DYNAMICS OF PLANT RESIDUE DECOMPOSITION AND NUTRIENT RELEASE

By

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Abstract

Proper management of soil organic matter (SOM) contributes to increasing plant productivity and reducing dependency on mineral fertilizers. Organic matter is widely regarded as a vital component of a healthy soil as it plays an important role in soil physical, chemical and biological fertility. Plant residues are the primary source of SOM. Therefore, proper SOM management requires a better understanding of plant residue decomposition kinetics in order to synchronize nutrient release during decomposition and plant uptake and prevent nutrient losses. In natural and managed ecosystems, residues are added frequently to soil, in the form of dead roots and litter fall of plant species with different C/N ratios. However, in most studies on residue decomposition, residues with different C/N ratios are added once and the effect of the presence of plants on residue decomposition is rarely investigated. In this project, four experiments were carried out with different objectives in order to close these knowledge gaps.

The aim of the first experiment was to investigate the effect of frequent wheat residue addition on C mineralization and N dynamics. The experiment consisted of five treatments with different frequency of residue addition (2% w/w of wheat residues in total): once (100%W), every 16 days (25%), every 8 days (12.5%) or every 4 days (6.25%) and noresidue addition (control) with four replicates. The results showed that increasing frequency of low-N wheat residue addition increased C mineralization. Compared to 100%W, cumulative respiration per g residue at the end of the incubation (day 80) was increased by 57, 82 and 92% at 25%W, 12.5%W and 6.25%W, respectively. Despite large increases in cumulative respiration, frequent residue addition did not affect inorganic N or available N concentrations, microbial biomass C and N or soil pH. It is concluded that experiments with single residue additions may underestimate residue decomposition rates in the field because with several additions, soil microbes respire more of the added C (and possibly native soil C) per unit biomass but that this does not change their N requirements or the microbial community composition.

In the second experiment, the effect of mixing of high and low C/N residues at different times during incubation was investigated. There were 4 addition times; 25% of a total of 2% (w/w) residue was added either as wheat (high C/N) or lupin (low C/N) residue. Wheat residue was added to lupin residues on days 16 (LW-16), 32 (LW-32) or 48 (LW-48). Additional treatments were 100%L (added 25% of lupin residues on days 0, 16, 32

and 48) and 100%W (added 25% of wheat residues on days 0, 16, 32 and 48) and 0% (the control) with four replicates. Adding high C/N residues into decomposing low C/N ratio residue strongly decreased the respiration rate compared to the addition of low C/N residues, and lowered the availability of inorganic N, but significantly increased soil pH and altered microbial community composition. By the end of the incubation on day 64, the cumulative respiration of LW-16, LW-32 and LW-48 was similar and approximately 30% lower than in the treatment with only lupin residue addition.

The third experiment studied the effect of spatial separation of high and low C/N residues on decomposition and N mineralization. Each microcosm consisted of two PVC caps of 70 mm diameter and 20 mm height with the open end facing each other separated by a 30 μm mesh. The caps were filled with soil mixed with either low or high C/N residue with three replicates. Contact of high and low C/N residues led to an increase in the decomposition rate of the high C/N residues at the interface whereas it decreased it in the low C/N residues. The results showed that N and soluble C compounds moved from the easily decomposable residues into the surrounding soil, thereby enhancing microbial activity, increasing inorganic N and significantly changing soil pH in the layer 0-5 mm from the interface compared to the 5-10 mm layer of the high C/N residues, whereas the movement of soluble C and N to high C/N residues decreased the decomposition of the low C/N residues.

The final experiment investigated the effect of living plants on decomposition of high and low C/N residues. Wheat was grown in pots with a 30 μm mesh at the bottom. After a root mat had formed (>50% root coverage), a PVC cap with soil with high and low C/N residues (2% w/w) was placed against the mesh. The presence of plant roots significantly increased the respiration rate, N immobilization and increased the soil pH in the 0-5 mm layer in the first 4 days compared to the 5-10 mm layer. This enhanced microbial activity (and probably microbial biomass) can be explained by root exudates. The microbial community composition of plant treatments differed significantly from treatments without plants and the effect was greater in the immediate vicinity of the roots.

