decomposition rate is reduced by the presence of high concentrations of polyphenols, cellulose and waxes due to enzyme inhibition and binding of mineralized N to insoluble organic compounds. Palm and Sanchez (1991) found that N mineralization was negatively correlated with polyphenol concentration ($r = -0.63$) and polyphenol: N ratio ($r = -0.75$). They found that plant residues high in polyphenols have low N mineralization rates because of the formation of stable polymers between poly-phenolics and amino groups within proteins. Oglesby and Fownes (1992) support

this conclusion finding that the initial polyphenol/N ratio was the best chemical index of N mineralization.

2.2.1.2. Physical properties

Separately from their biochemical composition, the physical properties of plant residues and their contact with the soil have a marked impact on N immobilization/mineralization turnover (Bending and Turner, 2004). Reducing the residue particle size increases the surface area available for colonization by soil microorganisms and allows a more uniform distribution of residues in the soil. Therefore, small-sized residues will decompose faster than residues of larger sizes. Singh *et al.* (2004) found that the particle size of canola residue had a significant effect on N immobilization, but interestingly did not significantly affect the C mineralization rate.

2.2.2. Effect of environmental factors on residue decomposition

2.2.2.1. Soil properties (clay, aeration, and pH)

Clay is one of the major soil texture components determining soil aeration and drainage and significantly affects residue decomposition rates. Clay concentration is closely associated with aggregate size and aggregate formation and was found to correlate negatively with potential N mineralization (Sylvia *et al.*, 2005). Negatively charged clays play a significant role in soil C, water and nutrient retention by chemically binding plant residues and cations such as NH_4^+, thereby reducing residue mass loss. Epstein *et al.* (2002) found that the rate of SOM decomposition increased as soil clay content decreased due to the increased O_2 levels, and that the accumulation of SOM was positively correlated with soil clay concentration. Sufficient soil aeration accelerates the decomposition of plant residues and the growth of microorganisms. Bacteria and fungi are the two main plant residue decomposers. Bacteria consist of aerobic and anaerobic organisms and both

groups are able to break down polymeric molecules such as lignin, celluloses and hemicelluloses; however, microbial populations increase faster and decomposition is greater under aerobic conditions because the energy yield of aerobic metabolism is higher than in anaerobic metabolism (Berg and McClaugherty, 2003).

Due to its direct effect on microbial physiology and mineral solubility, soil pH strongly influences plant residue decomposition processes. It also affects nutrient solubility and can change microbial community composition. Microbial activity is typically maximal at neutral soil pH values. According to Allison (1973), neutral pH and high N concentrations will favour multiplication of bacteria, while low pH and N concentrations will favour the growth of fungi. Marschner *et al*. (2005) showed that microbial community composition was more strongly affected by soil pH than other soil properties. Therefore, the survival and competitive ability of microbial species are strongly altered by soil pH. Plant residue decomposition may also induce alterations in pH through a stimulation of nitrification. Xu *et al.* (2006) concluded that the soil pH change after the addition of plant residues was dependent on plant residue type and initial soil pH. They indicated that the addition of legume residues first increased soil pH (due to NH_4^+ release), but then decreased it after a 42 day-incubation (as NH_4^+ was converted to NO_3^-), whereas soil pH remained comparatively constant after the addition of low N wheat residues.

2.2.2.2. Temperature and moisture

Temperature and moisture are important physical factors affecting the rate of residue decomposition as they directly affect soil microbial activity. Microbial activity increases with increasing temperature with an optimum of 30 to 45 °C, but the relationship between microbial activity and temperature is to some extent dependent on microbial species. Microbial respiration

is critically linked with temperature although it does depend to some extent on the adaptability of organisms to their soil habitat (Berg and McClaugherty, 2003). Under aerobic circumstances, the increase in residue decomposition with increasing temperature results in increased N release and a reduced rate of SOM accumulation (Eijsackers and Zehnder, 1990).

Soil moisture also has a big effect on residue decomposition. Sufficient moisture will increase the rate of decomposition and the growth of microorganisms as water is required for the breakdown of plant residues (ca. 25-80% water filled pore space). In contrast, high moisture levels (>80% water filled pore space) will result in anaerobic conditions and typically delay mineralization processes. Osono *et al*. (2003) found that the survival and activity of *Chamaecyparis obtusa* was positively correlated with moisture content, as well as with the concentration of soluble carbohydrate in the residue. In studies investigating the effect of residue properties on decomposition, it is therefore important to keep most or all environmental factors constant.

2.3. Dynamics of N during the decomposition of plant residues

2.3.1. Immobilisation

Mineralization and immobilization are the two major processes taking place during the decomposition of plant residues. The release of N from crop residues is dependent on the net balance between mineralization and immobilization and also the maintenance of residue N in SOM pools (Jansson and Persson, 1982). The products from mineralization comprise available forms of nutrients which can be readily absorbed by plants and living organisms. Likewise, the process of altering inorganic forms of nutrients into microbial biomass is known as immobilization, which helps decrease the loss of nutrients through leakage and erosion. N losses from the soil-plant system via leaching, de-nitrification and NH_3 volatilization can influence how much N from plant

residues is available for subsequent crops.

The key organic N compounds in soil are amino acids and amino sugars; amongst them, free amino acids are freely mineralized under aerobic conditions (Stevenson, 1982). During mineralization, organic N first deaminates to amino acids which are then converted into NH_4^+. The NH_4^+ generated within the cell can be incorporated into cell biomass, released from the cells and utilized by other microorganisms or nitrified to NO_3^- (Bolan and Hedley, 2003). The unending process of moving mineralized N into organic products and mineralization N back into inorganic forms is termed mineralization-immobilization turnover (Jansson and Persson, 1982). The difference between total N mineralization and immobilization from plant residues is known as "net N mineralization".

Net N mineralization is generally controlled by the C/N ratio of plant residues and soils. Net N mineralization from plant residues is based on the association between (1) gross N mineralization and respiration (C mineralization) and (2) gross immobilization and respiration (Berg and McClaugherty, 2003). Net N mineralization is strongly dependent on the C/N ratio of plant residues added. On average, the C/N ratio of microorganisms is 7:1, indicating that microbes need 7 parts of C for every 1 part of N for the maintenance of functionality, and of this one third of C is incorporated into their cells and two thirds are used as a source of energy. Consequently, plant residues with C/N ratios less than 21:1 will meet all the N demands of microbial mass and the extra N will be released or mineralized and N will flow from the organic into the inorganic pool (Walley and Yates, 2002). Therefore, the narrower the C/N ratio in plant material (less than 21:1), the more net mineralization will occur. According to Kachaka *et al*. (1993), a C/N ratio of residue < 25:1 (N content > 1.4%) leads to net mineralization, whereas net immobilization leads at a C/N ratio of residue > 25:1. When low N plant residues are first mixed into the soil, N immobilization will happen since these plant materials do not meet the N nutritional needs of soil

microbes. The N immobilized by living organisms will be released when microorganisms die off and are consumed by protozoa and other mesofauna (e.g. earthworms). In a in a tropical dry-land field study the influence of single and mixed plant residues with different C/N ratios found that adding Sesbania (C/N 16) + wheat straw (C:N 82) gave a higher level of microbial biomass N and N availability compared to single residue (Singh *et al.,* 2006).

When N is abundant, both microbes and plants assimilate NH_4^+, however, typically NO_3^- is not a preferred N source for microbes under these conditions (due to the extra energy required to assimilate NO_3^- in comparison to NH_4^+). After uptake into the cells, proteins, nucleic acids, cell wall components (e.g. peptidoglycan) and other organic N constituents are formed from the assimilated N.

2.4. Properties of the rhizosphere

The rhizosphere is defined in various ways, but the definition of Hiltner in 1904 is the most common: the volume of soil adjacent to and influenced by plant roots (Bertin *et al*., 2003). Plant roots affect the physiochemical properties of the soil. A study by Wang and Zabowski (1998) showed that the pH in the rhizosphere can differ by up to 2 pH units compared to the bulk soil. The rhizosphere is a favourable zone for the growth of soil microorganisms as a result of several factors. Of these, the release of root exudates appears to be most important. The rhizosphere is a habitat for microorganisms that differs substantially in its chemistry, biology and physics from that of the bulk soil. The microorganisms in the rhizosphere can stimulate (e.g. mycorrhizas) or reduce (e.g. fungal pathogens) plant growth.

2.4.1. Root exudation and the rhizosphere

Root exudation is a component part of rhizodeposition, and represents the main source of organic C lost by growing plant roots. Rhizodeposits are classified depending on their mode of arrival,

namely exudates, secretions, lysates and gases. The composition of root exudates generally consists of sugars, amino acids, organic acids, hormones, vitamins, and unidentified substances such as microbial growth stimulants and inhibitors (Lynch and Whipps, 1990). Though sugars offer a readily available source of C for microbial growth, amino acids are a readily available source of C and N (Baldock, 2007).

Root exudates are also known to stimulate the decomposition of SOM and plant residues (i.e. rhizosphere priming). Kuzyakov *et al.* (2007) simulated the rhizosphere by adding malate, glucose and glutamate at two different temperatures (15°C and 25°C) to soil with plant residues and found that the addition of these labile root exudates significantly increased plant residue decomposition, but the increase was regulated by temperature. Organic compounds released from roots affect the rate of residue decomposition and nutrient release through impacts on the activity and abundance of decomposer populations in the soil (Paterson *et al.,* 2006). They also contribute to greater aggregate stability as soluble root exudates can act as glue between clay particles. The release of organic substances from plant roots appears to be crucial in plant-microbe interactions as many act as signaling compounds which help coordinate the microbial community in the plant's favour (Lynch and Whipps, 1990). Ultimately, root exudates lead to major shifts in soil microbial community structure (Marschner *et al*., 2001) and therefore nutrient release.

Rhizosphere respiration can be regarded as a semi-quantitative indicator of the rate of root exudation owing to the direct correlation between microbial activity and root exudation. However, it should be noted that CO_2 production in the rhizosphere results from both root and microbial respiration which occur simultaneously (Haller and Stolp, 1985). Experimentally, it is difficult to separate these two fluxes (Kuzyakov *et al*., 2007). The quality and quantity of compounds exuded from roots varies greatly depending upon plant species, age (Lynch, 1990) and the availability of mineral nutrients (Eldhuset, 2005) and a range of external factors (e.g. temperature, moisture etc.).

Generally, young plants exude almost twice as much of their fixed C as root exudates as older plants.

Rhizodeposition typically accounts for 15-60% of total photosynthetic fixation (Curl and Truelove, 1986; Darrah, 1996). Further it has been calculated that 5-10 % of this loss occurs via root exudation (Jones *et al*., 2004). There are two classes of exudates: exudates which are lost as a result of passive diffusion, basal exudation, representing 3-5% of fixed C in photosynthesis (Pinton *et al*., 2001) and exudates which are released for a definite reason and over which the plant exerts direct control by the opening of membrane pores (Jones *et al*., 2004). C loss from the root has a large impact on microbial populations, population sizes and activities, all of which are elevated in the rhizosphere relative to the surrounding bulk soil. A steep exudate diffusion gradient is therefore maintained away from the root as a result of constant removal of exudates from the soil by microbial uptake and abiotic sorption processes (Kuzyakov *et al*., 2003).

Three rhizosphere divisions can be distinguished (Gobat *et al*., 2004): the endorhizosphere (interior of the root), the rhizoplane (surface of the root) and the ectorhizosphere soil that adheres to the root when the root system is physically removed from soil. In plants colonized by mycorrhizal fungi, the ectorhizosphere has an additional component, the mycorrhizosphere, which can extend for a considerable distance from the root. The volume of soil not influenced by roots is known as non-rhizosphere or bulk soil (Gobat *et al*., 2004). Rhizosphere microbial population densities are usually an order of magnitude higher than those found in bulk soil (Anderson *et al*., 1993). This increased microbial activity in the zone of soil surrounding the root has major implications for soil function. Rhizosphere effects have been found to influence C, N and P cycling (Blagodatsky and Richter, 1998a; Blagodatsky *et al*., 1998b and Schilling *et al*., 1998) and the rate of release of greenhouse gases (Bowen and Rovira, 1973; Paterson *et al*., 1997).

Root activity can significantly alter the physiochemical properties of its surrounding

environment which in turn influences the soil microflora in diverse ways: water and nutrient uptake can induce alterations in soil pH and redox potential as well as inducing nutrient stress on the rhizosphere microbial community (Frostegard *et al*., 1993; Baath and Anderson, 2003,). Typically, the O_2 concentration is also lower close to the root, which under extreme conditions can favour anaerobic microbial processes such as de-nitrification (Ghiglione *et al*., 2000).

Rhizosphere microbial communities can considerably influence pathogen growth (Schippers *et al*., 1987; Glick, 1995; Nehl *et al*., 1997), nutrient acquisition (Lynch, 1990), heavy metal resistance (Bradley *et al*., 1981) and the environmental fitness of plants (Parker, 1995). Moreover, roots can stimulate mucilage production by the soil microbial community leading to the formation of stable aggregates (Forster, 1990).

2.5. Dissolved organic nitrogen

Dissolved organic nitrogen (DON) plays a key role in the N cycle of many ecosystems and its availability and biodegradation are an important for plant growth, microbial metabolism and N transport in soils (Schmidt *et al.*, 2011). DON constitutes a major loss pathway from agro-ecosystems through leaching (Willett *et al.*, 2004; Van kessel *et al.*, 2009). DON plays a crucial role in soil C sequestration (Knicker, 2011) and represents an important direct source of N for plants (Hill *et al.*, 2011a, and Soper *et al.*, 2011).

DON and dissolved organic carbon (DOC) play a vital role in N and C cycles in all ecosystems. DON is increasingly recognized as a pivotal pool in the soil N cycle (Ros *et at.*, 2010), and a direct N source for plants and the dominant available N form in nutrient depleted soils (Delgado-Baquerizo *et al.*, 2011). Leakage of these compounds from soil results in loss of nutrients from the ecosystem and may ultimately lead to eutrophication of surface waters. N is the factor limiting net plant production in most ecosystems (Lambers *et al.*, 1998). While our

understanding of the factors regulating the production of NH_4^+ and NO_3^- in soil are very good (Jarvis *et al.,* 1996; Murphy *et al.,* 2003), our understanding of the processes upstream of NH_4^+ production within the N cycle are much more limited. This is surprising considering that in most soils and freshwaters, DON represents the major pool of soluble N.

DON can be collected in situ from soils using, for example, a zero-tension lysimeter, rhizon sampler, or ceramic suction sampler (Murphy *et al.,* 2000). The low MW fraction of DON is often considered to be a freely diffusible source of N which can potentially be taken up by soil microorganisms; it may also be used directly by some plants. Jones *et al*. (2004) argue that the conversion of insoluble organic N to low MW DON, but not of low MW DON to NH_4^+ or NH_4^+ to NO_3^- constitutes the main constraint on supply of N in soil. Jones *et al.* (2004) also found that NO_3^- accumulates rapidly in fertile agricultural grassland soil, but that the concentrations of NH_4^+, DON, and free amino acids (TFAA) in soil solution are low and do not appear to accumulate. It follows that they neither determine nor control the N mineralization rate.

DON can be derived from various sources including dry and wet deposition, root and microbial exudation, litterfall, animal urine and faeces, the turnover of roots and organisms, and organic fertilisers added to soil (Kalbitz *et al.,* 2000) and extended from LMW compounds such as amino sugars, amino acids, urea, and purines, to high MW complexes such as proteins, DNA, and chlorophyll (Antia *et al.,* 1991). It is present as a monomer units (e.g. amino acids), however, most DON enters soil as polymers e.g. in the form of protein and peptides (Jones *et al*., 2004a). No elaborative studies on the response of amino acid concentration/turnover in soil to agriculture management regime are available (Jones *et al*., 2005b). In addition, while plants provide the primary input of DON and DOC in soil, little effect of individual grass species on DON and DOC concentrations in soil solution were found by Khalid *et al.* (2006).

DON can be viewed as a sub-pool of extractable organic N (EON) (Gjettermann *et al.,* 2008;

Qualls, 2000). Mineralization, immobilization, desorption, adsorption solubilisation and precipitation are all controlled by the quality and quantity of DON and EON in soil (Kalibitz *et al.*, 2000; Qualls, 2000). The DON concentration is affected by microbial activity more than EON. The rate of DON turnover is also faster than the turnover of the EON pool because EON is in part physically protected (Zsolnay, 2003). Ros *et al.* (2009) argue that the EON and DON pools are neither analogous nor organized by similar factors.

In the context of low-input sustainable agriculture, the role of direct uptake of DON by plants may be extremely important. However, the relative importance of DON and NH_4^+/NO_3^- (DIN) in temperate environments has been difficult to ascertain experimentally as DON fluxes in soil are complex and dynamic. At present, only uni-directional pulse label ^{15}N isotope studies have been conducted to verify that DON uptake from soil into plants can occur (Näsholm *et al*., 1998; Lipton *et al*., 1999; Owen and Jones, 2001). However, amongst other fluxes, rhizodeposition (i.e. the root release of DON) must also be considered alongside microbial-root competition/uptake for labile N.

Dissolved organic matter (DOM) is a generic term that describes both DOC and DON. It is composed of humic materials as well as other organic compounds, like amino acids, carbohydrates and hydrocarbons. DOM plays a vital role in the cycling of nutrients (e.g. N, P, C and S) within soil and the transport of these nutrients from terrestrial to marine ecosystems. Soil solution DOM is operationally defined as the range of organic molecules that can pass a 0.45 μm filter. Several researchers have also used smaller filters (0.20 μm) to split actual DOM from colloidal substance which is not retained in 0.45 μm filters (Bolan *et al*., 2004). Within soils, DOM is supposed to be the more ecological/ immediate portion of SOM, because all microbial uptake systems require a water environment. DOM is also responsible for the transportation of metals (e.g. Al, Fe) as organometallic complexes in soil. It is also a substrate for microbial growth (Cronan

and Aiken, 1985; Zsolnay and Steindler, 1991; Qualls and Haines, 1992). In cultivated soils, crop residues offer the major source of DOM whereas in forest soils, waste, plant residues and throughfall serve as the key source. Important changes in the quality and quantity of DOM take place mostly after snowmelt, in the spring after plant growth has started and in the autumn after leaf fall (Antweiler and Drever, 1983). Overall, DOM in soil solution is higher in summer in comparison to winter (DOM sorbs to several different surfaces in soil with the amount of sorption dependent on pH, nature of the surface and the average MW of DOM (sorption of DOM increases with declining pH).

In the soil, N can be present in solid, gaseous or dissolved forms. Solid forms include N bound to soil organic matter (SOM) and N associated with the soil exchange complex, while gaseous forms are e.g. NH_3 and N_2O. Dissolved forms of N may include organic or inorganic N forms (mainly NH_4^+ and NO_3^-). Large proportions of dissolved nutrients in the soil are present as organic forms (e.g. Kalbitz *et al.*, 2000; McDowell, 2003), particularly in forest ecosystems (Michalzik *et al.*, 2001).

Although the dissolved form only contributes little to total soil N (0.1-3%, Haynes, 2005), it plays a crucial role in the soil N cycle because it is mobile and may provide nutrients and energy for microorganisms (Brooks *et al.*, 1999). Dissolved organic matter (DOM) in general also contributes to nutrient and contaminant transport (Zsolnay, 2003) and is important for soil structure and formation (Stevenson, 1994; Lundström *et al.*, 1995). DON can be a substrate for microbial growth and a product of microbial activity (exudation or cell lysis) and compared to the size of the mineral N and soluble organic N (SON) pools there is a considerably large flux of N through the microbial biomass (Murphy *et al.*, 2000). Uptake by microbes results in an increase in particulate organic N (PON). Other possible pathways in the soil solution are heterotrophic nitrification to NO_3^- or direct mineralization to NH_4^+.

DON is often defined as the organic N in solution that is measured by leaching methods or suction cups (Murphy *et al.,* 2000; Zhong and Makeschin, 2003; Chen *et al.,* 2005), while water-extractable organic nitrogen (WEON) or SON is sometimes used for describing laboratory extractions of soil with water (Zsolnay, 2003; Ros *et al.,* 2009). Recently, the term extractable organic N (EON) was proposed to be used instead of SON (Xiang *et al*., 2008; Ros *et al.,* 2009) to emphasize that the solutions are obtained by extraction procedures. The reason for the need to distinguish between these forms of soluble organic N lies within the different chemical composition of these solutes. Various extractants (Ros *et al.,* 2009) as well as field methods (reviewed in Weihermüller *et al.,* 2007) yield solutes with differing chemical composition. However, the terms WEON, SON, EON and DON are not used consistently among studies. If it is clearly stated, whether soluble organic material has been derived from the field or extracted in the lab this discrimination is not essential. In this study, DON is used as term for both field and laboratory-derived solutes.

DOC, which is also part of DOM, cannot be distinguished chemically from DON, as e.g. amino acids contain both C and N in their chemical structure. There-fore, DOC can serve as a rough proxy for DON (Neff *et al.*, 2003). Many studies have found, that DOC and DON behave similarly in soils (Cleveland *et al.,* 2004), while a substantial temporal and spatial variation of DOC/DON ratios in soils has also been reported (Prechtel *et al.,* 2000). This indicates that findings on DOC are not necessarily true for DON. Depending on the chemical composition, DOC and DON can behave differently in soils and findings on DOC for one ecosystem cannot be easily transferred to DON.

In contrast to DIN, DON is not a single compound or a single class of compounds, but a structurally complex mixture of materials with very different chemical properties (Neff *et al.,* 2003). DOM consists of a small labile and a large more stable pool (Gregorich *et al.,* 2003). The

latter is of high molecular weight recalcitrant nature (Yu *et al.,* 2002), but the exact chemical composition of both DON and DOC remains unknown. Only 44% of DOC and 47% of DON in soil solution under temperate coniferous plantations could be characterized chemically in terms of total free amino-acid N, protein N and total phenolic C (Jones *et al.,* 2008).