Chapter 1: General Introduction

Soil degradation, a current concern world-wide, results from improper land-use and cultivation, leading to soil nutrient deficiency, low plant productivity and ultimately low standards of living for farmers. Currently agriculture often results in exploitation of soil productivity without attention being paid to ameliorating the fertility of worn-out soils. Additionally, as the world population is projected to increase by nearly 35% in 2020 compared to the 1995 population, soil degradation is now regarded as an obstacle when tackling the need for a great increase in world food supply (UN 1996). In order to meet future world food needs it is possible to breed new cultivars with expected traits, but their yield potential may not be realized in over-exploited soils. This can be addressed if the benefits of soil organic matter decomposition are optimized. For long-term sustainable agricultural development, conservation of natural resources, especially soil organic matter (SOM), the product of decomposition of organic compounds added to soil and a good indicator of soil quality, is considered a key measure for ameliorating deteriorated soils (FAO 2003). Hence, addition of plant residues has now become a pivotal strategy for soil fertility improvement and sustainable of land use (FAO 2003). Plant residue composition and its breakdown rate affect soil physical properties such as the soil structure and porosity; water infiltration rate and moisture holding capacity of soils; biological properties (the diversity and biological activity of soil organisms); and chemical properties (plant nutrient availability) (FAO 2005). Knowledge of the processes involved in the plant residue decomposition is critical to integrated and sustainable agricultural management (Angers and Caron 1998). It is therefore important to understand the dynamics of decomposition and nutrient release patterns of plant residues as an important first step to better organic matter management.

It is possible to increase organic matter 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). Plant residue decomposition is very complex and affected by a wide range of factors. Many studies have been carried out on the dynamics of plant residue decomposition and factors affecting this process in different ways, such as adding crop residues or root

exudate-like substances and mixing nutrients, soil and plant residues, in order to obtain an in-depth understanding of decomposition mechanisms (Chintu *et al.* 2004; Potthoff *et al.* 2005; Kuzyakov *et al.* 2007). In these studies, plant residues were mainly added once and only one type of residue was used. However, in agricultural and natural systems, plant residues have been added into soil continuously often in mixtures of residues from different plant species. Additionally, since plants are often growing in soils during residue decomposition, another area that needs further research is the effects of roots on high and low C/N residue decomposition. Currently, the mechanisms of nutrient accumulation and the decomposability of plant residues with different attributes when mixed and frequently added in the presence or absence of plants are not thoroughly explored. Understanding the dynamics of decomposition and nutrient release patterns of frequent plant residue additions is critical to optimize the positive effects of SOM and sustain long-term productivity.

By assessing respiration, nutrient release and microbial community structure, this project aims to:

(i) investigate the effect of frequent plant residue addition;

(ii) study how low and high C/N residues interact spatially and temporally; and

(iii) investigate the decomposition rate of low and high C/N at different distances from the plant roots.

As outlined in Table 1.1, this thesis will review relevant literature (Chapter 2), describe materials and methods used to analyze given variables (Chapter 3), report on and discuss experimental results (Chapter 4, 5, 6 and 7) and provide a general discussion (Chapter 8).

Table 1.1 Thesis structure

Chapter	Content
Chapter 1	General introduction
Chapter 2	Review of the literature on plant residue decomposition and factors affecting it.
Chapter 3	Analytical methods used to measure microbial respiration rate, microbial community composition (PLFA), microbial C and N, inorganic N and soil pH.
Chapter 4	The effect of frequent wheat residue addition on C mineralization and N dynamics
Chapter 5	The effect of mixing of high and low C/N residues on C mineralization, microbial community composition and N dynamics.
Chapter 6	The effect of spatial separation of high and low C/N residues on C mineralization, microbial community composition and N dynamics.
Chapter 7	The effect of plants on decomposition of high and low C/N residues.
Chapter 8	General discussion and future research

Most of the information included in Chapter 4 has been published in *Soil Biology and Biochemistry*:

Duong TTT, Baumann K, Marschner P (2009) Frequent addition of wheat straw residues to soil enhances carbon mineralization rate. *Soil Biology & Biochemistry* **41**, 1475-1482.