Due to the fact that the exact chemical determination of compounds present in DOM is difficult, DOM has sometimes been described on the basis of fractionation techniques separating DOM into two operationally defined fractions, the so called hydrophilic and hydrophobic fraction (Aiken and Leenheer, 1993). The hydrophobic acid fraction includes the humic substances along with the amino acids and phosphate esters intimately bound to them, while the hydrophilic acids may consist of humic like substances with lower molecular size and higher COOH:C ratios, oxidized carbohydrates with carboxylic acid groups, low molecular size carboxylic acids, and sugar phosphates. Hydrophobic acid fraction includes lipids and some pigments, while hydrophilic neutrals may consist of simple and complex carbohydrates. The phenols (i.e., weak hydrophilic acids) include tannins and flavonoids without carboxylic acid groups. Finally, the base fraction is comprised of free amino acids and free proteins. The most abundant fractions in soils are hydrophobic and hydrophilic acids (Hongve *et al.*, 2000). The chemical composition of DON in forest soils can vary with tree species and degree of decomposition. Litter from different plant species differs not only in the composition but also in concentrations of water extractable compounds (Suominen *et al.*, 2003; Wardle *et al.,* 2003; Don and Kalbitz, 2005). In leaf litter, there are more easily degradable hydrophilic compounds such as sugars, amino acids and aliphatic acids (Hongve *et al.,* 2000), while coniferous trees have higher concentrations of tannins and other phenolics (Kraus *et al.,* 2004). These chemical differences lead to differences in decomposition rates among different substrates. During biodegradation, concentrations of water-soluble compounds decrease, while a concentration of slowly decomposable compounds increase (Berg,

2000; McTiernan *et al.,* 2003). As the layers of the forest floor represent different stages of decomposition, concentrations in water-extractable compounds also decrease with depth in organic layers (Fröberg *et al.,* 2003).

2.6. Soil microbial activity

Microorganisms play a key role in nutrient cycling by decomposition and mineralization of organic material and releasing and transforming inorganic nutrients (Marschner *et al.,* 2011). Microorganisms effect plant growth and nutrient uptake by release of growth stimulating or inhibiting substances that effect root growth (Ryu *et al.,* 2005; Govindasamy, *et al.,* 2009). Growth and activity of soil microorganisms are limited by C availability (Demoling *et al.,* 2007).

The release of root exudates results in higher microbial density and metabolic activity in the rhizosphere than the bulk soil (Soderberg and Bååth 1998). The experiment results provide evidence that microbial immobilization rather than sorption is the key factor limiting the movement of neutrally changed amino acids and peptides in soil (Abbas *et al.*, 2012). In addition, it also supports that DON loss from grazed pasture soils occur mainly in the form of recalcitrant DON compounds rather than labile low MW DON like amino acids and oligopeptides (Roberts and Jones, 2012).

SOM is composed of the "living" (microorganisms) the "dead" (fresh residues) and the "very dead" humus in various states of decomposition together with a stable humus portion (Gobat *et al*., 2004) which accounts for about one third of the soil's CEC, which increase soil aggregate strength (Brady, 1990). It has been suggested that 80-90% of reactions in soil are caused by microbes (Coleman and Crossley, 1996). In agriculture, bacteria are responsible for myriad activities that affect soil fertility and plant health including nutrient cycling, organic matter composition and decomposition, soil composition and plant growth (Kennedy, 1999). The

existence of microorganisms in the soil will depend on the number and quantity of available microhabitats and bacterial activity to the total of available metabolic substrates found in those microhabitats (Stotsky, 1997; Nannipieri *et al.*, 2003). Soil containing numerous different microhabitats generally show increased bacterial diversity. In an aggregated soil, 1g of soil typically contains several thousand microbial species (Torsvik *et al.*, 1990). The bacterial predation by bacteriophages, protozans or nematodes facilitates the re-mineralization of nutrients (Griffiths and Bardgett, 1997). Sextone *et al*. (1985) reported that regions in the soil where microbial activity is increased are called hot spots (e.g. the rhizosphere; Kuzyakov, 2002).

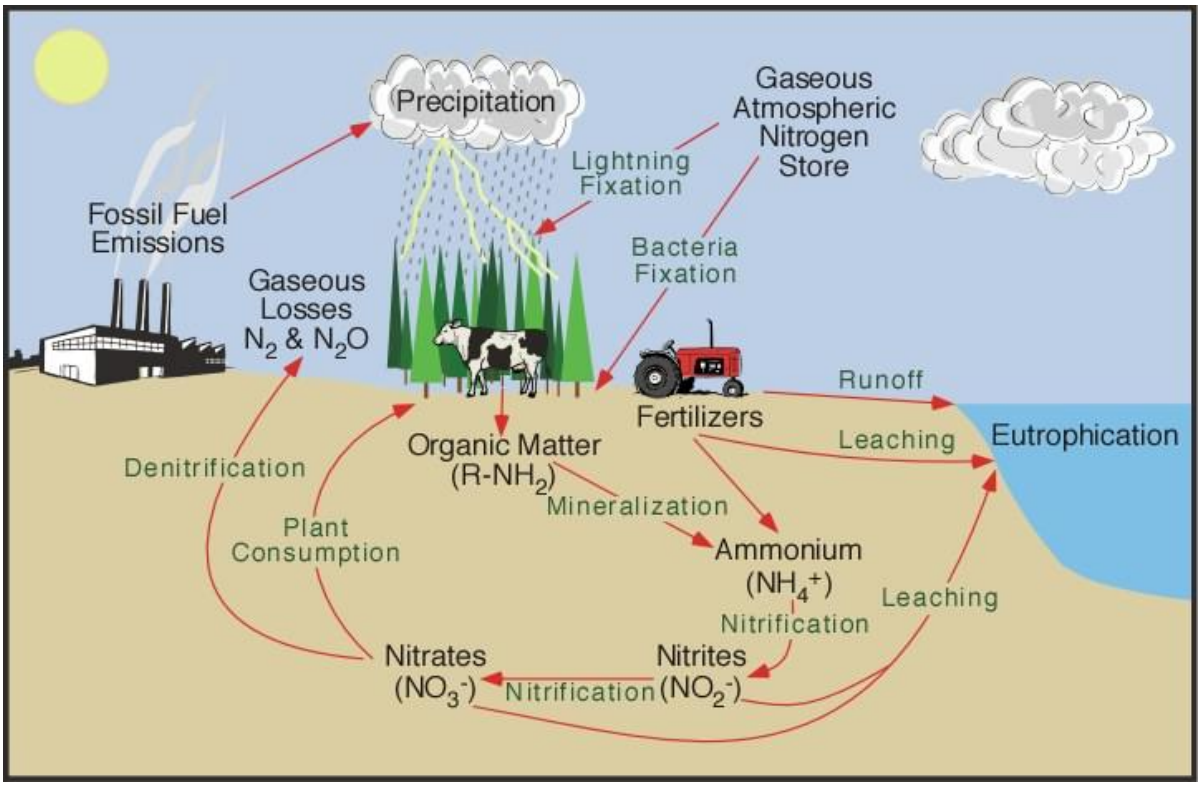

Figure 2.1. Schematic diagram illustrating the main features of the terrestrial N cycle.

2.7. Literature cited

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Chapter 3

2. Investigating the impact of plant residues on dissolved organic nitrogen cycling in soil

3.1. Introduction

Many plant species with and without mycorrhizal associations are able to take up DON directly (Öhlund and Näsholm, 2004), especially in highly N-limited ecosystems. The absorption rate of amino acids, which contribute to DON, can be as fast as or even faster than that of DIN (Persson *et al.,* 2003). Therefore, plants in such ecosystems do not need to rely on the mineralization of DON to DIN by microbes to meet their N demand. This finding also challenges the traditionally inorganic N-centered view of the terrestrial N cycle (Schimel and Bennett, 2004), but whether DON represents a significant N source for trees in temperate forests is still unknown. Jones *et al.* (2005b) concluded that root uptake of amino acids will only be of minor importance in soils with high mineralization rates or with high anthropogenic N inputs Finzi and Berthrong (2005) showed that temperate tree species took up amino acid N, NH_4^+ and NO_3^- in proportion to their presence in the soil.

Plants and microbes compete for DON and at low added concentrations, microorganisms effectively outcompete plants for amino acids in the soil (Bardgett *et al.,* 2003) while at high soil concentrations plants probably become more competitive (Jones *et al.,* 2005b). The extent, to which biotic uptake relies on DON should vary with the degree of N-limitation and DOM quality (Kaushal and Lewis, 2003; Neff *et al.,* 2003).

The ability to take up amino acids is omnipresent in microorganisms (Anraku, 1980).Soil organisms use DON as substrate (Zsolnay and Steindl, 1991; Qualls and Haines, 1992; Nelson *et*

al., 1994) but at the same time, DON is the product of microbial activity. This fact makes it difficult to quantify gross mineralization in soils. Al-though the biodegradation of DON by microorganisms is an important process in soils, it has rarely been investigated for the entire class of compounds of DON (Neff *et al.,* 2003), but rather for single compounds that are known to be part of DON, such as amino acids or amino sugars (Jones, 1999; Vinolas *et al.,* 2001; Roberts *et al.,* 2007). In contrast, more information is available on the biodegradability of DOC (Yano *et al.,* 2000; Kalbitz *et al.,* 2003; Marschner and Kalbitz, 2003; Qualls, 2005; Schwesig *et al.,* 2003; Don and Kalbitz, 2005), which can serve as a rough proxy for DON (Neff, *et al.,* 2003). This assumption is supported by laboratory studies that found DON not to decay faster than DOC (Cleveland *et al.,* 2004; Kiikkilä *et al.,* 2005). However, in agricultural soils and aquatic systems, the biodegradation of DON was often greater than that of DOC (Gregorich, *et al.,* 2003; Wiegner and Seitzinger, 2004; Kaushal and Lewis, 2005; Petrone *et al.,* 2009), which in turn supports the idea, that findings on DOC cannot be transferred to DON.

For forest soils, there are only few results concerning DON Biodegradation. Kiikkilä *et al.* (2005) showed that DON biodegradation was related to tree species and was higher in water extracts of coniferous than deciduous species. They also added NH_4Cl to their samples to investigate the effect of mineral N in solution, but as their samples were pre-incubated, conclusions regarding the actual degradability of DON are difficult to draw.

There are several measures for DOM biodegradability, e.g. the difference in concentration before and after incubation (e.g. Kiikkilä *et al.,* 2005), CO_2 production during the incubation time in the headspace of incubation flasks (for DOC, Kalbitz *et al.,* 2003). In this study, the term biodegradation is referred to as the sum of mineralization (break-down of organic compounds to obtain energy and nutrients) and microbial uptake. While biodegradable DON is defined as the quantity of DON that is actually utilized by microorganisms, bioavailability only describes the

ingestion and retention of organic compounds (Marschner and Kalbitz, 2003).

DON represents a significant pool of soluble N in most ecosystems, but little is known about the factors that control its concentration and fate in soil in comparison to dissolved inorganic N (NO_3^- and NH_4^+) (Murphy *et al.,* 2000; Neff *et al.,* 2003; Christou *et al.,* 2005). DON represents the substrate from which NH_4^+ and ultimately NO_3^- is produced in soil. DON concentrations show little seasonality, while NO_3^- concentrations vary dramatically with temperature and, consequently, biological activity (Willett *et al.,* 2004).

Among these, root exudation and turnover are the most dominant in agricultural systems where inorganic fertilisers are added (Casper *et al.,* 2003). DON typically constitutes between 30-40% of soluble N in agricultural soils (Christou *et al.,* 2005).

The major amino acids (AA) that are the source of DON are: alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine, and valine. Plants can only take up low MW DON in the form of amino acids and peptides (Jones *et al.,* 2005a) implying that most of the soil's DON is not plant-available. Amino acid turnover in soil has been shown to be rapid in the field as well as in laboratory assays. The low concentrations of amino acids in soil solution may be due to slow rates of production or high rates of microbial consumption. This rapid rate of microbial assimilation of amino acids is supported by the high concentrations of NO_3^- and lack of NH_4^+ in grassland soil solutions which have suggested that the rate of ammonification and nitrification is rapid (Jones *et al.,* 2005b). Owen and Jones, (2001), Bardgett *et al.* (2003) and Jones *et al.,* (2005c) have reported that a strong competition exists between roots and the soil microbes for amino acid N, especially when amino acid concentrations are low in soil. Plants can better compete for NO_3^- with microbes, and thus it is likely that plants are more dependent on inorganic NO_3^- than amino acids as a source of N, as NO_3^- diffuses faster than amino acids in most soils (Wilson *et al.,* 1988; Hunter and Ruffner, 1997;

Jones *et al.*, 2005a).

The rhizosphere is the zone of soil surrounding the roots (up to a radial distance of ca. 2 mm), and plays an important role in DON availability to plants, and competition for DON between roots and microorganisms. Roots release a great variety of organic substances from roots into the soil (termed root exudates or rhizodeposition), which either directly or indirectly influences the quality and quantity of microorganisms and DON in the root region. Enzymatic activity of plant roots and the surrounding microbial community is also high in this zone, often resulting in an accelerated breakdown of SOM.

Soil organic matter (SOM) plays an important role in ecosystems by retaining and supplying plant nutrients, improving soil aggregation, reducing soil erosion, and enhancing water holding capacity (Brady and Weil, 2002). Grassland soils are generally thought to be rich in organic matter, but poor management, vegetation shifts, and changes in climate have decreased SOM stocks in many of the world's grasslands (Asner *et al.,* 2004; Bai *et al.,* 2008). The large global extent of grasslands with depleted organic matter stocks has focused attention on the management of these ecosystems for C sequestration to help mitigate climate change (Asner *et al.,* 2004; Bai *et al.*, 2008; Conant *et al.*, 2011; Follett, 2001; Lipper *et al.*, 2010; Morgan *et al.*, 2010). Management induced soil C sequestration has been studied in agricultural systems in the context of conservation tillage, cropping, organic and synthetic fertilization, and residue incorporation (Lal, 2002; Kaiser *et al.,* 2007; Kong *et al.,* 2009; Lugato *et al.,* 2010). In contrast, information regarding the impact of grassland management on soil C sequestration is much more limited (Conant *et al.,* 2001; Follett, 2001; Lal, 2002; Derner and Schuman, 2007).

Organic matter amendments have been proposed as a means to increase C storage in soils (Cabrera *et al.,* 2009; Powlson, *et al.,* 2012); this can occur directly from the C inputs in the amendment and indirectly from increased plant production (Ryals and Silver, 2013). Organic

matter amendments to soils have been related to enhanced soil water holding capacity (Pandey and Shukla, 2006), decreased bulk density (Lynch *et al.,* 2005), and improved soil fertility (Mader *et al.,* 2002). Amendments may also increase organic N in soils, which could act as a slow release fertilizer enhancing net primary productivity (Ryals and Silver, 2013). Furthermore, organic matter amendments could provide opportunities for greenhouse gas offsets if materials are diverted from high emissions sources, such as food waste from landfills (Powlson *et al.,* 2012; DeLonge *et al.,* 2013).

However, the fate of organic matter amendments in grassland soils remains unclear. Most organic matter amendments are applied to the soil surface. The proportion of this material that is incorporated and retained in soils over time is unknown, but is likely a function of the chemical composition of the material added and the specific soil and environmental conditions of the site. Several mechanisms can act to stabilize added C and N including physical protection via soil aggregation by, for example, microbial production of binding agents in the course of organic matter decomposition (Gulde *et al.,* 2008). Detecting and interpreting changes to soil C and N pools can be difficult, particularly in ecosystems with relatively large standing stocks of bulk soil C and N. If organic matter that is applied to the surface is not sorted or separated when soils are sampled and analyzed, increases in soil C and N stocks may be greatly exaggerated.

Turnover of SOM is governed, in part, by the accessibility of organic substrates to decomposers (Dungait *et al.,* 2012), and also by the chemical quality of the material (Jastrow *et al.,* 2007; Conant *et al.,* 2011). Assessments of SOM chemical characteristics are commonly used to infer its potential reactivity (Kögel-Knabner *et al.,* 2008). By combining physical fractionation and chemical characterization, it is possible to identify fractions with different SOM stabilization potentials (Sohi *et al.,* 2001; Poirier *et al.,* 2005) to assess their relevance for long-term soil C and N storage.

3.1.1. Objectives of the study

Plant residues are frequently added to agricultural soils from the turnover of roots, ploughing in of crop residues and green manures or during re-seeding of grasslands. This represents a major addition of organic N to the soil and I hypothesize that it will cause a large increase in soil DON concentrations. The aim of this study was therefore to investigate the impact that incorporating plant residues into soil has on DON cycling.

3.2. Materials and methods

3.2.1. Experimental site description

The study site was located in Abergwyngregyn, Gwynedd, North Wales (53°14' N, 4°01'), and consisted of ungrazed, temperate oceanic agricultural grassland. The mean annual air temperature is 11°C and the mean annual rainfall is 1250 mm. The soil is classified as a fine, loamy textured brown earth derived from glacial till of Ordovician origin. The grass sward predominantly consists of perennial ryegrass (*Lolium perenne* L.) and clover (*Trifolium repens* L.), and the site is subject to continuous sheep grazing (ca. 10 ewe ha^{-1}).

3.2.2. Soil and plant sample collection

Soil samples (ca. 1 kg) were taken from the surface Ah horizon (0-15 cm) of the Eutric cambisol (Fig 3.1a), while *Lolium perenne* shoot samples were collected from three randomized sites at the same location. A bulk air-dry sample of barley (*Hordeum vulgare* L.) straw grown the previous year at the site was taken from the store (Fig. 3.1b). Soil was removed using a spade and stored in CO_2 permeable polypropylene bags for immediate transport to the laboratory. In the laboratory, the soil was sieved (<5 mm) to remove earthworms, above-ground vegetation and large masses of

roots. The soil was then sub-divided into three bags for use in the experiments (i.e. essentially pseudoreplicates of one large bulk soil sample). The field-moist soil and grass and air-dry straw were stored at 4°C prior to use.

3.2.3. Decomposition study

Three experimental treatments were used to alter the quality and quantity of organic matter inputs to soil. These included: (1) soil amended with grass shoots (low C/N ratio), (2) soil amended with barely straw (high C/N ratio), and (3) non-amended soil (control).

Prior to experimentation the grass and straw samples were cut into 1 cm lengths by hand. Replicate batches of soil (100 g) contained in polyethylene bags were then amended with either 5 g of grass or straw and mixed by hand. The samples were then placed in the dark at 10°C. After incubation for either 0, 1, 7, 14, 21, or 28 d, individual bags were removed and soluble N quantified as described below. Residue moisture content were determined by oven drying (80°C, 24 h).

3.2.4. Soil solution extraction

Soil solution was extracted by the centrifugal-drainage technique of Giesler and Lundström (1993). Briefly, soil from individual replicates was centrifuged (30 min, 4000 rev min^{-1}) enabling the recovery of 10-15 ml of soil solution representing 30-40% of the soil water. The soil solutions were then stored frozen at -20°C in polyethylene vials for further chemical analysis.

3.3. Soil and solution analysis

Soil moisture content was determined gravimetrically after oven drying (105°C, for 24 h). Maximum water holding capacity (MWHC) was determined by saturating the soils for 24 h and

then measuring the moisture content as described previously. Soil pH and electrical conductivity (EC) were determined in a 1:1.25 (w/v) soil: distilled water extracts using an Orion 410A pH meter calibrated with pH 4 and 7 buffers every 20 samples and with every 10 sample replicated. pH was measured for both topsoil samples and sub soil samples. And Jenway 4520 conductivity meter (Jenway, Dunmow, UK).

Soil solution NH_4^+ was determined colorimetrically using a PowerWave-XS® microplate spectrometer at 667 nm (Bio-Teck Instruments Inc, Miami, FL, USA) according to the salicylate-nitroprusside, Na_2EDTA, and Na-hypochlorite method of Mulvaney (1996). The principle of this assay is reduction of nitrate by vanadium (III) combined with detection by the acidic Griess reaction. This assay is sensitive to 0.5 mM NO_3^- and is useful in a variety of fluids including cell culture media, serum, and plasma. S-Nitrosothiols and L-arginine derivatives were found to be potential interfering agents. However, these compounds are generally minor constituents of biological fluids relative to the concentration of nitrate/nitrite. This report introduces a new, convenient assay for the stable oxidation products of nitrogen oxide chemistry in biological samples. NO_3^- was determined colorimetrically on the same analyzer at 540 nm using the vanadium reduction methods of Miranda *et al.* (2001) detail of methods are given below:

REAGENTS

- Prepare saturated VCl_3 solution
- Dissolve 400 mg VCl_3 in 50 ml of 1 M HCl
- Remove excess solid with nylon syringe filter
- Store in the dark at 4^0C for max. of 2 weeks
- Prepare Greiss reagent
- 0.1% (w/v) NEDD in H20 = 0.02g NEDD in 20 ml water
- 2% (w/v) Sulfanilamide in 5% HCl or H_3PO_4 (v/v) = 0.4g Sulfanilamide in 20 ml 5% HCl
- Store in the dark at 4^0C for max of 2 weeks

METHOD

- Add 100 µl sample or standard to each well
- Add 100 µl VCl_3
- NB: Step 3 & 4 can be done together by mixing equal quantities of NEDD & Sulfanilamide in a reagent well and adding 100 µl of the combined Greiss reagent. Dispose of after use.
- Add 50 µl of NEDD
- Add 50 µl of Sulfanilamide and mix
- Allow pink colour to develop for ~15 mins at room temperature.
- Read absorbance at 540 nm on the plate- reader.
- If values obtained for samples are greater than those of the top standard, dilute samples as appropriate and repeat from step 1.

Calculations:

- Prepare a standard curve from recorded reading (absorbance vs. concentration) of standard and read as µg NH_4^+N ml-1 in extract. Results are calculated as follows:
- NO_3^-N in moist soil (µg g-1) = NO_3^-N in extract µg ml-1) x volume of extractant (ml)/weight of moist soil (g)
- Moisture factor = Moist soil (g)/Oven-dried soil (g)
- NO_3^-N in oven-dried soil (µg g-1) = NO_3^-N in moist soil (µg g-1) x moisture factor

Soluble P was determined using the spectrophotometric molybdate blue-ascorbic acid method of Murphy and Riley (1962).

Dissolved organic C (DOC) and total dissolved N (TDN) were determined with a TOCV-TNM1 (Shimadzu Crop., Japan; Fig. 2.1c). The analyzer injects 50 µl of soil solution into a combustion furnace held at 720°C, after which the CO_2 produced is detected by an IR detector and the N_2O using a chemiluminescence detector. The limit of detection for C and N in the samples was 50 µg l^{-1}. DON was calculated from the difference between TDN and the combined NH_4^+ and NO_3^- reading. Total free amino acid concentrations were determined according to the β-

mercaptoethanol-*o*-phthialaldehyde fluorometric procedure of Jones *et al.* (2002). Individual free amino acids were determined by gas chromatography using an EZ: faast^{TM} kit (Phenomenex Inc, California).

Total soluble phenolic concentration was determined according to the methodology of Swain and Hillis (1959) using the Folin-Ciocalteu reagent calibrated with a phenol standard. The procedure for total phenol analysis in freshwaters, soil solutions and soil extracts.

Extraction procedure

- Weigh out 10 g of soil
- Add 10 ml of distilled water
- Shake for 30 mins
- Centrifuge 1.5 ml in eppendorf high speed centrifuge at 18,000g
- Retain supernatant for analysis

Assay procedure for spectrophotometer

- Make up some saturated sodium carbonate (Na_2CO_3). To do this keep adding solid Na_2CO_3 to distilled water until no more will dissolve. Store at room temperature. Crystals will appear over time, however, this is nothing to worry about as long as you take the liquid layer off the top for analysis.
- Add 240 µl of your sample or standard to an eppendorf
- Add 15 µl of Folin and Ciocalteau's Reagent (stored in fridge: made by Sigma)
- Let stand for 5 minutes
- Add 30 µl of saturated sodium carbonate solution (Na_2CO_3)
- Mix well and let stand for 5 mins.
- Spin at 14,000 g for 5 mins

- Add 200 µl to a microplate well
- A blue colour should develop if you have phenols. Blanks should go colourless. Read absorbance at 725 nm
- Prepare standards with phenol in the following range (0-20 µg ml). The solid phenol is stored on the shelf in the chemical store. Be careful not to spill this when weighing out as it is pretty toxic. It is best to make up a 2 mg ml-1 stock first (i.e. 0.04 g phenol to 20 ml of distilled water) which can then be stored in the fridge for a long time. To make up 5 mL of each standard solution make the following dilutions into 20 mL polypropylene scintillation vials:

Method reference

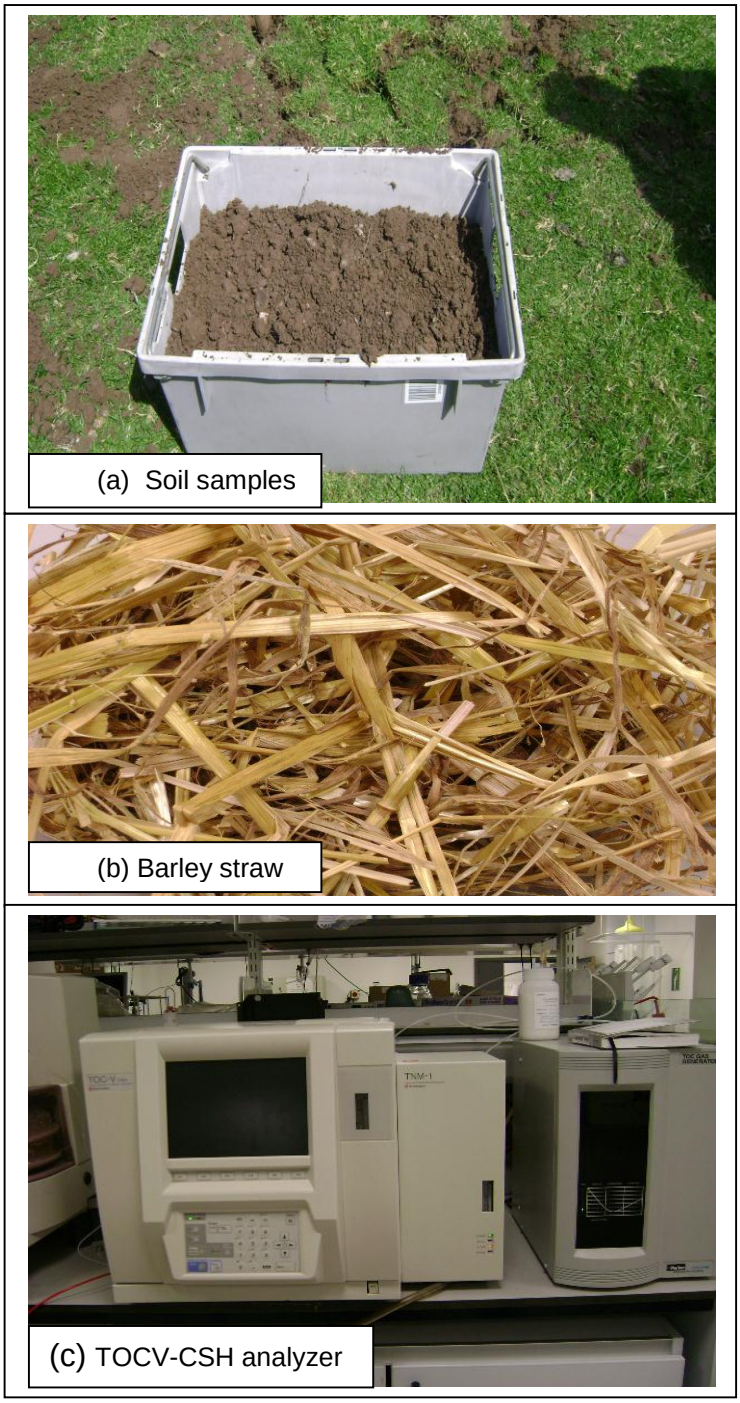
Swain, T., Hillis, W.E. 1959. The phenolic constituents of Prunus domestica. I. The quantitative analysis of phenolic constituents. Journal of Agriculture and Food Science **10:** 63‑68.

To support the phenolic measurements, the absorbance of the soil solution in the UV (200 nm) and visible range (400 nm) were made on the microplate reader. The latter provides a measure of the brown coloration of solution and is used as an indicator for the presence of humic substances.

3.4. Statistical analysis

Statistical analysis (linear regression, ANOVA with Tukey post-hoc comparison) was undertaken with SPSS v14 (IBM Inc., Portsmouth, UK) using $P < 0.05$ as the level for statistical significance. Graphs were prepared with SigmpPlot v 8.0 (Systat Software Inc., San Jose, USA).

Figure 3.1. Photographs of the Eutric Cambisol soil (panel a), the barley straw used in the experiment (panel b), and the TOCV-CSH analyzer used to measure DOC and TDN (panel c).



3.5. Results

3.5.1. Soil solution N dynamics

Results indicated that both soil solution NO_3^- and NH_4^+ accumulated in the grass shoot-amended soil in contrast to that amended with either straw or in the un-amended control soil (Figs. 3.1. and 3.2). Overall, the straw-amended soil immobilized DIN in solution. DON in the grass shoot amended soil increased from day 14 to 21 but sharply decreased thereafter whilst the straw amended soil and control DON concentrations remained relatively constant throughout the duration of the experiment. Contrary to expectation, the results indicate that addition of organic matter did not cause a large rise in DON relative to that of DIN. This suggested that in this high fertility agricultural soil the microorganisms rapidly mineralize DON contained in N rich organic residues to DIN. For N poor residues DON appears to represent a more important source of N, however, its availability to plants remains as yet unknown.

Figure 3.1 provides clear evidence for the rapid rate of nitrification in this soil with NO_3^- concentrations being many folds higher than that of NH_4^+ after 28 d when incubated with grass shoots. The results also provides strong evidence showing that the rate of ammonification is also rapid in this soil but that the effect is hidden due to the rapid microbial conversion of NH_4^+ to NO_3^-. Contrary to expectation, the low amount of nitrate in the straw treatment is consistent with ammonium immobilization and the lack of substrate for nitrification. Comparison to the control also indicated that the straw treatment also induced immobilization of nitrate by the microbial community.

The results presented in Figure 3.3 indicate that addition of organic matter did not cause a large rise in DON relative to that of DIN. This suggests that in this high fertility agricultural soil the microorganisms rapidly break down DON contained in N rich organic residues leading to the production of DIN.

Further, it was noticed that there was significant variation between treatments for DON, NO_3^- and NH_4^+ concentrations in soil solution (Table 3.1). Multiple Tukey pairwise comparisons showed significant differences in DON concentrations between the Control treatment and the Grass and Straw treatments and between the Grass and Straw treatments (Table 3.2). Regarding NO_3^-, significant differences were apparent between the Control and the Straw treatment and between the Grass and Straw treatments. Similarly for NH_4^+, significant variation was observed between the Grass and the Control and Straw treatments (Table 3.2).

3.5.2. Other changes in soil solution

It was apparent from the results of the analysis of variance that there were highly significant variations for the pH, EC, NH_4^+, NO_3^-, TN, DIN, DON and MC, DOC and Phosphorus (Table 3.1). pH showed significant variation between Control with Grass and Grass with Straw (Table 3.3). Multiple comparisons were undertaken and the results showed that significant variation in EC was observed between Control with Grass and Grass with Straw. Significant variation was observed between Control with straw and Grass and Grass with Straw for TN. Similarly for DOC, the Control treatment showed significant variation with straw and Grass with Control and straw.

Figure 3.4 shows that with incubation time the control treatment has maintained a relatively constant pH. The pH in the straw treatment had a linear decreasing trend with increasing incubation time. The grass treatment also showed a linear decreasing trend like straw, which was less acidic as compared to straw. It is observed from the Figure 3.5 that EC of grass amended soil was remained unchanged till first day and then increased gradually and reached maximum until day 21, after which it decreased sharply. Moisture content was higher in straw amended soil on day one after incubation and decreased on day 7 to day 14 thereafter remain constant. In case of grass amended soil moisture content gradually increased. In control moisture content increased on day

1 and remained same up to14 afterward it decreased (Fig. 3.6). For further detail see appendix 7.1.

pH of soil solution from three treatments decreased after 28 days of incubation time. From a One-Way ANOVA, we see that after 28^{th} days of experiment pH of soil solution from control, grass and straw differ significantly ($p = 0.004$). Further post hoc test (Tukey) showed that pH of grass solution is significantly lower than control ($p = 0.023$). Although soil solution pH within the straw treatment was higher than control, the variation was not significant ($p = 0.223$). On the other hand, pH of the straw treatment was higher than in the grass treatment by 1.5 units (highly significant, $p = 0.003$).

Electrical conductivity (EC) of soil solution from three treatments decreased after 28 days of incubation. From a One-Way ANOVA, we see that after 28^{th} day of experimentation, the EC of soil solution from control, grass and straw differ highly significantly ($p < 0.001$). Further post hoc test (Tukey) showed that EC of grass solution is significantly higher than the control treatment ($p < 0.001$). Although the EC of the straw treatment was lower than the control, the difference was not significant ($p = 0.297$). On the other hand, EC of the straw treatment was lower than the corresponding grass treatment (highly significant, $p < 0.001$).

Moisture content (MC) of soil solution from the three treatments decreased after 28 days of incubation. From a One-Way ANOVA, we see that after the 28^{th} day of experimentation MC of soil solution from control, grass and straw treatments differ significantly ($p = 0.045$). Further post hoc test (Tukey) showed that MC of grass soil solution was significantly lower than in the control treatment ($p = 0.080$). Although MC of straw soil solution was lower than control, the difference proved non-significant ($p = 0.951$). On the other hand, MC of straw soil solution was significantly lower than for the grass ($p = 0.054$).

Ammonium of soil solution from three treatments decreased after 28 days of incubation. From a One-Way ANOVA, we see that after 28 days of experimentation NH_4^+ of soil solution

from control, grass and straw differ significantly ($p = 0.007$). Further post hoc test (Tukey) showed that NH_4^+ of grass solution was significantly lower than the control treatment ($p = 0.006$). Although NH_4^+ of straw was higher than the control, the difference was not significant ($p = 0.271$). On the other hand, NH_4^+ of straw was lower than grass (highly significant, $p = 0.003$).

Nitrate of soil solution from three treatments decreased after 28 days of incubation. From a One-Way ANOVA, we see that after 28 days of experimentation, the NO_3^- concentration in soil solution from control, grass and straw differed significantly ($p < 0.001$). Further post hoc test (Tukey) showed that NO_3^- of grass solution was significantly lower than the control ($p < 0.001$). The NO_3^- of straw soil solution was lower than the control, the variation being highly significant ($p = 0.007$). On the other hand, NO_3^- of straw was lower than grass ($p < 0.001$).

Total nitrogen of soil solution from three treatments decreased after 28 days of incubation. From a One-Way ANOVA, we see that after 28 days of experimentation, TN of soil solution from control, grass and straw differ highly significantly ($p < 0.001$). Further post hoc test (Tukey) showed that TN of grass solution is significantly different to the control ($p < 0.001$). In addition, the TN of straw was lower than the control ($p = 0.033$). On the other hand, TN of straw was lower than grass ($p < 0.001$).

Dissolved inorganic nitrogen of soil solution from three treatments decreased after 28 days of incubation. From a One-Way ANOVA, we see that after 28 days of experimentation, DIN of soil solution from control, grass and straw differ highly significantly ($p < 0.001$). Further post hoc test (Tukey) showed that DIN of grass solution is significantly higher than the control treatment ($p < 0.001$). The DIN of straw was lower than control, the variation was highly significant ($p = 0.007$). On the other hand, DIN of straw was lower than grass (highly significant, $p = 0.001$).

Dissolved organic nitrogen of soil solution from three treatments decreased after 28 days of incubation. From a One-Way ANOVA, we see that after 28 days of experimentation DON of

soil solution from control, grass and straw differed significantly ($p = 0.004$). Further post hoc test (Tukey) showed that DON of grass solution is significantly higher than the control ($p = 0.005$). Although DON of straw was higher than the control, the variation was not significant ($p = 0.525$). On the other hand, DON of straw was lower than grass ($p = 0.033$).

Dissolved organic carbon of soil solution from three treatments decreased after 28 days of incubation time. From a One-Way ANOVA, we see that after 28 days of experimentation DOC of soil solution from control, grass and straw differ significantly ($p = 0.018$). Further post hoc test (Tukey) showed that DOC of grass solution is significantly higher than control ($p = 0.959$). The DOC of straw was significantly higher than the control ($p = 0.024$). The DOC of the straw treatment was higher than the grass treatment (significant, $p = 0.033$).

Phosphorous of soil solution from three treatments decreased after 28 days of incubation time. From a One-Way ANOVA (Table 3.1), we see that after 28 days of experimentation P of soil solution from control, control, grass and straw differ highly significantly ($P < 0.001$) (Table 3.3). Further post hoc test (Tukey) showed that P of grass soil solution was significantly higher than the control ($P = 0.001$). The P concentration in the straw treatment was also higher than in the control ($P < 0.001$). On the other hand, P of straw was not significantly higher than in the grass treatment ($P = 0.469$).

Amino acids results are presented in Table 3.4 which reveals that there is no significant variation for amino acids except two amino acids i.e. (GLY and HYL) that showed significant difference at $P < 0.05$ level of probability.

3.5.3. Relationship between measured parameters

pH showed strong significant negative correlation between EC ($r=-0.712$), NO_3^- ($r=-0.486$)

and DON (r=-0.779) (Table 3.2). EC showed a strong significant positive correlation with NO_3^- (r=0.841), NH_4^+ (r=0.470), and DON (r=0.972). NO_3^- showed significant association with NH_4^+ and DON (r=0.336 and 0.523, respectively). NH_4^+ showed a significant correlation DON (r=0.393).

Table 3.1. Significant differences in a range of soil quality indicators after incubation of straw, grass residues in soil for up to 28 d in comparison to an un-amended control soil treatment. From One-Way analysis of variance (ANOVA) and mean performance of different components. * indicates p < 0.001, ** indicates p < 0.01 and * indicates p < 0.05. NS indicates no significant difference (p > 0.05).**

		Sum of Squares	df	Mean Square	F	Sig.
pH	Between groups	3.591	2	1.795	16.361	**
	Within groups	0.658	6	0.11		
EC	Between groups	5.1E+07	2	2.6E+07	320.198	***
	Within groups	479415	6	799002		
MC	Between groups	322.3	2	161.15	5.455	*
	Within groups	177.259	6	29.543		
NH_4^+	Between groups	0.784	2	0.392	12.565	**
	Within groups	0.187	6	0.031		
NO_3^-	Between groups	482482	2	241241	292.587	***
	Within groups	4947.06	6	824.51		
TN	Between groups	587564	2	293782	233.502	***
	Within groups	7548.94	6	1258.16		
DIN	Between groups	483531	2	241766	291.695	***
	Within groups	4972.99	6	828.831		
DON	Between groups	5898.79	2	2949.4	15.382	**
	Within groups	1150.43	6	191.739		
DOC	Between groups	51211.2	2	25605.6	8.443	NS
	Within groups	18197.2	6	3032.86		
Phosphorus	Between Groups	4880	2	2440	50.165	**
	Within Groups	292	6	48.64		

Table 3.2. Summary table showing the Tukey ANOVA multiple comparison results comparing the three different treatments (Control, Grass and Straw amended soil).

Dependent Variable	(I) Treatment	(J) Treatment	Sig.
NH_4^+	Control	Grass	0.006
		Straw	0.271
	Grass	Control	0.006
		Straw	0.042
	Straw	Control	0.271
		Grass	0.042
NO_3^-	Control	Grass	0.001
		Straw	0.007
	Grass	Control	0.001
		Straw	0.001
	Straw	Control	0.007
		Grass	0.001
		Grass	0.001
DON	Control	Grass	0.005
		Straw	0.525
	Grass	Control	0.005
		Straw	0.015
	Straw	Control	0.525
		Grass	0.015
DOC	Control	Grass	0.959
		Straw	0.024
	Grass	Control	0.959
		Straw	0.033
	Straw	Control	0.024
		Grass	0.033

Table 3.3. Pairwise comparisons with Tukey between the three different treatments to show significance differences (Control, Grass and Straw amended soil).

Dependent Variable	(I) Treatment	(J) Treatment	Sig.
pH	Control	Grass	0.023
		Straw	0.223
	Grass	Control	0.023
		Straw	0.003
	Straw	Control	0.223
		Grass	0.003
EC	Control	Grass	0.001
		Straw	0.297
	Grass	Control	0.001
		Straw	0.001
	Straw	Control	0.297
		Grass	0.001
MC	Control	Grass	0.800
		Straw	0.951
	Grass	Control	0.080
		Straw	0.054
	Straw	Control	0.951

Table 3.3. (Continued)

Dependent Variable	(I) Treatment	(J) Treatment	Sig.
TN	Control	Grass	0.001
		Straw	0.033
	Grass	Control	0.001
		Straw	0.001
	Straw	Control	0.033
		Grass	0.001
DIN	Control	Grass	0.001
		Straw	0.007
	Grass	Control	0.001
		Straw	0.001
	Straw	Control	0.007
P	Control	Grass	0.001
		Straw	0.000
	Grass	Control	0.001
		Straw	0.469
	Straw	Control	0.000

Table 3.4. Significant differences in a range of soil quality indicators after incubation of straw, grass residues in soil for up to 28 d in comparison to an un-amended control soil treatment. From a One-Way analysis of variance (ANOVA) of different amino acids. * indicates $p < 0.05$. NS indicates no significant difference ($p > 0.05$).