Chapter 2: Literature Review

2.1. Plant residue decomposition

Soil organic matter (SOM) decomposition is generally considered as a substantial contributor to nutrient availability in the soil. Incorporating animal manure and other organic materials into the soil is recognized to have beneficial effects on soil physical, chemical as well as biological properties. Plant residues are the primary source of soil organic matter. According to NRCS (n.d.), SOM consists of different pools, humus, fresh plant residues (active fraction or particulate organic matter) and root exudates. These pools are linked by chemical and biochemical processes and differ in decomposition rate with rates decreasing in the following order: root exudates>fresh residues>humus. These components are the food source for the community of heterotrophic organisms. The significance of SOM in soil quality improvement makes it important to have a good understanding of how it decomposes, how plant residue nutrients are released and the factors affecting decomposition. Decomposition processes are affected by identified and unidentified factors of which nutrient availability, soil microorganisms, physical environment, crop residue quality, root exudation and rhizosphere priming effects are some of the determining factors (Singh *et al.* 2004). Environmental factors such as soil texture, moisture and temperature are very important because they can modify decomposition rates due to their effects on microbial activity. The quality of plant residues added to soils determines both the rate of decomposition and the nutrient dynamics. Residue quality i.e. chemical composition, namely carbon/nitrogen ratio (C/N ratio) and carbon/phosphorus ratio (C/P ratio) as well as the concentration of simple and complex compounds have been shown to be important. Typical green plant residues comprise: cellulose (45%), hemicellulose (20%), lignin (20%), proteins (8%), sugars and starches (5%) and fats and waxes (2%) (NRCS 2000).

When plant residues are returned to the soil, its organic compounds undergo microbial decomposition. Plant residue decomposition is a biologically driven process, i.e. the breakdown is accomplished by metabolic activities of soil organisms. Its rate is largely determined by three main factors: soil organisms, physical environment (temperature, moisture, soil textures and oxygen levels) and the quality of the plant residues (C/N ratios and other chemical properties). Chemical, physical and biological agents transform complex organic compounds into accessible organic and inorganic compounds available for absorption by plants, thus improving soil biological, chemical

and physical status, as shown in Figure 2.1. These activities then positively influence soil productivity and crop yields via improving soil structure and nutrient cycling. Plant residue composition changes during decomposition**.** The final products of plant residue decomposition include carbon dioxide, water, energy, microbial biomass, inorganic nutrients and re-synthesized organic carbon compounds such as humus, phenolics, celluloses, hemicelluloses and lignin (Baldock 2007). Humus has a very important effect on soil properties, as soil becomes darker, soil aggregation and aggregate stability are increased and it serves as a slow-release storage pool of N, P and other nutrients.