		SS	df	MS	F	Sig.
ALA	Between Groups	0.000	2	0.000	0.000	1.000
	Within Groups	0.000	24	0.000		
SAR	Between Groups	0.000	2	0.000	0.125	0.883
	Within Groups	0.004	24	0.000		
GLY	Between Groups	0.001	2	0.001	4.000	0.032
	Within Groups	0.004	24	0.000		
ABA	Between Groups	0.000	2	0.000	0.000	1.000
	Within Groups	0.000	24	0.000		
VAL	Between Groups	0.026	2	0.013	1.163	0.330
	Within Groups	0.273	24	0.011		
b-VAL	Between Groups	0.000	2	0.000	0.000	1.000
	Within Groups	0.000	24	0.000		
IS	Between Groups	0.000	2	0.000	.	.
	Within Groups	0.000	24	0.000		
LEU	Between Groups	0.000	2	0.000	0.000	1.000
	Within Groups	0.000	24	0.000		
aILE	Between Groups	0.000	2	0.000	0.000	1.000
	Within Groups	0.000	24	0.000		

Table 3.4. (Continued)

ILE	Between Groups	0.000	2	0.000	0.000	1.000
	Within Groups	0.000	24	0.000		
THR	Between Groups	0.000	2	0.000	0.000	1.000
	Within Groups	0.000	24	0.000		
SER	Between Groups	0.000	2	0.000	0.000	1.000
	Within Groups	0.000	24	0.000		
PRO	Between Groups	0.000	2	0.000	0.000	1.000
	Within Groups	0.000	24	0.000		

Table 3.4. (Continued)

		SS	df	MS	F	Sig.
ASN	Between Groups	0.000	2	0.000	0.000	1.000
	Within Groups	0.000	24	0.000		
TPR	Between Groups	0.000	2	0.000	0.000	1.000
	Within Groups	0.000	24	0.000		
ASP/MET	Between Groups	0.064	2	0.032	0.585	0.565
	Within Groups	1.309	24	0.055		
HYP	Between Groups	0.000	2	0.000	0.000	1.000
	Within Groups	0.000	24	0.000		
GLU/PHE	Between Groups	0.000	2	0.000	0.870	0.432
	Within Groups	0.002	24	0.000		
AAA	Between Groups	0.000	2	0.000	0.000	1.000
	Within Groups	0.000	24	0.000		
APA	Between Groups	0.000	2	0.000	0.000	1.000
	Within Groups	0.000	24	0.000		
APA/GLN	Between Groups	0.000	2	0.000	0.000	1.000
	Within Groups	0.000	24	0.000		
ORN/GPR	Between Groups	0.001	2	0.001	0.967	0.395
	Within Groups	0.016	24	0.001		
LYS	Between Groups	1.394	2	0.697	1.280	0.296
	Within Groups	13.068	24	0.545		

Table 4.4. (Continued)

HIS	Between Groups	0.002	2	0.001	0.337	0.717
	Within Groups	0.063	24	0.003		
HYL	Between Groups	0.031	2	0.015	3.632	0.042
	Within Groups	0.101	24	0.004		
TYR	Between Groups	0.001	2	0.001	2.626	0.093
	Within Groups	0.006	24	0.000		
PHP	Between Groups	0.000	2	0.000	0.000	1.000
	Within Groups	0.000	24	0.000		
TRP	Between Groups	0.000	2	0.000	0.000	1.000
	Within Groups	0.000	24	0.000		
CTH	Between Groups	0.000	2	0.000	0.000	1.000
	Within Groups	0.000	24	0.000		
C-C	Between Groups	0.000	2	0.000	0.000	1.000
	Within Groups	0.000	24	0.000		

Table 3.5. Correlation between soil pH, EC, moisture content (MC), NO_3^-, NH_4^+, DOC and DON across all three experimental treatments (control, straw and grass). * and ** indicate significant relationships at the P < 0.05 and P < 0.01 level respectively.

	EC	MC%	NO_3^-	NH_4^+	DOC	DON
pH	-0.712**	-0.288*	-0.486**	-0.206	0.264	-0.779**
EC		0.376**	0.481**	0.470**	-0.261	0.972**
MC%			0.273*	0.006	-0.066	0.392**
NO_3^-				0.366**	-0.109	0.523**
NH_4^+					0.13	0.384**
DOC						-0.322*

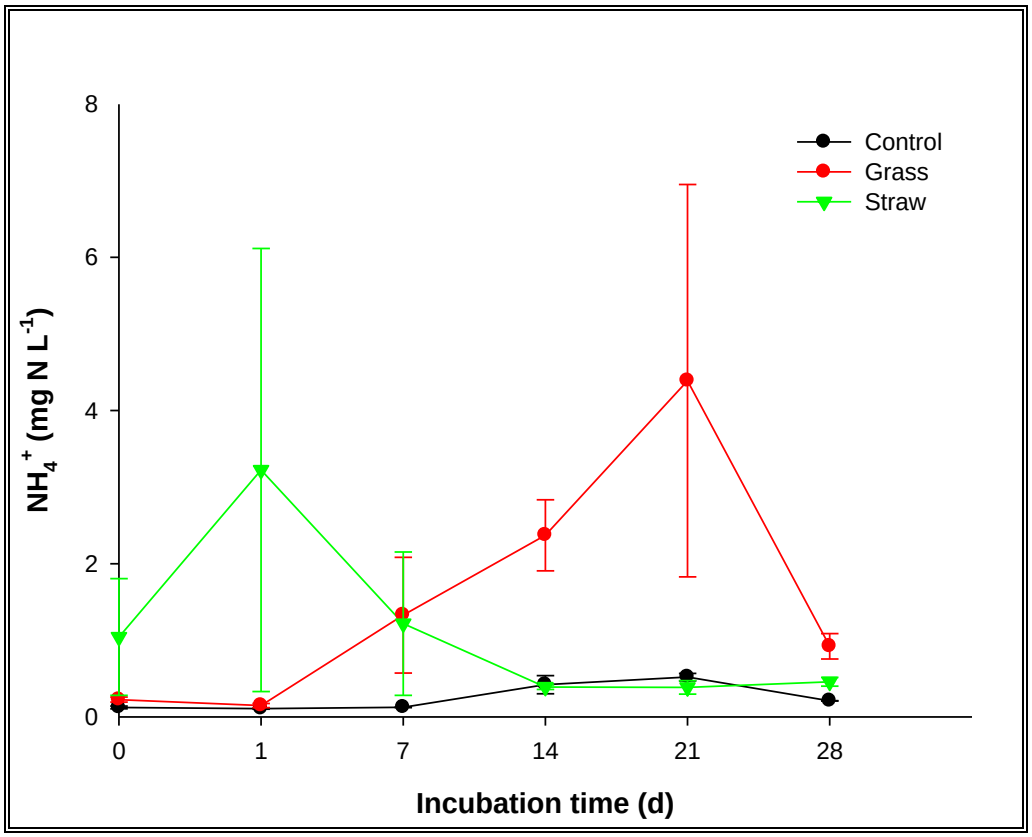


Figure 3.1. Soil solution NH_4^+ concentrations during the incubation of grass and straw in an agricultural grassland soil. Values represent means ± SEM (*n* = 3).

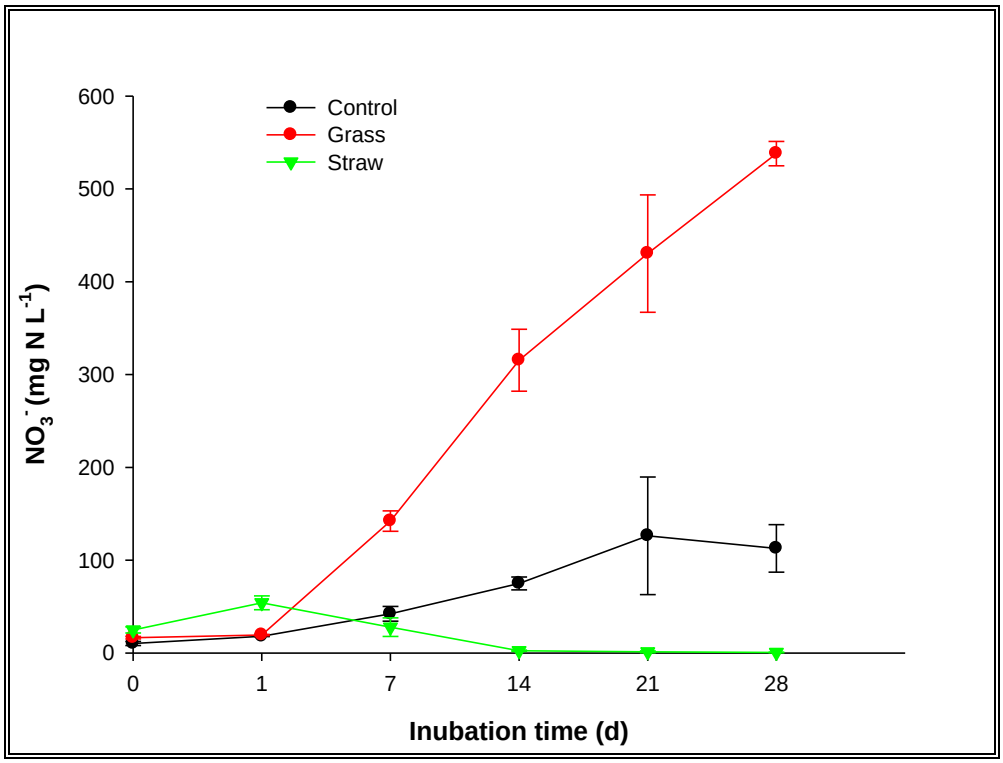


Figure 3.2. Soil solution NO_3^- concentrations during the incubation of grass and straw in an agricultural grassland soil. Values represent means ± SEM (*n* = 3).

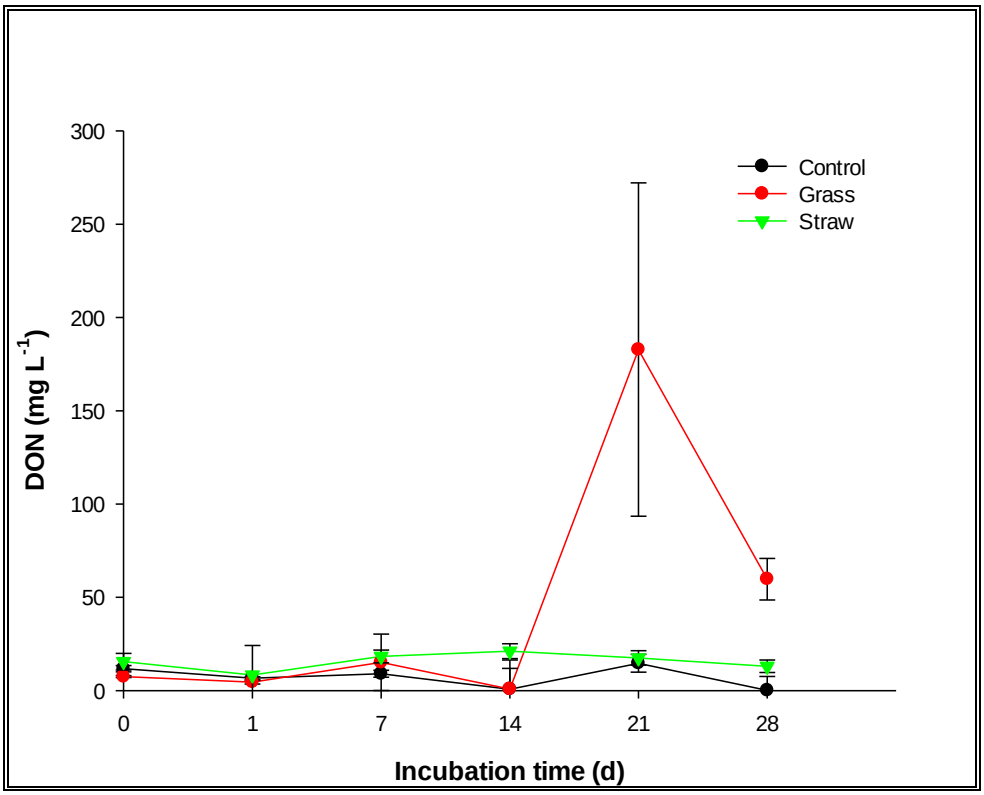


Figure 3.3. Soil solution DON concentrations during the incubation of grass and straw in an agricultural grassland soil. Values represent means ± SEM (*n* = 3).

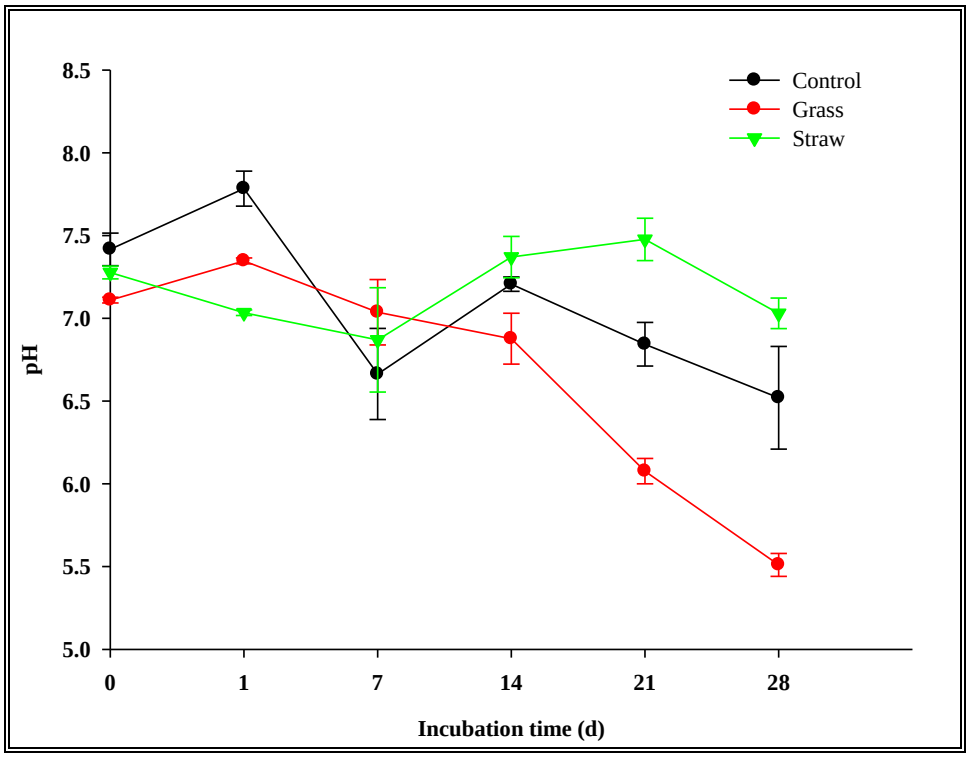


Figure 3.4. Soil solution pH concentrations during the incubation of grass and straw in an agricultural grassland soil. Values represent means ± SEM (*n* = 3).

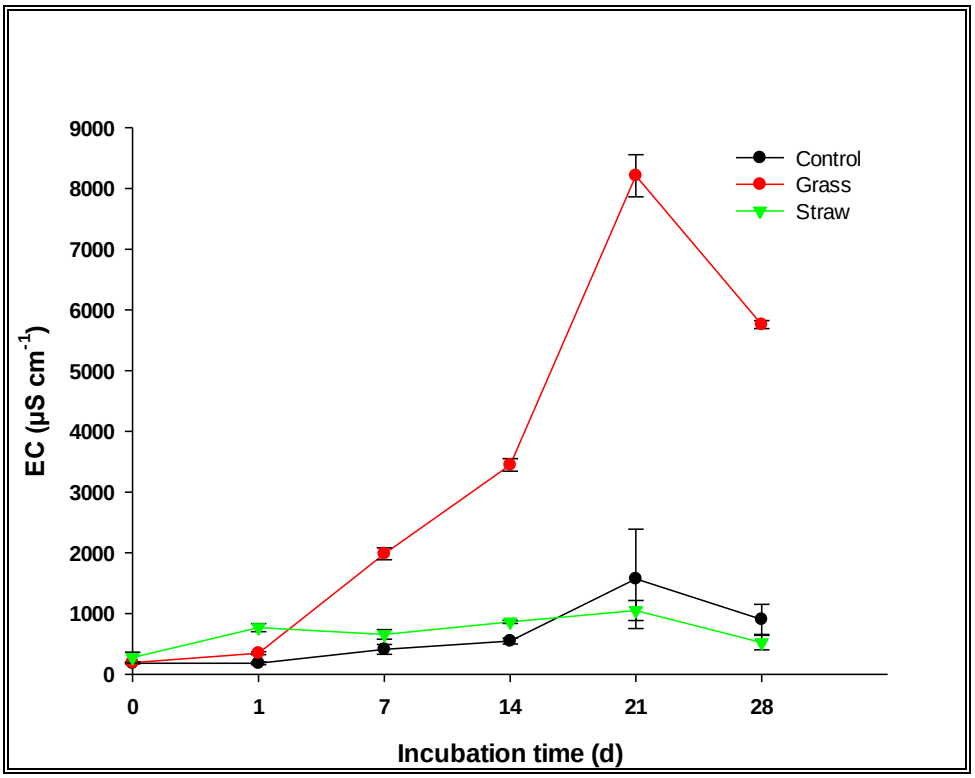


Figure 3.5. Soil solution EC concentrations during the incubation of grass and straw in an agricultural grassland soil. Values represent means ± SEM (*n* = 3).

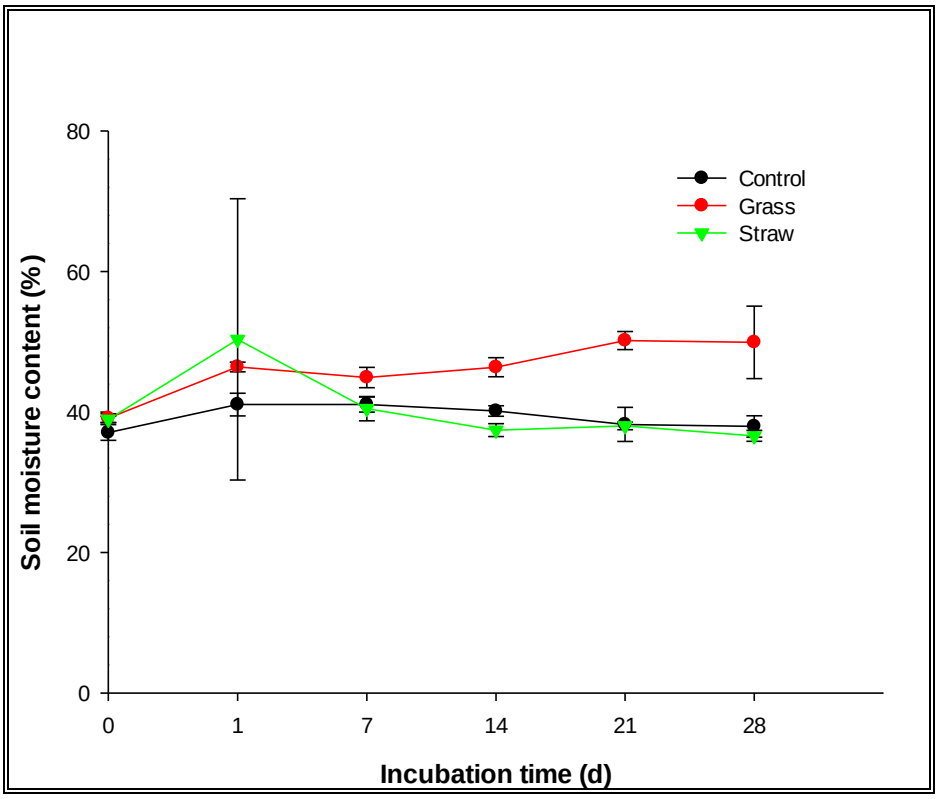


Figure 3.6. Soil moisture content during the incubation of grass and straw in an agricultural grassland soil. Values represent means ± SEM (*n* = 3).

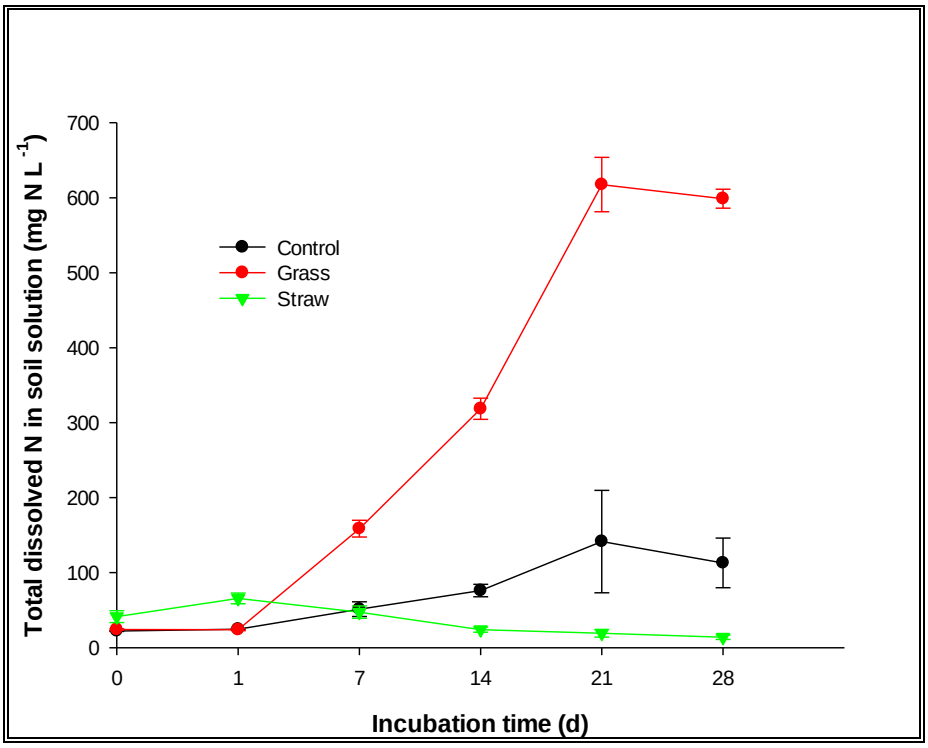


Figure 3.7. Soil solution total dissolved N (TN) concentrations during the incubation of grass and straw in an agricultural grassland soil. Values represent means ± SEM (*n* = 3).