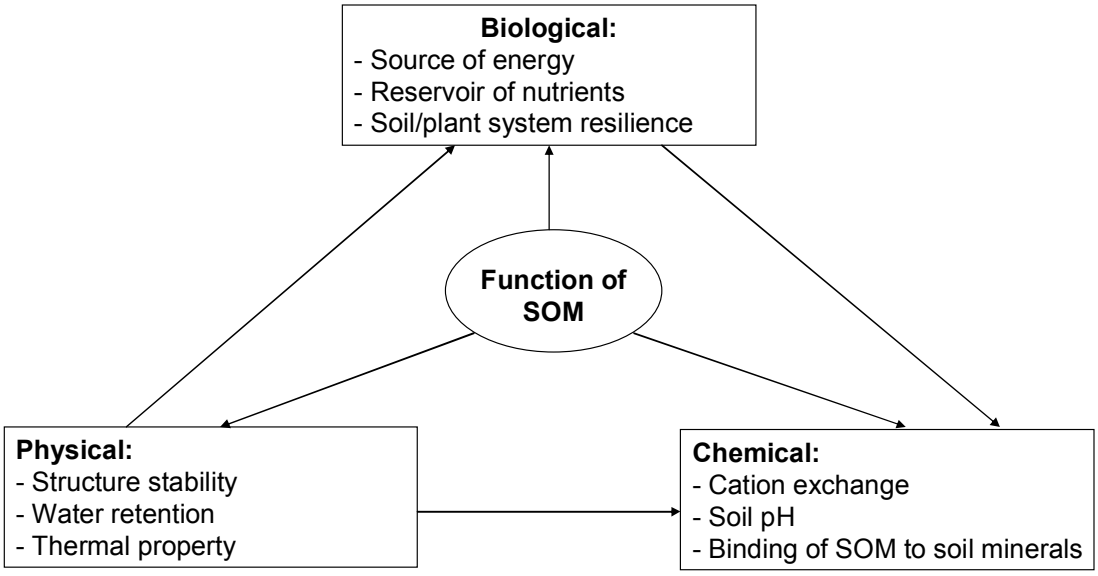


Figure 2.1 Function of SOM in soil quality improvement
(Planet Power n.d.)

The classification of organic compounds in residues is based on their molecular sizes, solubility and primary constituents, as seen in Table 2.1. When plant residues enter the soil, some components decay quickly, whereas others decay slowly. Simple compounds such as sugars, amino acids and low molecular weight phenolics are quickly decomposed, while polymeric molecules such as celluloses, hemicelluloses and lignin are decomposed slowly (Berg and McClaugherty 2003). The decomposition rate of plant residue is normally high initially because simple/soluble compounds are utilised by a large number of microorganisms.

Table 2.1 Representative compositions of corn, soybean and wheat residues (g kg^{-1} dry mass)

Components	Corn	Soybean	Wheat
Soluble components	293	557	288
Hemicellulose	268	90	184
Cellulose	284	222	361
Lignin	56	119	141
Ash	93	64	84
Nitrogen	10	22	9

Rapid decomposition of plant residue leads to residue mass loss. This is due to leaching of decomposed organic compounds and CO_2 release. Leaching is the loss of nutrients and incompletely decomposed compounds caused by water movement during the decay processes. The release of CO_2 is a result of microbial activity under aerobic conditions; under anaerobic conditions, organic acids or methane may be produced instead of CO_2 (Wood 1989).

The decomposition of plant residues has been studied by measuring a number of different parameters, such as carbon dioxide (CO_2) evolution or respiration, nutrient release and residue carbon. As plant residues are decomposed, microbial populations increase rapidly, evidenced by increased release of CO_2 leaving the soil via respiration. By measuring CO_2 evolution, the rate of mineralization of carbon in residues can be determined. Measuring residue carbon content during the decomposition process is another method that is used to determine decomposition rate. Nutrient release during decomposition is particularly interesting in terms of nutrient availability to soil microorganisms as well as plants (Trinsoutrot *et al.* 2000; Corbels *et al.* 2003; Baggie *et al.* 2005).

Soil respiration is a good indicator for assessing the decomposition rate of plant residues and respiratory activities of plant roots. These processes are accelerated or retarded by environmental factors due to their effect on metabolic activities of plants and microorganisms (Hu *et al.* 2006). Several studies have shown that temperature, water availability and pH are key factors affecting the rate of soil respiration (Hobbie 1996; Osono *et al.* 2003; Marschner *et al.* 2005). According to Nikliska and Klimer

(2007), temperature has a substantial effect on soil respiration and on the loss of plant residues directly and indirectly via its effect on water loss. The components released during decomposition are also driven by the properties of the plant residues. The respiration rate of plant residues with a high C/N ratio is much lower than that of low C/N plant residues as they do not contain sufficient N to satisfy the requirements of the soil organisms. In addition, they often contain large amounts of poorly decomposable C compounds such as celluloses or lignin. The respiration rate of mixed plant residues (mixture of high and low C/N residues) is normally higher than that of high C/N residues, whereas N release is higher. Aita and Giacomini (2003) evaluated the dynamics of decomposition and N release of cover crop residues as mixed and single applications: (a) black oat; (b) common vetch; (c) oilseed radish; (d) black oat + common vetch, and (e) black oat + common vetch. Their findings were that mixed residues of common vetch-oat gave a lower decomposition rate than the vetch alone but released higher amounts of N. Additionally, Jensen (1997) found that mixing plant residues in soil is a strong stimulus for the soil microbial biomass on and around the residue particles, which is regarded as a source for nutrients and as a catalyst for decomposition. However, respiration rate, the mechanisms of nutrient release when plant residues are added frequently and the effect of living plants are not 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