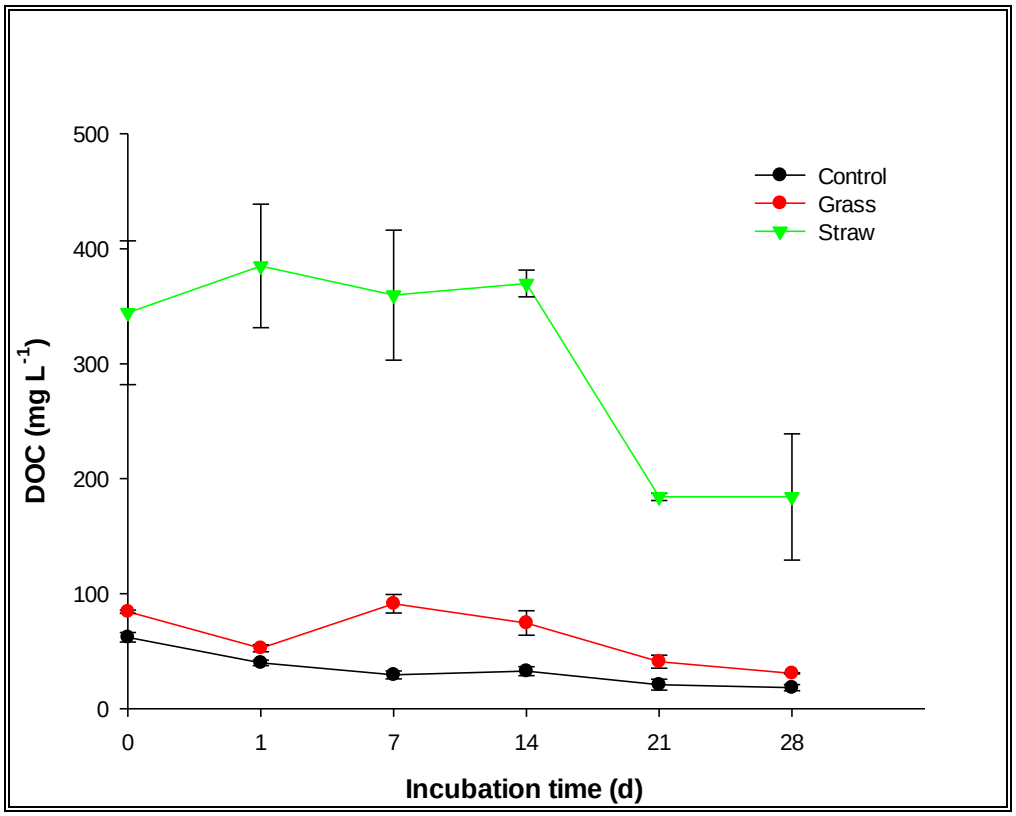


Figure 3.8. Soil solution DOC concentrations during the incubation of grass and straw in an agricultural grassland soil. Values represent means ± SEM (*n* = 3).

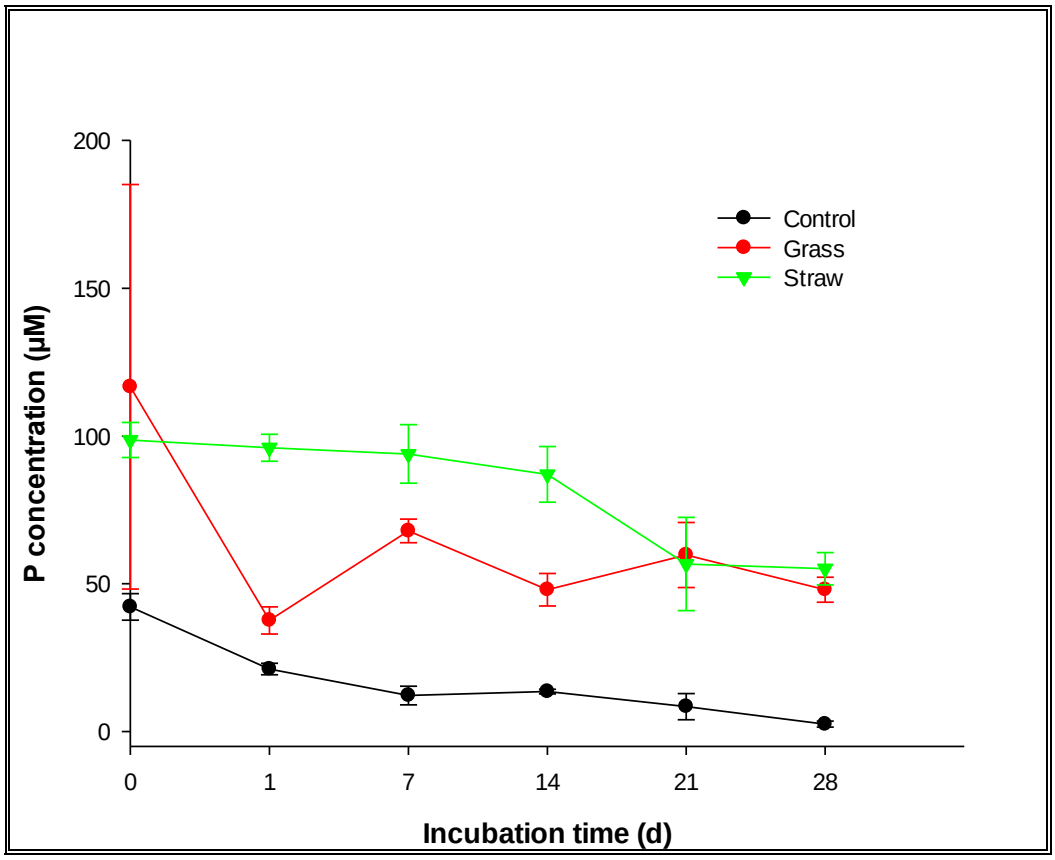


Figure 3.9. Soil solution P concentrations during the incubation of grass and straw in an agricultural grassland soil. Values represent means ± SEM (*n* = 3).

3.6. Discussion

In this study DON was significantly higher in grass treatment than either control or straw treatments after two weeks, which quantified an important part of the dissolved N pool particularly when grass treatment added to the soil. Multiple studies have shown that DON accounts for a significant fraction of N losses to streams of pristine or forested catchments (Hedin *et al*., 1995; Lajtha *et al*., 1995; Campbell *et al*., 2000; Neff *et al*., 2003). In the review of van Kessel *et al.* (2009), it was found that the average loss of DON in diverse agricultural systems with mostly light textured soils accounts for 26% of total soluble N.

The results presented here showed that soil solution NO_3^- and NH_4^+ accumulated in the grass-amended soil in contrast to that amended with straw or in the un-amended control soil. DON in the grass amended soil increased from day 14 to 21 and sharply decreased thereafter whilst the straw amended soil and control remained relatively constant. Contrary to expectation, the results indicate that addition of organic matter did not cause a large rise in DON relative to that of DIN. This suggests that in this high fertility agricultural soil the microorganisms rapidly break down DON contained in N rich organic residues to DIN. For N poor residues DON appears to represent a more important source of N, conversely, its availability to plants remains as yet unknown.

Due to the same organic matter origin and similar organic components, DON is often closely linked to the C cycle (Campbell *et al*., 2000; Goodale *et al.,* 2000; Willett *et al*., 2004; Cooper *et al*., 2007; Ghani *et al*., 2007; van Kessel *et al*., 2009). Accordingly, it is assumed that DON and DOC should be equally influenced by soil type or land use. Accordingly, Dawson *et al.* (2008) concluded that the export rate of DOC depends on the carbon soil pool and therefore the organic fraction. Jones *et al.* (2005b) reported that DON was turned over rapidly particularly in the

top soil in European agricultural systems (e.g. grasslands, arable soil).

Marschner and Römheld (1983) and Paul *et al.* (2001) reported that pH changes at the soil-root interface in relation to cation-anion uptake differ between plant species, nutrient supply and depend on the pH buffering capacity of the soil. Marschner *et al*. (1986) concluded that the rhizosphere pH may be as much as 2 units higher or lower than the pH of the bulk soil. Here we show a decrease in soil pH from day 16 to 30 which is probably due to nitrification. Although there was no consistent decrease in NH_4^+ concentration or increase in NO_3^- concentration in green shoot treatment that would support this explanation, it should be noted that here only net NH_4^+ and NO_3^- concentrations were measured.

3.7. Conclusions

The major findings of this study are as follows:

- DON is an important soluble N pool in soil but is less dynamic than soluble inorganic N
- Only a small proportion of the DON was higher in grass treatment than either control or straw treatments after two weeks.
- DIN is more influenced by organic residue addition to soil than DON.

3.8. Literature cited

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Chapter 4

4. Influence of root nitrogen content on nitrogen release during root turnover in soil.

4.1. Introduction

Maize (*Zea mays* L.) represents the third most important cereal crop in the world following wheat and rice (David and Adams, 1985), providing nutrients for humans and animals. Approximately 8 to 10% of the world's maize production is used for human consumption (Muhammad *et al.,* 1990). Maize is grown broadly in many countries of the world. In Pakistan, maize is cultivated on an area of 880.8 thousands hectares. The annual production is 128.3 thousand tones, and an average grain yield of 1445 kg per hectare (Bismillah Khan *et al.,* 2001).

Nitrogen (N) is one of the nutrients essential to living organisms. In boreal forest ecosystems available nitrogen in the soil is the nutrient most strongly limiting the growth of trees (Nilsson and Wiklund, 1995). It is generally well-known that N mineralization plays a crucial role in supplying nitrogen to plants. Nitrogen is considered as an important element for development and yield of the crop. After green revolution, farmers are responsible to maximize N fertilization to increase crop yield (Hirel *et al*., 2007). Ever since the need for additional food production increases, the worldwide utilization of inorganic and organic has increased at greater rate (Tilman *et al*., 2001). High N-fertilizer use also causes environmental disturbances, through excess N lost by leaching into ground water and overflow into surface water, plus ammonia (NH_3) volatization and production of NOx gases from de-nitrification polluting the atmosphere (Kaye and Hart, 1997; Galloway *et al*., 2008; Gruber and Galloway, 2008; Conley *et al*., 2009). Small amount of N fertilizers is costly to subsistence farmers who rely on their crops to yield enough food to feed their family nutritiously. Insufficient N supply causes reduced plant growth and create morphological changes for example increased root growth in relation to shoot growth to investigate a larger soil

volume. Therefore, in various parts of the world, low usage of N fertilizers, with increasing its efficiency was felt.

In low-input agriculture, the role of direct uptake of dissolved organic nitrogen (DON) by plants may be extremely important. However, the relative importance of DON and ammonium/nitrate (NH_4^+/NO_3^-) dissolved inorganic nitrogen (DIN) in temperate environments has been difficult to ascertain experimentally as DON fluxes in soil are complex and dynamic. At present, only unidirectional pulse label ^{15}N isotope studies have been conducted to verify that DON uptake from soil into plants can occur (Näsholm *et al*., 1998; Lipton *et al*., 1999; Owen & Jones, 2001). However, amongst other fluxes, rhizodeposition must also be considered alongside microbial-root competition for labile N. The aim of this study proposal is to help resolve the relative importance of DON uptake in comparison to DIN and, by so doing, explore the mechanism by which priming-induced vigour affects N-use efficiency in crop plants in the temperate climate.

Complex interactions between litter quality and microbial communities regulate the magnitude of this C flux and determine the trajectory of decay (Berg and Mc Claugherty, 2008). For instance, the same litter exposed to different microbial communities frequently displays pronounced differences in chemistry, even after substantial mass loss (Wallenstein *et al.,* 2011; Wickings *et al.,* 2011, 2012). Additionally, the complexity and diversity of litter chemical composition and its impact on microbial community function may explain why diverse plant litter mixtures often follow different decay trajectories than the average of the component species alone (Meier and Bowman, 2010). However, we lack detailed data on how microbial communities respond to labile and recalcitrant litter types at progressive stages of decomposition under field and laboratory conditions. Decomposition rates of the same litter vary widely across terrestrial ecosystems.

Variations in quality of plant litter, soil organic matter content and pH, and even wind

velocity can alter microbial activity and decomposition rates (Berg and McClaugherty, 2008). Filamentous decomposers (i.e., actionmycetes and fungi) influence decomposition by physically integrating substrates that differ in C and N availability, thus overcoming local nutrient limitation through translocation (Boberg *et al.,* 2010). Therefore, the magnitude of decomposer responses to litter quality under field conditions is not consistent across sites due to the variable effects of biotic and abiotic factors on microbial-substrate interactions and C flux rates (Carreiro *et al.,* 2000; Treseder, 2008; Snajdr *et al.,* 2011).

N availability has been hypothesized to be a key regulator of net primary productivity and vegetation successional gradients in forest systems (Vance and Chapin, 2001). The role of dissolved organic and inorganic N compounds in plant and microbial nutrition remains controversial (Owen and Jones, 2001; Schimel and Bennett, 2004). Evidence suggests that a range of plants may be capable of by passing the mineralization step of the nitrogen cycle by directly taking up low molecular weight (MW) DON such as amino acids and peptides thereby obviating the need for the soil microbial community to process organic N to NH_4^+ and NO_3^- (Jones *et al.,* 2005a; Kielland *et al.,* 2006b; Hill *et al.,* 2011). In many forest systems the soil microbial community is dominated by mycorrhizal fungi which can provide roots with an additional mechanism to short circuit the N cycle. Further, mycorrhizal fungi are capable of degrading a range of complex soil organic N forms; although the direct transfer of this N to roots has rarely been demonstrated (e.g. polyphenol bound protein etc. Jones *et al.,* 2005a; Rains and Bledsoe, 2007). Competition for DON between the intrinsic microbial population and roots with associated mycorrhizas can be expected to be great in the most N limiting ecosystems (Andresen, *et al.,* 2008). Studies have shown that while the size of the amino acid pool in soil is low, with concentrations typically ranging from 1 to 50 mM, the flux through this pool can be extremely rapid (Jones and Kielland, 2002; Jones *et al.,* 2009). Studies with isotopic C tracers have indicated that the half-life

of the amino acid pool in soil is in the region of 1-6 h indicating that the free amino acid pool turns over hundreds of times annually in black spruce forests (Kielland *et al.,* 2007). The impact of this rapid cycling through the low MW DON pool on subsequent NH_4^+ and NO_3^- production, however, remains largely unknown. When adding the N-rich amino acid arginine to soil, Jones and Kielland (2002) demonstrated a rapid production and excretion of NH_4^+ into the soil concomitant with the release of CO_2 from the amino acid. This result suggested that the soil microbial community might be using free amino acids not as a source of N but for their energy generating capacity.

Previous work in a wide range of global biomes has indicated that both these pathways (catabolism and anabolism) operate simultaneously with approximately 30-40% of amino acid-C used in respiration with the remaining amino acid-C used for cell biomass production and maintenance (Jones *et al.,* 2005b, 2009). Whether 30-40% of the N associated with the amino acid-C that is respired gets consistently excreted into the soil, however, remains unknown.

Current evidence suggests that while amino acid turnover is rapid in forests soils, the levels of inorganic N are extremely low with very little accumulation of NO_3^- (Kielland *et al*., 2006a). This lack of NO_3^- accumulation has led to the hypothesis that there is a nitrification block in black spruce soils possibly induced by the low pH of these ecosystems which suppresses nitrifying bacteria. An alternative explanation, however, is that in these N limiting environments NO_3^- fails to accumulate due to the lack of nitrification precursors due to rapid removal of NH_4^+ by plants and microorganisms. Similarly, nitrification could be rapid but continual removal of NO_3^- by plants and microorganisms would also prevent accumulation. The aim of this study was therefore to evaluate the points in the breakdown pathway of DON that limit inorganic N production in black spruce soils

4.1.1 Objectives

- To study the uptake of DON, in comparison with DIN by plant root system.
- To investigate the impact of plant residues on DON cycling when incorporated to soils.
- To investigate the influence of a rhizosphere on the transformation of DON in soil.

4.1.1.1. The objective of this experiment is to:

1. To quantify the DON uptake into the soil microbial community and plant roots and shoots over time.
2. Assess the biochemistry of soil with and without root residue.

4.1.1.2. Hypotheses to be tested

a) That the labile low molecular weight DON pool in soil increases after organic residue addition and that this constitutes an important source of N to plants in comparison to inorganic N.

b) The production of dissolved inorganic N (DIN) is limited by the transformation of complex DON to labile DON and not by the transformation of labile DON to DIN.

4.2. Materials and methods

A hydroponic experiment was conducted to address the hypothesis for assessing the biochemistry of soil with and without root presence. Maize plants were grown for 24 days in nutrient solution with low nitrogen (LN^{-}) and high nitrogen (HN^{+}) levels in this study.

Maize (*Zea mays* L. cv. KWS) seeds were aerated pre-germination in water for 24 hrs. After aeration seeds were sown in trays for germination. After 3 days germination single seedling was then transferred to individual test tube and a total 30 tubes were placed in each hydroponic

tank containing 3.3 l^{-1} 50% full Strength Long Ashton Nutrient Solution Table 4.1 (Hewitt, 1966). Macronutrients (mol dm^{-3}) were used (KNO_3, $Ca(NO_3)_2.4H_2O$, 0.67 $NaH_2PO_4.2H_2O$, 0.75 $MgSO4.7H_2O$, 0.1 EDTA.FeIII.Na) and micronutrients ($13x10^{-3}$ $MnSO_4.4H_2O$, $1x10^{-3}$ $CuSO_4.5H_2O$, $1x10^{-3}$ $ZnSO_4.7H_2O$, $50x10^{-3}$ H_3BO_3, $0.55x10^{-3}$ $Na_2MoO_4.2H_2O$ and 0.1 NaCl). pH was adjusted 6.0 in a controlled environment chamber. Hydroponic tanks were kept in a growth cabinet with photoperiod 16 hrs. (06:00 – 22:00 hrs.) and 8 hrs. darkness, day/night temperature 25/20°C, relative humidity 70%.

After one week nutrient solution was changed / tank and in addition 50 µl of $Fe(OH)_3$ was added to each tank. On the third week two treatments were given as (T1= HN^+ and T2 = LN^-), nutrient solution was changed in two tanks full nutrients were given in (T1) Table 4.1 and in remaining two tanks KNO_3, Ca $(NO_3)_2$. $4H_2O$ were stopped (T2) Table 4.2. In addition, 100 µl of Fe $(OH)_3$ was added to each tank. This procedure was repeated daily for further 8 days. After 24 days, the crop was harvested and data was recorded from 10 randomly selected plants in each treatment, plant height was measured from the surface of tube to the tip of plant, root and shoot length was measured cm by ruler. The leaf chlorophyll content was measured with portable equipment (Soil Plant Analysis Development) SPAD Minolta 502. The youngest leaf of each maize plant was measured for chlorophyll content. A SPAD meter (SPAD–502 Minolta Co., Ltd. Japan) was used to compare relative chlorophyll content.

5 g of fresh root and shoot samples were taken and dried at 80 °C for 48 hrs. in an oven and re-weighed to determine their dry weight. The time moisture content was also recorded. Samples were frozen at 10 °C for further chemical analysis to study the DON and DOC dynamics present in the soil.

4.2.1. Decomposition study

Field soil was collected from the Henfaes Research Center and then moist soil was passed through a 5–mm aperture sieve and divided into three parts and placed in separate bags. Roots of maize from HN^+ and LN^- were cut into 1 centimeter lengths. Three experimental treatments were established with different organic inputs made to the soil. Non-amended (control), soil amended HN^+ residue and soil amended with LN^-. Soil solution was extracted at different times after amendment to study DON and DIN dynamics. 100 g soil (control), 100 g soil + add 10 g roots of maize (HN^+) and 100 g soil + add 10 g roots of maize (LN^-). These treatments were sampled six times i.e. day–0, d1, d7, d14, d21 and d28. The amended samples were frozen at -10 °C for further analysis.

4.2.2. Soil solution extraction

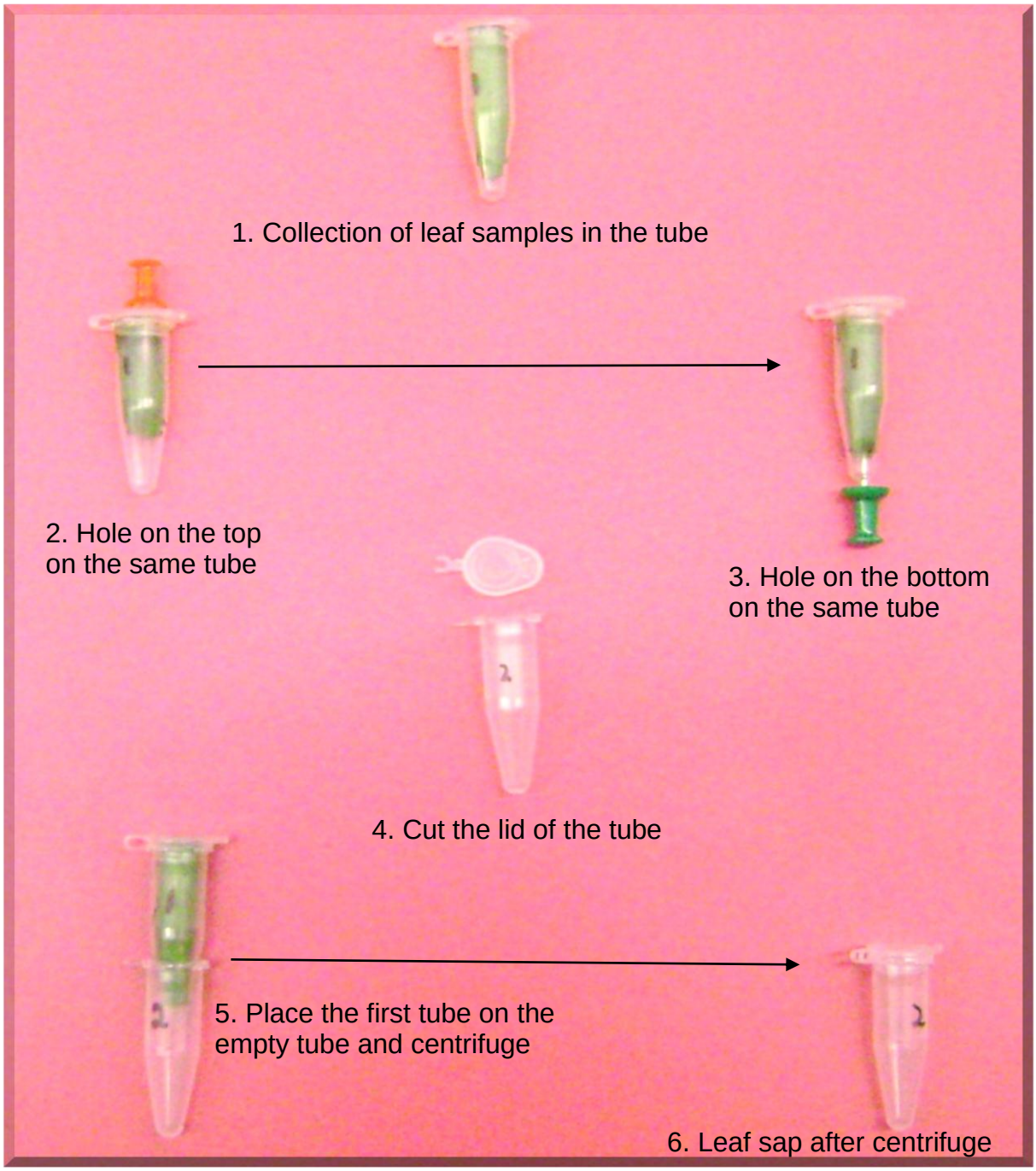
Soil solution was extracted following the centrifugation drainage technique of Giesler and Lundström (1993). About 100 g of field-moist soil were taken into the nylon centrifugal extraction cups; the soil was centrifuged for 30 min at 4000 rpm (~3.6 MPa, 4 °C; Beckman J2–21 centrifuge with a JA 14 rotor). On average, 10–15 ml of soil solution was obtained from each soil sample, the soil solutions were stored frozen at –20 °C in polyethylene vials for further chemical analysis. Moreover, moisture content was recorded from the soil and plant residues samples drying in oven at 80 °C overnight.