A range of chemical residue properties are good predictors of the evaluation of the rate and course of residue degradation. Residues typically consist of three fractions which differ in decomposition rate: 1) easily decomposable sugars and amino acids; 2) slowly decomposable compounds comprising cellulose and hemicellulose; and 3) recalcitrant materials such as lignin (Van Veen *et al.* 1984). Many studies have revealed that the initial concentration of N (Jensen 1997), P (Soon and Arshad 2002), lignin (Müller *et al.* 1988), polyphenols (Constantinides and Fownes 1994) and soluble C (Kachaka *et al.* 1993) are good indicators for plant residue quality and residue decomposition rates.

The initial N content of plant residues is one of the crucial factors accelerating or inhibiting residue decay, as it determines the turnover of the microbial mass mineralizing the residues (Heal *et al.* 1997). The N content of plant residues is positively correlated with the percentage of N mineralization. The soil C/N ratio is 12 and that of microorganisms is 8. Whereas the optimum C/N ratio for microbial growth is around 25, the C/N ratio of crop residues ranges from 20 to 500 and depends 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 the C/N ratio less than 40. Low C/N plant materials will meet the N requirements of soil microbial population and extra N will be mineralized and becomes available for plant uptake. Normally, plant residues of the *Poaceae* family such as wheat, oat, and barley have a high C/N and the *Fabaceae* or leguminous family such as vetches, lupin, soybean and mung bean have a low C/N. The study conducted by Soon and Arshad (2002) revealed that the decomposition rate of straw of three crops was, in order: pea>canola>wheat with residue N contents of 7.09, 7.04 and 5.06 mg g^{-1} straw and C/N ratios of 66, 71 and 97, respectively.

The lignin/carbohydrate ratio also affects the rate of plant residue degradation (Heal *et al.* 1997). Lignin plays an important role in plant cell wall structure and makes the cell walls resistant to microbial breakdown. The decomposition of plant cell walls is crucial in the breakdown of plant residues because it allows microbial access. Herman *et al.* (1997) stated that 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 easily decomposable compounds are depleted, lignin decomposers will be dominant and regulate the course of degradation (Berg and McClaugherty 2003).

Plant parts with different biochemical composition show different C mineralization kinetics. Generally, the organic carbon 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 as soil organic matter (NRCS 2000). According to Reinertsen *et al.* (1984), 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 studied (Palm and Sanchez 1991; Oglesby and Fownes 1992). According to Sivapalan *et al.* (1985), plant residue decomposition rate is decreased by the presence of high concentrations of polyphenolics, celluloses and waxes due to enzyme inhibition and binding of mineralized N to insoluble organic compounds. In addition, 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 concluded that plant residues high in polyphenols have low N mineralisation because of the formation of stable polymers between polyphenolics and amino groups. This conclusion was strengthened by Oglesby and Fownes (1992), who found that the initial polyphenol/N ratio was the best chemical index of N mineralisation.

2.2.1.2. Physical properties

Apart from their biochemical composition, the physical properties of plant residues and their contact with the soil have a pronounced impact on N immobilisation/mineralisation turnover (Bending and Turner 2004). Reducing particle size increases the surface area available for colonisation by soil micro-organisms and allows a more uniform distribution of residues in the soil. Hence, small-sized residues will decompose faster than residues of larger sizes. A study by Singh *et al.* (2004) showed that the particle size of canola residue had a significant effect on mineral N immobilization, but did not significantly affect the C mineralisation rate. They also found that canola particle size had no effect on microbial C and N during incubation.

However, little is known about the residue decomposition of high and low C/N residues when they are added repeatedly, separately and/or mixed in the soil and/or at different distances from the interface between high and low C/N residues.