For the determination of DON similar procedure of experiment no–1 was followed. The roots of maize from HN^+ and LN^- have been mixed with soil to study the decomposition of soil determination of DON. 1g of fresh root and shoot were taken and sap was extracted. Total dissolved nitrogen (TDN) was measured from these saps by TOC Analyzer.

4.2.2.1. Sap extraction

Leaf, petiole and stem samples (approximately 1.5 g) of each genotype were cut and placed immediately in 1.5 cm^3 Eppendorf micro-centrifuge tubes. Cell sap was extracted according to (Gorham *et al.,* 1984b). The tubes were frozen at –20 °C in a commercial freezer. After that the tubes were taken out and thawed at room temperature (approximately 20 °C) while still sealed. (To avoid condensation of moisture from the air on the cold plant material). Two holes were made (Fig. 2.3), one at top and one in the bottom of the original tube. The lids of other tubes were removed and labeled. Each original micro centrifuge-tube was placed in one of the lid-less tube (Fig 2.3). The tubes were centrifuged (Eppendorf, Centrifuge 5810 R) at 15,000 *g* for 5 minutes. The upper tubes were discarded and the sap collected in the lower tube. This was stored in the freezer at –20 °C until further analysis.

Figure 4.1. Procedure of sap extraction of leaf tissues for chemical analysis



4.2.3. Chemical analysis

The pH and electrical conductivity (EC) was determined in a 1:1.25 soil: deionised water and values were determined using a pH meter and EC meter. Moisture content was determined by drying at 105 °C for 24 h.

Ammonium (NH_4^+–N) was determined colorimetrically by using the salicylate-nitroprusside, Na_2EDTA and Na–hypochlorite method of Mulvaney (1996) on a spectrophotometric analyzer. Nitrate (NO_3^-–N) was also determined colorimetrically by using the vanadium a rapid, simple method for simultaneous detection of nitrate method of Miranda *et al*. (2001) on a spectrophotometric analyzer.

Solution concentrations of dissolved organic carbon (DOC) and TDN were determined with Shimadzu total organic carbon (TOC)–V–CSH analyzer (Shimadzu Crop., Kyoto, Japan). The TOC analyzer injects 50 µl of soil solution or extract into a combustion furnace held at 720 ^{0}C after which the CO_2 produced is detected by an infrared gas analyser and the subsequent detection of N_2O using a chemiluminescence detector. Potassium nitrate (KNO_3) and sodium nitrate ($NaNO_3$) were used as standards. The limit of detection in the samples was 50 µg l^{-1} for soil solutions. Dissolved Organic Nitrogen (DON) was calculated from the difference between TDN and combined NH_4^+ –N and NO_3^- –N reading dissolved inorganic nitrogen (DIN).

$DIN = NH_4^+\text{-}N + NO_3^-\text{-}N$

$DON = TDN - DIN$

Nitrogen and carbon percentage (%) was analyzed from 5 g of root and shoot after drying and grinded with pestle and mortars using the LECO CHN 2000 analyzer, model, TruSpec® Series.

4.3. Statistical and data analysis

Data was analyzed with SPSS statistical package 14 which involve analysis of variance, mean performance, standard deviation, standard error and simple correlation. Graphical analysis was done using Sigma Plot v8.0.

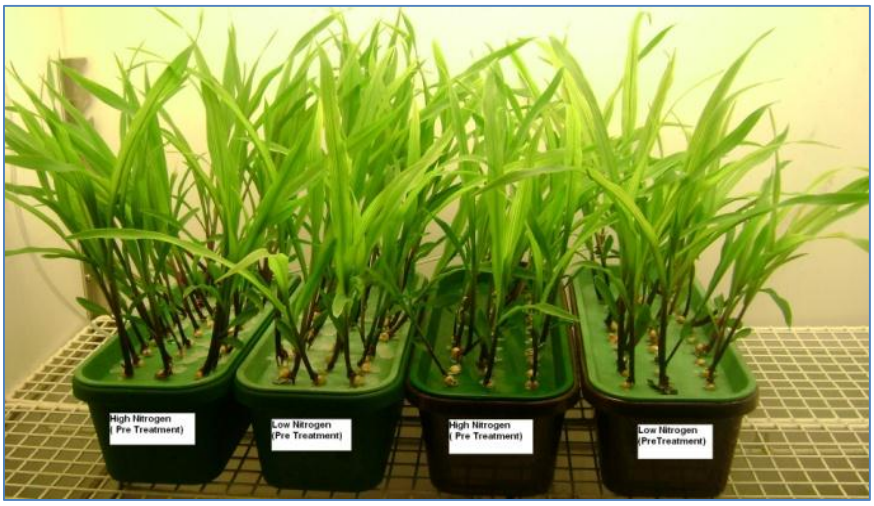
Table: 4.1. Fifty percent (50%) full strength Long Ashton nutrient solution for maize crop grown in hydroponic culture (high nitrogen).

Stock solution	Name of elements	To prepare 500 cm^3 stock sol. Use (g)	Stock sol. Conc. (mol dm^{-3})	For final full strength sol. Use (cm^3 dm^{-3})
1	KNO_3 (Potassium nitrate	50.5	1	4
2	$Ca(NO_3)_2.4H_2O$ Calcium nitrate (tetrahydrate)	236	2	2
3	$NaH_2PO_4.2H_2O$ Sodium dihydrogen orthophosphate (dihydrate)	52	0.67	2
4	$MgSO_4.7H_2O$ Magnesium sulphate (heptahydrate)	92	0.75	2
5	EDTA.FeIII.Na (EDTA Ferric monosodium salt)	18.65	0.1	1
6	**Micronutrients solution:**			1
	a). $MnSO_4.4H_2O$ (Manganous sulphate (tetrahydrate)	1.125	$13x10^{-3}$	
	b). $CuSO_4.5H_2O$ (Cupric sulphate (pentahydrate)	0.125	$1x10^{-3}$	
	c). $ZnSO_4.7H_2O$ (Zinc sulphate (heptahydrate)	0.145	$1x10^{-3}$	
	d). H_3BO_3 (Boric acid)	1.55	$50x10^{-3}$	
	e). $Na_2MoO_4.2H_2O$ (Sodium molybdate (dihyrate)	0.06	$0.55x10^{-3}$	
	f). NaCl (Sodium chloride)	2.93	0.1	

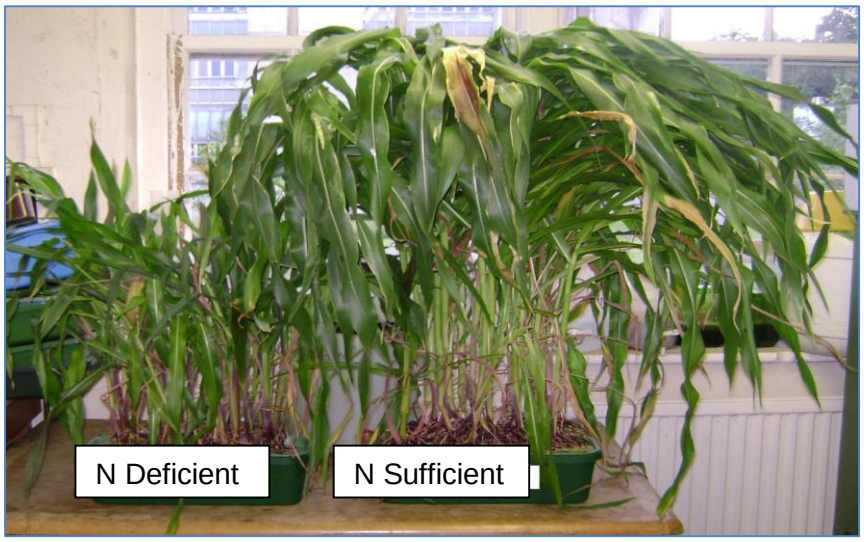
Table: 4.2. Fifty percent (50%) full strength long Ashton nutrient solution for maize crop grown in hydroponic (low nitrogen).

Stock solution	Name of elements	To prepare 500 cm^3 stock sol. Use (g)	Stock sol. Conc. (mol dm^{-3})	For final full strength sol. Use (cm^3 dm^{-3})
3	$NaH_2PO_4.2H_2O$ (Sodium dihydrogen orthophosphate (dihydrate)	52	0.67	2
4	$MgSO_4.7H_2O$ (Magnesium sulphate (heptahydrate)	92	0.75	2
5	EDTA.FeIII.Na (EDTA Ferric monosodium salt)	18.65	0.1	1
6	Micronutrients solution:			1
	a). $MnSO_4.4H_2O$ (Manganous sulphate (tetrahydrate)	1.125	$13x10^{-3}$	
	b). $CuSO_4.5H_2O$ (Cupric sulphate (pentahydrate)	0.125	$1x10^{-3}$	
	c). $ZnSO_4.7H_2O$(Zinc sulphate (heptahydrate)	0.145	$1x10^{-3}$	
	d). H_3BO_3 (Boric acid)	1.55	$50x10^{-3}$	
	e). $Na_2MoO_4.2H_2O$ (Sodium molybdate (dihyrate)	0.06	$0.55x10^{-3}$	
	f). NaCl (Sodium chloride)	2.93	0.1	

Figure 4.2. Effect of low and high nitrogen on growth of maize plant grown in hydroponic culture.



Pre treatment



Post treatment

4.4. Results

4.4.1 Soil solution N dynamics

An experiment was conducted to investigate the effect of low and high nitrogen maize root content on their subsequent rate of decomposition in soil. A summary of the statistical results are presented in Table 4.2. The statistical analysis revealed significant differences between the high and low N treatments and a zero root addition (control) treatment for the soil solution traits NH_4^+, NO_3^- and DOC ($p < 0.001$). However, DON showed no significant difference for the different nitrogen levels.

Post hoc comparison test was performed by Tukey and the results revealed that there was a significant difference for NH_4^+ between the control treatment and the N+ and N- treatments (Table 4.3; $p<0.001$). In the case of NO_3^- , Tukey comparison showed that there was significant difference between the control and high and low nitrogen treatments. Similarly for DOC a significant difference was observed between the control and the high and low nitrogen treatments ($p< 0.001$).

Results for ammonium are presented in Figure 4.3 which showed that NH_4^+ content of soil decreased over time. At day 7, NH_4^+ concentrations were very low whilst NO_3^- concentrations were at a maximum. This drastic decrease in NH_4^+ may be attributed to the turnover of NH_4^+. This may be also due to volatilization of NH_4^+ as on day 7 the moisture content was maximal (35.75%) in high N soil as compared to control and low N soil. High soil moisture content results in volatilization of ammonium and N- concentration in soil (Al-Kanani *et al* 1991). From the One-Way ANOVA, we see that after 28 days NH_4^+ concentrations in soil solution within the control treatment were significantly different to the N+ and N- treatments **(**$P = 0.031$) (Table 4.2). In addition, NH_4^+ concentrations in the N- treatment was not significantly higher than in the N+

treatment (P = 0.109).

Nitrate (NO_3^-) concentration of soil increased in case of low N- treatment as compared to control and high N+ treatment. These results if compared with NH_4^+ content of soil indicate that NH_4^+ is converted to NO_3^- with the passage of time Figure 4.4. Nitrate in soil solution from the three treatments decreased after 28 days of incubation. From a One-Way ANOVA, we see that after 28 days, NO_3^- in soil solution from the control, N+ and N- treatments differed significantly (P < 0.001) (Table 4.2). Further post hoc test (Tukey) showed that NO_3^- of N+ solution was significantly lower than the control (P < 0.001) (Table 4.3). NO_3^- within the N- treatment was significantly higher than the control (P < 0.001). On the other hand, NO_3^- of the N- treatment was not higher than the N+ treatment (not significant, P = 0.488).

The concentration of DON during 28 days is shown in figure 4.5. The concentration of DON did not increase. In case of low N- soil, DON concentration decreased to (0 to –15) which was well below the values of high N+ and soil. The decrease in DON concentration may be due to the fact that DON is usually composed of wide range of compounds. The lower content of these compounds in DON might have decreased the DON concentration in soil. From a One-Way ANOVA, we see that after 28 days, DON of soil solution from the control, N+ and N- treatments were not significantly different from each other (P = 0.226) (Table 4.2). Further post hoc test (Tukey) showed that DON of N+ solution was not significantly lower than in the control (P = 1.000). Although DON in the N- treatment was lower than the control, the difference was not significant (P = 0.282). Similarly, DON in the N- treatment was not lower than in the N+ treatment (P = 0.287).

As presented in Figure 4.6, dissolved organic carbon (DOC) in the high nitrogen (N+) and low nitrogen (N–) treatment decreased with time. It was observed that DOC content of soil applied with N+ increased for the 1st day but decreased in the soil having N–. This pattern was observed

throughout the 28 day incubation period. The results indicate that with the passage of time, the organic carbon containing compounds of soil decreased due to breakdown due to microbial mineralization. From a One-Way ANOVA, we see that after 28 days DOC of soil solution from the control, N+ and N- differed significantly (P = 0.008) (Table 4.2). Further post hoc test (Tukey) showed that DOC of N+ solution was significantly higher than the control (P = 0.031) (Table 4.3). The DOC of soil solution in the N- treatment was also higher than the control (P = 0.008). On the other hand, DOC in the N- and N+ treatments were not significantly different from each other (P = 0.675).

4.4.2. Other changes in soil solution

One way analysis of variance was done along with Para wise comparison with Tukey. Results showed that there was a significant variation for the pH, EC, MC, TN and DIN (Table 4.2). Significant variation for pH was between control and low nitrogen $p< 0.01$ (Table 4.4). Significant variation was observed between controls with high and low nitrogen and high nitrogen control and low nitrogen for EC. For moisture content significant variation was observed for control and high and low nitrogen level $p<0.001$. Similarly for TN and DIN significant Variation was due to variation in control with high and low nitrogen $p<0.001$.

Figure 4.4 showed that pH of root cell sap is around pH 6.5 and this may have caused the initial increase in soil solution in addition, when the organic acid anions (e.g. $citrate^{3-}$) are released from roots into soil they complex H^+ from the soil (i.e. $H_2.citrate^{1-}$) and thus raise the pH.

It was revealed from the Figure 4.5 that with the passage of time electric conductivity (EC) of the control soil remained constant or unchanged where no N+ or N– was put in the soil. The EC of the soil where roots (N+) was mixed unchanged till 1^{st} day and then increased gradually and reached maximum till 14^{th} day. After that it's decreased sharply. Higher in roots due to release of

nutrient ions into solution from the roots (e.g. K, Na, Ca, Mg) and also the breakdown of organic N leading to the production of NO_3^-.

Amino acids (AA) in soil solution from the three treatments decreased after 28 days of incubation. From a One-Way ANOVA, we see that after 28 days, AA of soil solution from the control, N+ and N- treatments differed significantly ($P < 0.001$) (Table 4.2). Further post hoc test (Tukey) showed that AA of N+ solution is significantly higher than the control treatment ($P = 0.017$) (Table 4.4). The AA of the N- treatment was also higher than in the control treatment ($P < 0.001$). On the other hand, the AA content of soil solution in the N- was not significantly higher than in the N+ treatment ($P = 0.357$).

Data presented in Table 4.5 revealed that there was a significant difference between the nitrogen levels ($p<0.001$) for shoot height. Mean shoot height was higher in the N+ in comparison to the nitrogen deficient plants (Table 4.5). In the case of chlorophyll content, there no significant difference was observed between treatments. However, slightly higher chlorophyll content was recorded in the sufficient nitrogen treatment (Table 4.5). Similarly, shoot dry weight was significantly different between the two nitrogen levels (Table 4.4). The highest shoot dry was recorded in the sufficient nitrogen treatment (Table 4.5).

It was revealed from the Table 4.5 that nitrogen levels showed no significant difference for shoot total carbon content. Regarding shoot total nitrogen content, a significant difference was observed ($p<0.05$) with shoot nitrogen being highest in the sufficient nitrogen treatment (Table 4.5).

In case of shoot carbon-to-nitrogen ratio, it was noticed that there was highly significant variation between nitrogen treatment ($p<0.001$). The highest C/N ratio was shown in the nitrogen deficient treatment compared to those plants receiving sufficient nitrogen (Table 4.5).

Data presented in Table 4.5 revealed significant differences for the total C and N content

of roots. It was found that the nitrogen sufficient treatment showed the highest root total nitrogen content (Table 4.5). Highly significant difference was observed between nitrogen levels for root carbon-to-nitrogen ratio. It was noticed that highest root C/N ratio was recorded in the N deficient roots. There was no significant difference observed for root soluble carbon and root soluble nitrogen (Table 4.5).

4.4.3. Correlations

Correlations was calculated and presented in Table 4.7 which revealed that pH showed highly significant positive correlation with MC%, NH_4^+ and DOC. EC showed highly significant correlation with MC%, NO_3^-, TN and DOC. However, it showed highly significant negative correlation with DON. MC% had highly significant correlation with NH_4^+, NO_3^-, TN and DOC. NH_4^+ showed highly significant positive correlation with DOC. Similarly NO_3^- had highly significant positive correlation with TN and highly significant negative correlation with DON. Finally TN showed highly significant negative correlation with DON.

Table 4.3. Significant differences in a range of soil quality indicators after incubation of high and low N containing roots in soil for up to 28 d in comparison to an unamended control soil treatment. From a One-Way analysis of variance (ANOVA) and mean performance of different components. * indicates p < 0.001, ** indicates p < 0.01 and NS indicates no significant difference (p > 0.05).**

		Sum of Squares	df	Mean Square	F	
NH_4^+	Between Groups	277.043	2	138.522	6.652	**
	Within Groups	1436.92	69	20.825		
NO_3^-	Between Groups	612185	2	306092	38.169	**
	Within Groups	553338	69	8019.4		
DON	Between Groups	596.928	2	298.464	0.751	NS
	Within Groups	27416.3	69	397.338		
DOC	Between Groups	70648.8	2	35324.4	30.557	**
	Within Groups	79765.5	69	1156.02		
pH	Between Groups	1.154	2	0.577	4.255	**
	Within Groups	9.36	69	0.136		
EC	Between Groups	7.2E+07	2	3.6E+07	168.862	**
	Within Groups	1.5E+07	69	214249		
MC	Between Groups	1308.89	2	654.444	42.985	**
	Within Groups	1050.53	69	15.225		
TN	Between Groups	640603	2	320302	50.286	**
	Within Groups	439500	69	6369.57		
DIN	Between Groups	637980	2	318990	42.607	**
	Within Groups	516594	69	7486.86		

Table 4.4. Summary table showing the Tukey ANOVA multiple comparison results comparing the three different treatments (Control, High and Low N root content amended soil).

Dependent Variable	(I) Treatment	(J) Treatment	Sig.
NH_4^+	Control	N+	0.018
		N-	0.003
	N+	Control	0.018
		N-	0.805
	N-	Control	0.003
		N+	0.805
NO_3^-	Control	N+	0.000
		N-	0.000
	N+	Control	0.000
		N-	0.966
	N-	Control	0.000
		N+	0.966
DON	Control	N+	0.798
		N-	0.829
	N+	Control	0.798
		N-	0.442
	N-	Control	0.829
		N+	0.442
DOC	Control	N+	0.000
		N-	0.000
	N+	Control	0.000
		N-	0.106
	N-	Control	0.000
		N+	0.106

Table 4.5. Pairwise comparisons with Tukey between the three different treatments to show significance differences (Control, high and low N root amended soil).

Dependent Variable	(I) Treatment	(J) Treatment	Sig.
pH	Control	N+	0.113
		N-	0.017
	N+	Control	0.113
		N-	0.705
	N-	Control	0.017
		N+	0.705
EC	Control	N+	0.000
		N-	0.000
	N+	Control	0.000
		N-	0.032
	N-	Control	0.000
		N+	0.032
MC	Control	N+	0.000
		N-	0.000
	N+	Control	0.000
		N-	0.981
	N-	Control	0.000
		N^+	0.981

Table 4.5. (Continued)

Dependent Variable	(I) Treatment	(J) Treatment	Sig.
TN	Control	N+	0.000
		N-	0.000
	N+	Control	0.000
		N-	0.846
	N-	Control	0.000
		N+	0.846
DIN	Control	N+	0.000
		N-	0.000
	N+	Control	0.000
		N-	0.972
	N-	Control	0.000
		N+	0.972
AA	Control	N+	0.017
		N-	0.000
	N+	Control	0.017
		N-	0.357
	N-	Control	0.000
		N+	0.357

Table 4.6. Properties of the N sufficient and N deficient plants used in the experiments. Values represent means ± SEM (n = 4). * P< 0.05 probability level. ** P<0.01 probability level. ± Standard error of four replications.

	N deficient	N sufficient	P value
Shoot height (m)	0.75 ± 0.03	1.38 ± 0.03	**
Leaf chlorophyll content (µg l^{-1})	23.6 ± 2.0	28.2 ±1.66	NS
Total shoot DW (g $plant^{-1}$)	12.2 ±1.06	48.8 ±1.28	**
Shoot total C (%)	47.9 ± 0.18	48.4 ± 0.06	NS
Shoot total N (%)	2.0 ± 0.14	3.1 ± 0.05	*
Shoot C:N	24.0 ± 0.7	16.0 ± 0.1	**
Root total C (%)	47.2 ± 0.25	45.9 ± 0.09	*
Root total N (%)	1.5 ± 0.03	2.2 ± 0.01	**
Root C:N	32.0 ± 0.9	21.0 ± 0.2	**
Root soluble N (mg N l^{-1})	242.9 ± 25.2	1369.1 ± 582.5	NS
Root soluble C (mg N l^{-1})	986.1 ±184.9	1718.1 ± 507	NS

Table 4.7. Shows correlation between pH, EC, MC%, NH_4^+, NO_3^-, TN, DOC and DON of maize in low and high nitrogen. Values represent mean ± SEM (n = 4).

	EC	MC%	NH_4^+	NO_3^-	TN	DOC	DON
pH	0.084	0.478**	0.589**	-0.153	-0.142	0.553**	0.002
EC (µS cm^{-1})	1	0.569**	0.164	0.899**	0.921**	0.489**	-0.354**
MC %		1	0.381**	0.396**	0.441**	0.593**	-0.033
NH_4^+ (mg N l^{-1})			1	-0.142	-0.075	0.763**	0.206
NO_3^- (mg N l^{-1})				1	0.986**	0.138	-0.529**
TN (mg N l^{-1})					1	0.213	-0.386**
DOC (mg N l^{-1})						1	0.171
DON (mg N l^{-1})							1

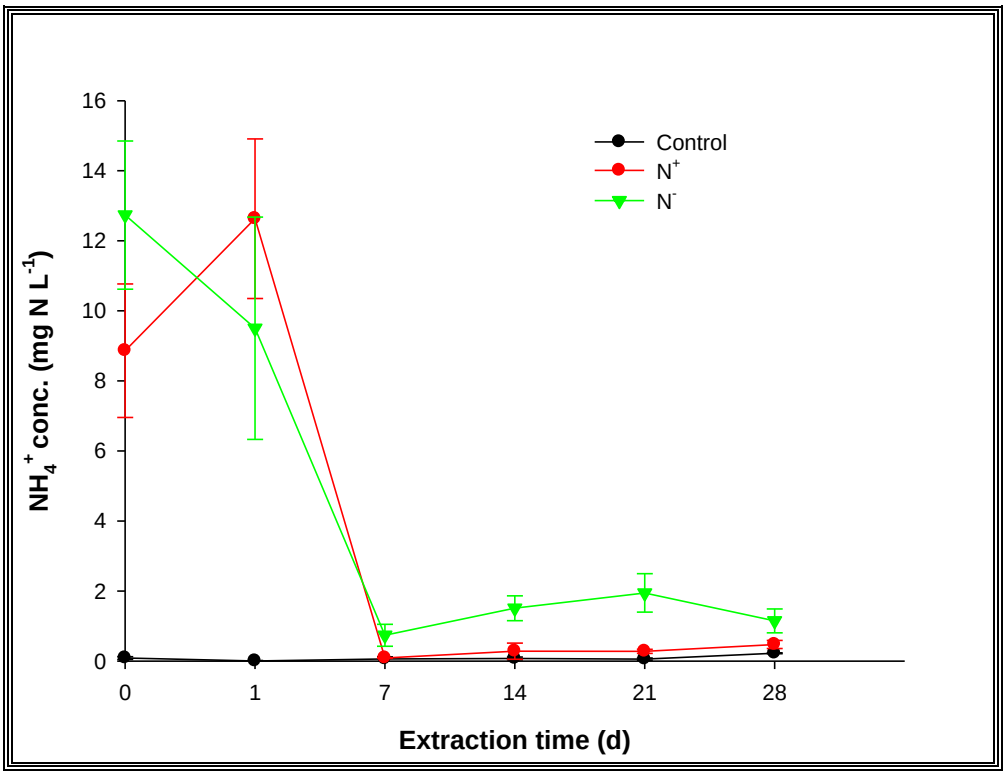


Figure 4.3. Showing soil solution ammonium during the incubation of roots (high nitrogen and low nitrogen) amended soil. Error bars represents ± se.

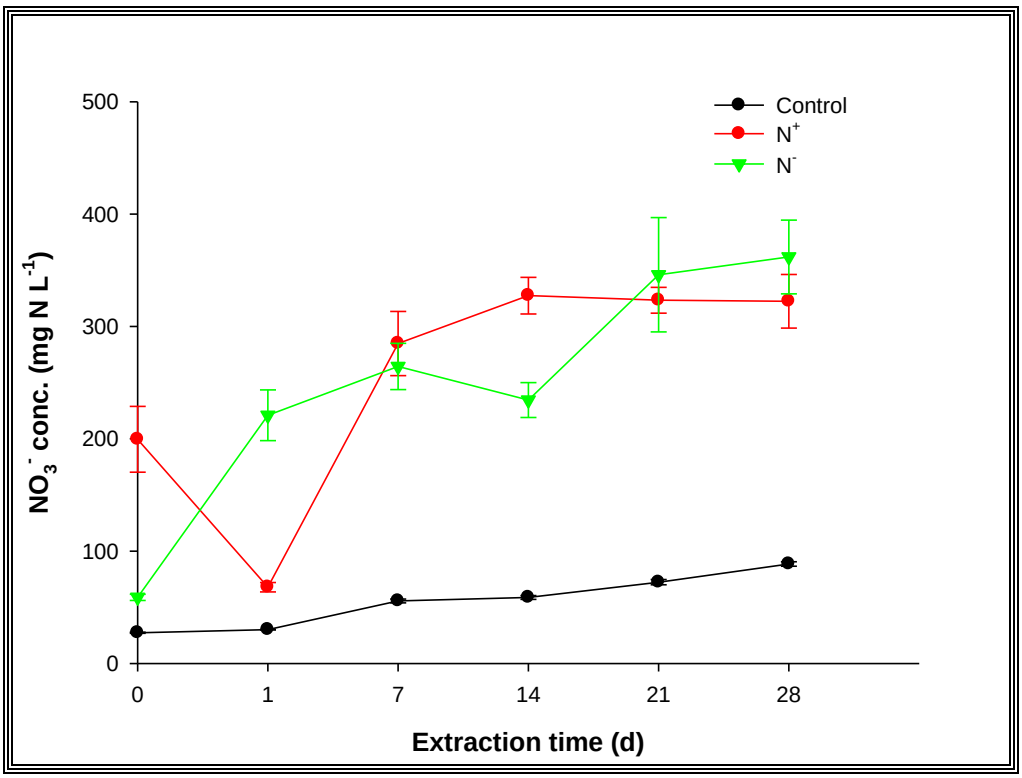


Figure 4.4. Graph showing soil solution Nitrate pools during the incubation of roots (high nitrogen and low nitrogen) amended soil. Error bars represents ± se.

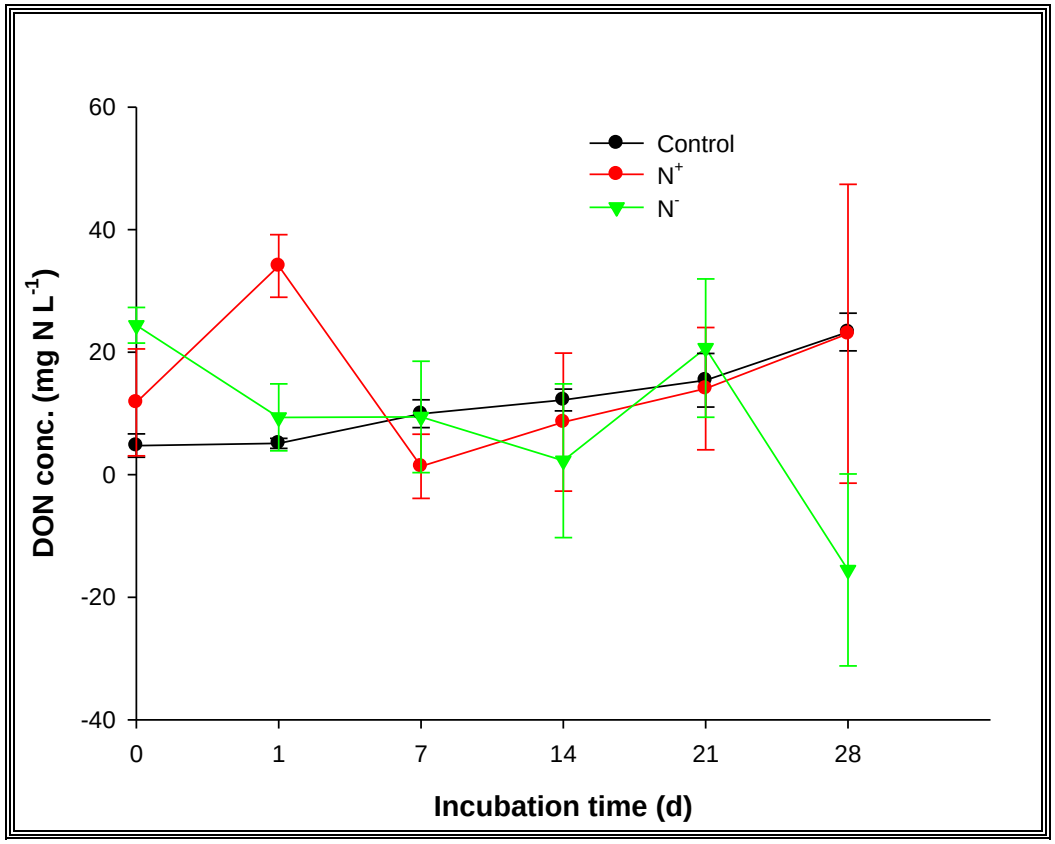


Figure 4.5. Showing DON during the incubation of roots (high nitrogen and low nitrogen) amended soil. Error bars represents ± se.

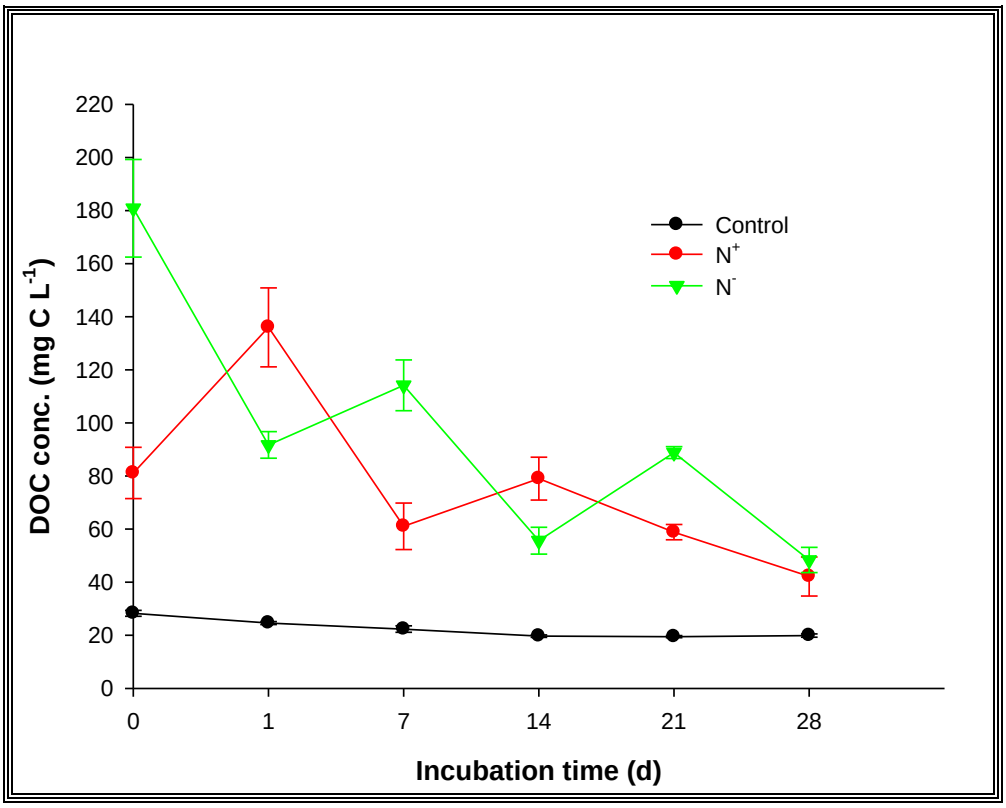


Figure 4.6. Showing DOC during the incubation of roots (high nitrogen and low nitrogen) amended soil. Error bars represents ± se.

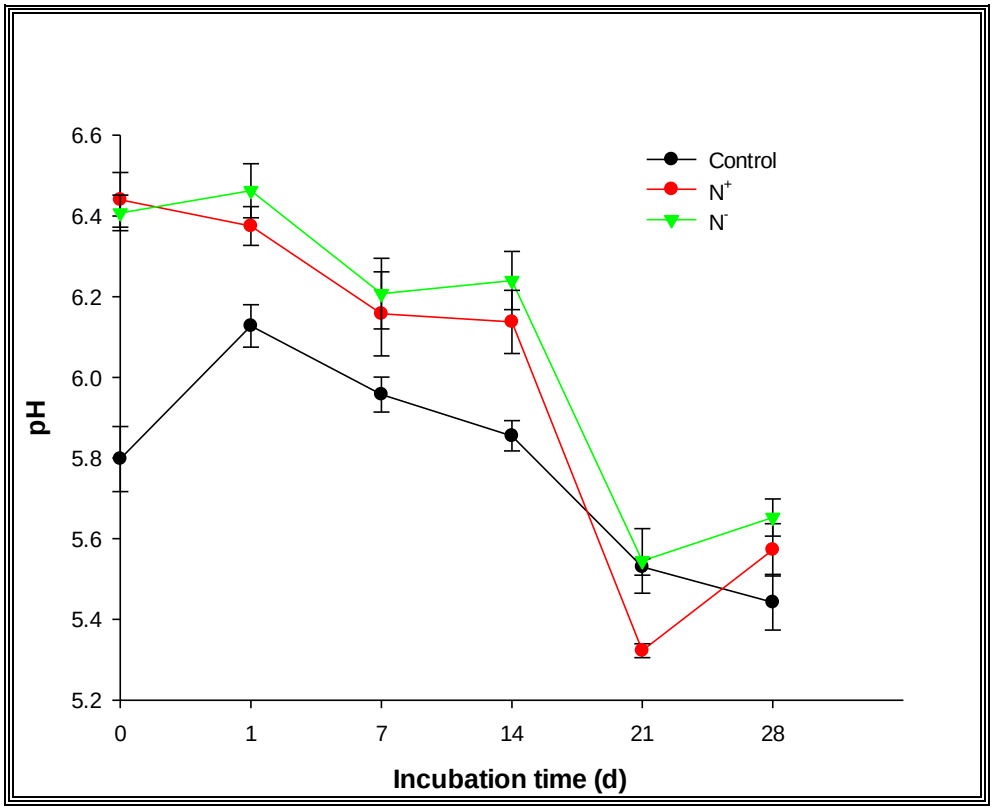


Figure 4.7. Showing soil solution pH during the incubation of roots (high nitrogen and low nitrogen) amended soil. Error bars represents ± se.

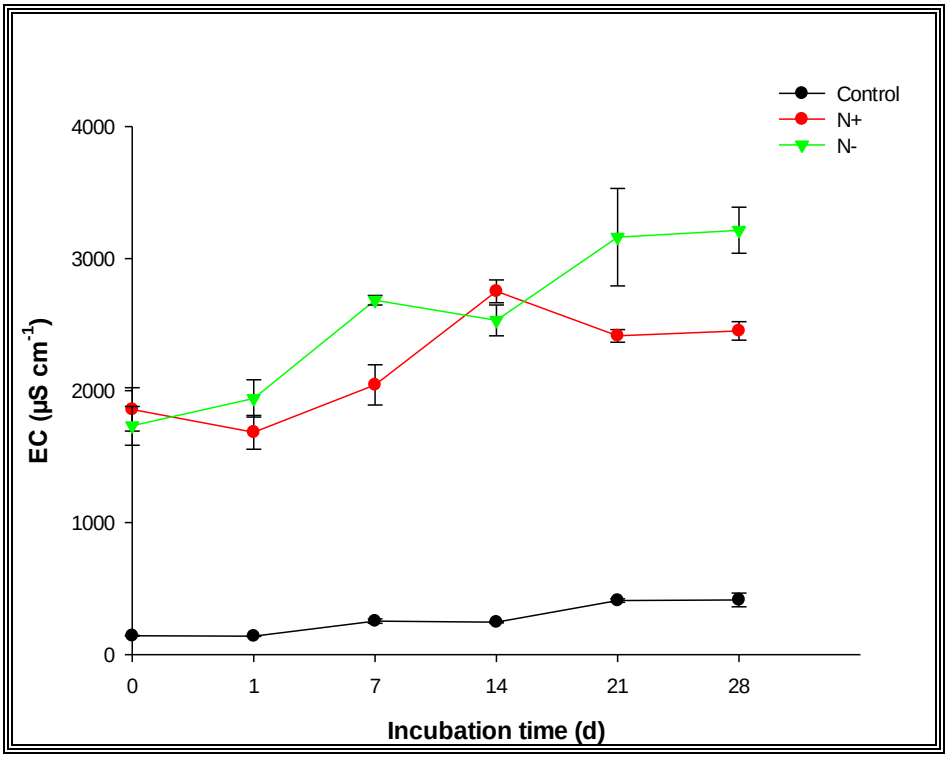


Figure 4.8. Showing soil solution EC during the incubation of roots (high nitrogen and low nitrogen) amended soil. Error bars represents ± se.

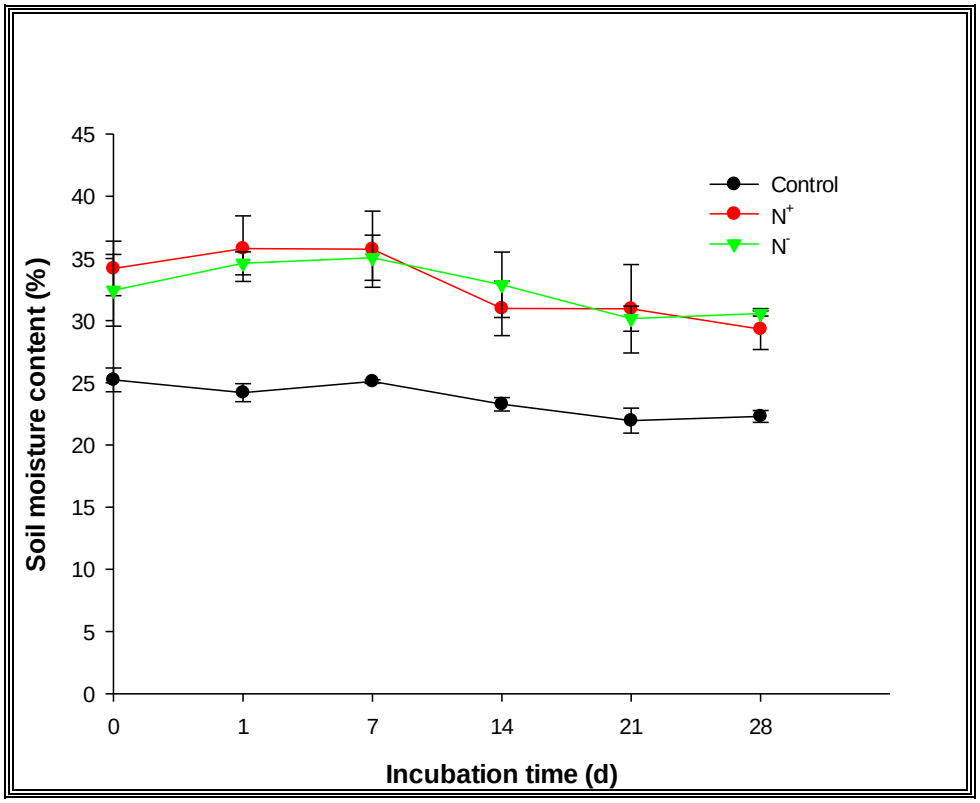


Figure 4.9. Showing soil solution moisture content during the incubation of roots (high nitrogen and low nitrogen) amended soil. Error bars represents ± se.

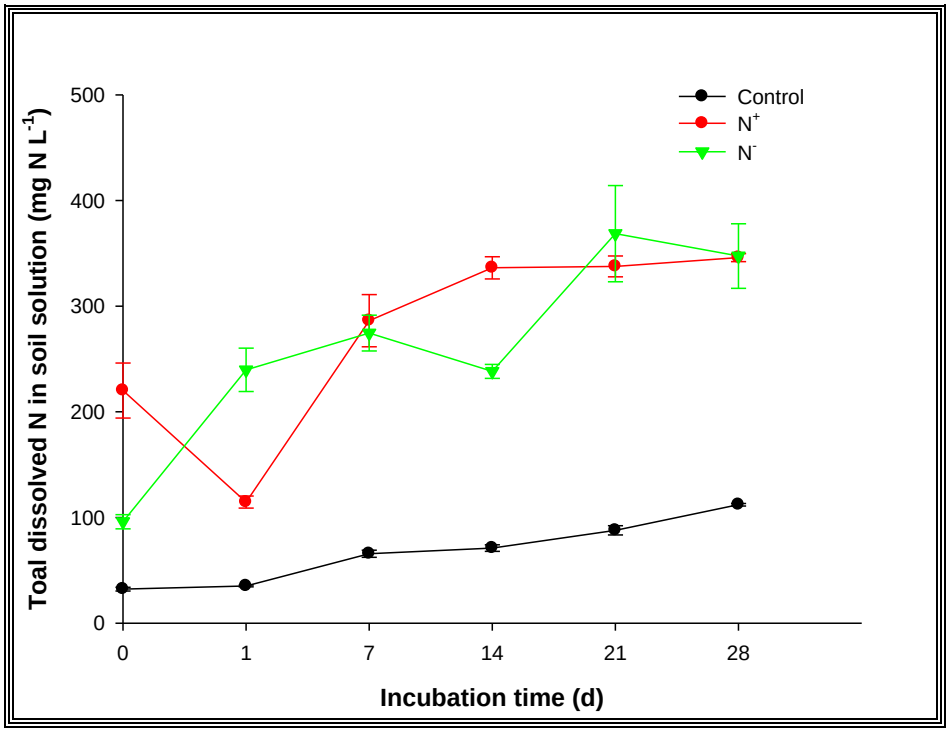


Figure 4.10. Showing soil solution total dissolved nitrogen during the incubation of roots (high nitrogen and low nitrogen) amended soil. Error bars represents ± se.

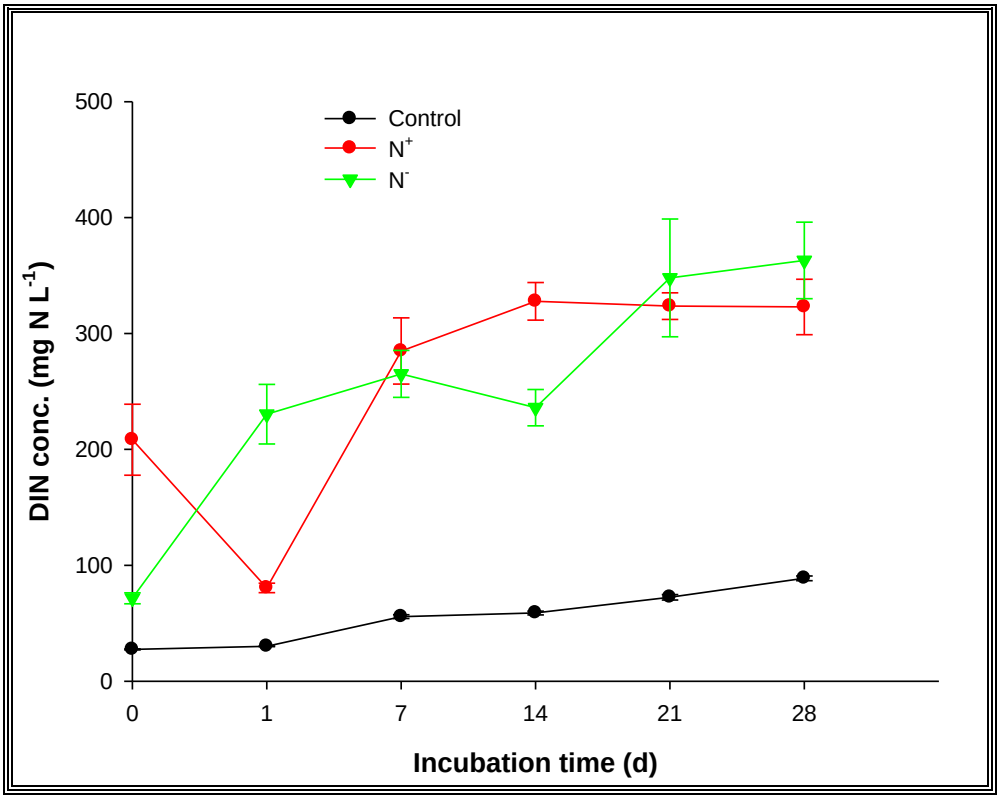


Figure 4.11. Showing soil solution dissolved inorganic nitrogen during the incubation of roots (high nitrogen and low nitrogen) amended soil. Error bars represents ± se.

4.5. Discussion

Incorporation of crop residue sustains soil fertility and crop production (Kwabiah *et al.* 2003).It is well-known that microbes look for and utilize all nutrient sources available in the soil to satisfy their nutrient needs and the closer the nutrient patches are, the higher the microbial density (Gaillard *et al.* 1999; Kandeler *et al.* 1999; Gaillard *et al.* 2003). High C/N residues (cereal straw residues) are, in contrast to low C/N residues, poor in easily utilizable sugars and N, but rich in cellulose and hemicelluloses. As a result, the decomposition rate is slow, but long-lasting.

The use of two crop residues with contrasting biochemical composition was expected to provide better insights into how far soluble organic compounds moved from the residue interface into the adjacent soil and how this movement affects respiration rate, microbial community and N concentration. This could be due to greater shoot/root N content, or DON and DIN in soil with passage of time.

The low C/N ratio residues contain higher concentrations of water soluble C and N compounds which can be utilized by microbes in adjacent nutrient poorer areas. The addition of crop residues with different C/N ratios had a clear impact on the spatial distribution of residue-derived N at different distances from the interface via two mechanisms, namely diffusion and transport of N by fungal hyphae (Gaillard *et al.* 1999; Frey *et al.* 2003).

The initial increase in soil solution pH might be associated with higher release of organic acid anions ($citrate^{3-}$) from roots into soil they complex H^+ from the soil (i.e. $H_2.citrate^{1-}$) and thus elevate the pH. The higher soil EC up to two weeks could be associated with to release of nutrient ions into solution from the roots (e.g. K, Na, Ca, Mg) and also the breakdown of organic N leading to the production of NO_3^-. The higher NO_3^- concentration of soil in the low N- treatment might be associated with conversion of ammonium to nitrate with increasing incubation period, which also

have resulted in decreasing NH_4^+ content of soil. The higher soil moisture content resulted in volatilization of ammonium and hence low concentration in soil of NH_4^+ might be other possible reasons.

Dissolved organic carbons (DOC) of soil have high nitrogen (N+) and low nitrogen (N–) treatment decreased with the passage of time. The decomposition of organic carbon containing compounds of soil might have decreased the DOC. Total dissolved nitrogen (TDN) of soil increased with the increasing incubation as compared to control. This might be due to release of TDN from organic residue.

The decrease in DON concentration may be due to the fact that DON is usually composed of wide range of compounds. The lower content of these compounds in DON might have decreased the DON concentration in soil.

5. Overall discussions

Poor availability of nutrients commonly constrains crop production in marginal environments of developing countries. Degraded soils and poor access to fertilizers limit the yields that can be produced by resource-poor farmers. In such circumstances, farmers need to use management techniques that maximize nutrient use efficiency of their crops. There are various techniques available, such as use of legumes in crop mixtures or rotations, or careful placement and timing of fertilizer applications.

In low-input agriculture the role of direct uptake of dissolved organic N (DON) by plants may be extremely important. DON represents a significant pool of soluble nitrogen in most ecosystems. Some plants may possess a greater capacity to take up DON rather than inorganic nitrogen (DIN). DON is composed of many compounds which enter soil from a range of sources (e.g. dry and wet deposition, litterfall, root and microbial exudation, turnover of roots and organisms, urine and faeces, organic fertilizers). My aim was to investigate the impact of plant residues on DON cycling when incorporated into soils and to study the uptake of DON in comparison with DIN by the plant root system. In addition, the secondary aim was to investigate the influence of the rhizosphere on the transformation DON in soil.

Soil samples were taken from the Henfaes Experimental Station. Soil was sieved and stored field–moist at 4°C. Three experimental treatment were used to alter organic inputs: (1) Soil amended with straw (high C/N ratio), (2) Soil amended with grass residues (low C/N ratio), and (3) Non-amended (control). Total dissolved N (TDN) in solution was determined with a Shimadzu TOCV-CSH analyzer. NH_4^+ and NO_3^- in soil solutions were determined colorimetrically. DON was calculated as the difference between the TDN and the combined NH_4^+ and NO_3^- reading (DIN).

Soil solution NO_3^- and NH_4^+ accumulated in the grass-amended soil in contrast to that

amended with straw or in the un-amended control soil. Overall, straw immobilized DIN in solution. DON in the grass amended soil increased from day 14 to 21 and sharply decreased thereafter whilst the straw amended soil and control remained relatively constant. Contrary to expectation, the results indicate that addition of organic matter did not cause a large rise in DON relative to that of DIN. This suggests that in this high fertility agricultural soil the microorganisms rapidly break down DON contained in N rich organic residues to DIN. For N poor residues DON appears to represent a more important source of N, however, its availability to plants remains as yet unknown.

It can be seen from Experiment 1 that soil solution ammonium increased in the grass-amended soil in contrast to that in straw-amended and control soil. It indicated that ammonification was rapid in this grassland soil. The data presented in Experiment 1 also shows that nitrate in the grass-amended soil increased up to 28 and then declined gradually.

6. Suggestion for future research

Although results from the studies described in this thesis were generally in accordance with respective hypotheses, there were several limitations that might weaken the interpretation.

This would include an investigation of the role of amino acid and peptide N in the N nutrition of crop plants (wheat, maize, rice). Radioisotopically labeled DON compounds (e.g. ^{14}C labelled peptides, proteins and amino acids) could be injected into the soil to determine the competition for these introduced N resources by monitoring uptake into the soil microbial community and plant roots and shoots over time. Influence of inorganic N on the uptake and availability of DON in the rhizosphere could also be investigated.

Understanding ecological processes at a scale relevant to microbial interactions and the cycling of nutrients holds the promise for rhizosphere engineering to improve nutrient

acquisition, and in particular, for management of greenhouse gas emissions needs to be addressed. The conversion of complex macro molecules in to simple plant available form needs to understand the transformations complexity and linking the physical heterogeneity of soil to ecology are justified for the prime frontier to be focus in near future both for plant and soil. Identification of microbial form, discovering their habitats and knowing their activity is a major challenge for microbial ecologist, which could be accomplishing through linking soil physico-chemical heterogeneity and biological process, and thus unrevealing the complexity of microorganisms-plants interaction, decomposition of organic matter and cycle nutrients including the production of greenhouse gases required would be address in changing climate scenarios

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5. APPENDICES

Appendix 5.1. Shows the mean performance and standard error of different parameters of soil amended with grass, straw and without amended incubated for 0 to 28 days in experiment 1.

	pH_{H2O}			**EC (µS cm^{-1})**		
Time	Control	Grass	Straw	Control	Grass	Straw
0	7.41±0.09	7.11±0.01	7.27±0.03	182.03±10.02	191.7±13.65	274.76±88.67
1	7.78±0.10	7.34±0.01	7.03±0.01	182.73±24.63	347.0±24.0	768.66±65.99
7	6.66±0.27	7.03±0.19	6.87±0.31	411.0±80.88	1983.66±98.26	657.33±79.19
14	7.20±0.04	6.87±0.15	7.37±0.12	546.66±47.90	3446.66±102.68	862.33±26.16
21	6.84±0.13	6.07±0.07	7.47±0.12	1571.66±818.11	8210.0±345.89	1049.66±165.17
28	6.52±0.31	5.51±0.06	7.03±0.09	903.33±247.79	5760.0±66.58	522.0±118.62

Appendix 5.1. (continued)

	MC%age (g kg^{-1})			NH_4^+ (mg N l^{-1})		
Time	Control	Grass	Straw	Control	Grass	Straw
0	37.08±1.11	39.13±0.64	38.96±0.43	0.12±0.02	0.23±0.03	1.04±0.76
1	41.07±1.61	46.42±0.69	50.34±20.02	0.11±0.01	0.15±0.03	3.22±2.89
7	41.07±1.06	44.92±1.43	40.48±1.71	0.13±0.00	1.33±0.76	1.22±0.94
14	40.15±0.74	46.39±1.36	37.43±0.91	0.42±0.12	2.37±0.46	0.39±0.03
21	38.24±2.42	50.18±1.29	38.03±0.54	0.52±0.05	4.39±2.56	0.38±0.09
28	7.96±1.52	49.92±5.15	36.61±0.78	0.21±0.00	0.92±0.17	0.46±0.06
	NO_3^- (mg N l^{-1})			DON (mg N l^{-1})		
Time	Control	Grass	Straw	Control	Grass	Straw
0	10.28±2.18	16.49±1.33	24.87±3.33	11.73±1.73	7.49±0.47	15.51±4.43
1	17.95±0.22	19.40±0.74	54.08±7.46	6.61±0.83	4.49±1.01	8.37±15.82
7	42.19±7.97	142.17±11.08	27.87±9.93	9.03±1.88	15.13±15.11	18.31±3.41
14	74.93±6.93	315.38±33.35	2.45±0.66	0.74±11.17	0.84±15.57	21.10±3.99
21	126.31±63.36	430.38±63.27	1.32±1.05	14.64±4.80	182.80±89.36	17.49±3.90
28	112.68±25.54	538.06±13.12	0.52±0.33	0.11±7.49	59.71±11.16	13.03±3.34

Appendix 5.1. (continued)

	TN (mg N l^{-1})			DIN (mg N l^{-1})		
Time	Control	Grass	Straw	Control	Grass	Straw
0	22.13±0.59	24.21±0.94	41.42±7.92	10.41±2.17	16.71±1.31	25.91±4.08
1	24.67±0.84	24.04±1.35	65.68±7.05	18.06±0.22	19.55±0.77	57.30±8.79
7	51.35±9.74	158.63±11.17	47.39±8.23	42.32±7.96	143.50±10.56	29.09±9.52
14	76.09±8.30	318.60±14.15	23.94±3.36	75.35±6.95	317.76±32.89	2.84±0.64
21	141.47±68.19	617.57±36.27	19.20±5.01	126.83±63.40	434.77±65.25	1.71±1.12
28	113.0±33.03	598.70±12.59	14.01±2.97	112.89±25.54	538.99±13.28	0.98±0.37
	DOC (mg C l^{-1})			**Phosphorous (µM)**		
Time	Control	Grass	Straw	**Control**	**Grass**	**Straw**
0	62.09±4.14	84.35±1.38	344.40±62.55	42.16±4.48	116.65±68.43	98.66±5.93
1	39.83±2.39	52.56±3.03	385.10±53.72	21.14±1.93	37.59±4.59	96.04±4.58
7	29.41±3.47	91.28±8.04	359.73±56.51	12.19±3.16	67.88±3.98	93.93±9.92
14	32.67±3.88	74.51±10.62	369.87±11.63	13.53±0.78	47.95±5.51	87.02±9.41
21	20.88±4.70	40.83±5.65	184.20±3.18	8.42±4.42	59.74±10.99	56.66±15.75
28	18.30±2.72	30.73±0.51	184.17±55.00	2.51±1.01	47.95±4.21	55.08±5.46

Appendix 5.2. Mean performance of different amino acids with three treatments. µM in experiment 1.

Treatments	Control		Straw		Grass	
	Mean	SE±	Mean	SE±	Mean	SE±
ALA	0.010	0.000	0.010	0.000	0.010	0.000
SAR	0.091	0.003	0.067	0.011	0.079	0.010
GLY	0.010	0.000	0.025	0.008	0.010	0.000
ABA	0.010	0.000	0.010	0.000	0.010	0.000
VAL	0.062	0.023	0.158	0.050	0.149	0.051
b-VAL	0.010	0.000	0.010	0.000	0.010	0.000
IS (norvaline)	20.000	0.000	20.000	0.000	20.000	0.000
LEU	0.010	0.000	0.010	0.000	0.010	0.000
aILE	0.010	0.000	0.010	0.000	0.010	0.000
ILE	0.010	0.000	0.010	0.000	0.010	0.000
THR	0.010	0.000	0.010	0.000	0.010	0.000
SER	0.010	0.000	0.010	0.000	0.010	0.000
PRO	0.010	0.000	0.010	0.000	0.010	0.000
ASN	0.010	0.000	0.010	0.000	0.010	0.000
TPR	0.010	0.000	0.010	0.000	0.010	0.000
ASP/MET	0.310	0.086	0.373	0.101	0.254	0.082
HYP	0.010	0.000	0.010	0.000	0.010	0.000
GLU/PHE	0.041	0.016	0.015	0.005	0.010	0.000
AAA	0.010	0.000	0.010	0.000	0.010	0.000
APA	0.010	0.000	0.010	0.000	0.010	0.000
APA/GLN	0.010	0.000	0.010	0.000	0.010	0.000
ORN/GPR	0.047	0.024	0.030	0.020	0.010	0.000
LYS	0.450	0.260	0.074	0.020	0.639	0.342
HIS	0.106	0.027	0.099	0.032	0.061	0.029
HYL	0.102	0.050	0.018	0.008	0.025	0.008
TYR	0.052	0.022	0.015	0.005	0.010	0.000
PHP	0.010	0.000	0.010	0.000	0.010	0.000
TRP	0.010	0.000	0.010	0.000	0.010	0.000
CTH	0.010	0.000	0.010	0.000	0.010	0.000
C-C	0.010	0.000	0.010	0.000	0.010	0.000

Appendix 5.3. Shows the mean performance and standard error of different parameters of soil amended with control, high (N+) and low (N-) nitrogen incubated for 0 to 28 days in experiment 2.

	$pH_{H2}O$			**EC (µS cm^{-1})**		
Time	Control	N+	N-	Control	N+	N-
0	5.80±0.08	6.44±0.07	6.41±0.04	142.38±3.30	1857.75±164.79	1733.25±147.53
1	6.13±0.05	6.38±0.05	6.46±0.07	139.03±1.33	1684.00±128.64	1941.25±141.46
7	5.96±0.04	6.16± 0.10	6.21±0.09	253.75±17.04	2043.75±152.22	2685.00±35.94
14	5.86±0.04	6.14±0.08	6.24±0.07	245.75±5.99	2752.50±86.73	2532.50±117.14
21	5.53±0.02	5.32±0.02	5.55±0.08	409.25±14.04	2415.00±47.87	3162.50±369.11
28	5.44±0.07	5.57±0.06	5.65±0.05	413.75±51.54	2452.50±70.28	3215.00±174.62
	MC%age (g kg^{-1})			**NH_4^+ (mg N l^{-1})**		
Time	Control	N+	N-	Control	N+	N-
0	25.24±0.95	34.20±2.19	32.44±2.89	0.09±0.04	8.86±1.90	12.74±2.11
1	24.21±0.73	35.79±2.63	34.61±0.92	0.00±0.01	12.63±2.28	9.50±3.18
7	25.11±0.14	35.75±3.07	35.06±1.82	0.06±0.06	0.09±0.01	0.74±0.32
14	23.29±0.54	30.99±2.19	32.89±2.63	0.08±0.04	0.28±0.23	1.51±0.35
21	21.97±1.00	30.96±3.56	30.17±1.01	0.06±0.03	0.28±0.06	1.95±0.55
28	22.31±0.48	29.33±1.65	30.58±0.21	0.22±0.01	0.47±0.11	1.15±0.34

Appendix 5.3. (continued)

	NO_3^- (mg N l^{-1})			DON (mg N l^{-1})		
Time	Control	N+	N-	Control	N+	N-
0	27.36±0.47	199.52±29.25	58.87±2.77	4.76±1.90	11.81±8.72	24.39±2.90
1	30.13±0.33	67.89±4.20	220.90±22.59	5.12±0.82	34.07±5.10	9.36±5.45
7	55.65±1.68	284.81±28.57	264.42±20.58	9.95±2.27	1.37± 5.24	9.45±9.09
14	58.78±1.65	327.43±16.29	234.50±15.50	12.20±1.77	8.59±11.25	2.28±12.54
21	72.38±2.33	323.35±11.51	346.01±50.86	15.42±4.37	14.05±9.97	20.67±11.30
28	88.56±2.01	322.44±23.84	361.88±32.78	23.29±3.07	23.01±24.38	-15.55±15.67
	TN (mg N l^{-1})			DIN (mg N l^{-1})		
Time	Control	N+	N-	Control	N+	N-
0	32.21±1.77	220.19±26.02	96.00±6.72	27.45±0.45	208.38±30.62	71.60±4.72
1	35.25±0.84	114.59±5.68	239.76±20.47	30.13±0.32	80.52±4.12	230.40±25.70
7	65.66±3.35	286.28±24.64	274.60±16.92	55.71±1.65	284.90±28.56	265.15±20.29
14	71.05±3.07	336.30±10.52	238.29±6.60	58.85±1.64	327.71±16.24	236.01±15.63
21	87.85±4.38	337.68±9.88	368.63±45.55	72.43±2.31	323.62±11.50	347.96±50.77
28	112.08±1.20	345.93±3.79	347.48±30.59	88.78±2.00	322.91±23.87	363.02±33.02

Appendix 5.3. (continued)

	DOC (mg C l^{-1})			AA (mg N l^{-1})		
Time	Control	N+	N-	Control	N+	N-
0	28.26±1.12	81.15±9.63	180.83±18.38	0.084±0.015	0.287±0.077	0.081±0.009
1	24.61±0.56	136.01±14.88	91.71±5.03	0.063±0.009	0.115±0.032	0.104±0.017
7	22.33±1.22	61.07±8.75	114.18±9.56	0.066±0.008	0.115±0.015	0.237±0.022
14	19.69±0.48	79.01±8.07	55.64±5.08	0.056±0.013	0.127±0.015	0.192±0.039
21	19.49±0.40	58.86±2.92	88.85±2.24	0.052±0.009	0.040±0.002	0.236±0.011
28	19.91±0.60	42.16±7.36	48.35±4.75	0.053±0.001	0.063±0.011	0.078±0.014