2.2.2. Effects of environmental factors on decomposition

2.2.2.1. Soil properties (clay, aeration, pH)

Clay is one of the major soil texture components determining soil aeration and drainage and significantly affects residue decomposition rates. Clay is defined as soil particles less than 0.002 mm (2 μm). Clay concentration is positively correlated with aggregate size and aggregate formation and it was found to correlate negatively with potential N mineralization (Sylvia *et al.* 2005). Clay plays an important role in soil C, water and

nutrient retention by binding plant residues and chemical reactions between clay minerals (negatively charged) and cations such as ammonium (NH_4^+), thereby reducing residue mass loss. Epstein *et al.* (2002) concluded that the rate of SOM decomposition increased as soil clay content decreased due to the deceased oxygen levels in soils, and the accumulation of SOM was positively correlated with soil clay concentration.

Adequate soil aeration accelerates the decomposition of plant residues and the growth of micro-organisms. Sufficient oxygen stimulates soil organisms to convert organic compounds into inorganic compounds. 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 effect on microbial activity, soil pH influences plant residue decomposition processes. It also affects nutrient solubility and can change microbial community composition. Nutrient cycling is slowed or stopped if microbial populations are negatively affected (Bolan and Hedley 2003). Microbial activity is greatest at neutral soil pH. According to Allison (1973), high pH and nitrogen concentrations will favour multiplication of bacteria, while low pH and nitrogen concentrations will favour the growth of fungi. Results from a pot experiment using ten soil types by 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 altered by soil pH. In terms of the role of plant residues in soil pH changes, the degree of plant residue decomposition, the pattern of release of anions and cations and immobilization by microbes will affect soil pH. Additionally, plant residues with high concentrations of organic nitrogen such as proteins and amino acids will result in a higher mineralization of ammonium and/or nitrate in soil. Ammonification produces hydroxyl ions (OH^-) and increases soil pH, whereas nitrification produces protons H^+ and hence acidifies soils (Bolan and Hedley 2003) as illustrated in the chemical reactions (1) and (2). The study by Xu *et al.* (2006) showed that the soil pH change after the addition of plant residues was dependent on plant residue type and initial soil pH. They found that the addition of legume residues first increased soil pH,

but then decreased it after a 42 day-incubation, whereas soil pH remained relatively constant after the addition of low N wheat residues.

- Ammonification:

$$R\text{-}CO\text{-}NH_2 + 3H_2O \rightarrow 2NH_4^+ + \mathbf{2OH^-} + CO_2 \quad (1)$$

(R is defined as a carbon chain with indefinite length)

- Nitrification: $NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + \mathbf{2H^+}$ (2)

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 ^oC, but the relationship between microbial activity and temperature is dependent on microbial species. Microbial respiration is positively correlated with temperature and it depends on the adaptability of organisms to their soil habitat (Berg and McClaugherty 2003). Eijsackers and Zehnder (1990) concluded that, under aerobic conditions, the increase in residue decomposition with increasing temperature would result in increased N release and a reduced rate of SOM accumulation. Besides soil temperature, soil moisture also has a strong effect on residue decomposition. Adequate moisture will accelerate the rate of decomposition and the growth of microorganisms as water is required for the breakdown of plant residues. However, high moisture levels will result anaerobic conditions and may hinder processes. Osono *et al.* (2003) found that the survival and activity of *Chamaecyparis obtusa* was positively correlated with the 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.

2.2.3. 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. Due to the presence of root exudates

which are relatively easily decomposable, the rhizosphere is a habitat for microorganisms that differs substantially from that of the bulk soil. The microorganisms in the rhizosphere can stimulate or reduce plant growth.

2.2.3.1. Root exudation

Root exudation is an important stimulus for decomposition of plant residues. The root exudation is part of rhizodeposition, which is a main source of soil organic C exuded by growing plant roots. Rhizodeposits are classified depending on their mode of arrival, namely exudates, secretions, lysates and gases. Paterson *et al.* (2006) stated that organic compounds secreted from roots affect the rate of residue decomposition and nutrient release through impacts on the activity and abundance of decomposer populations in the soil. This also contributes to a greater aggregate stability as soluble root exudates can act as glue between clay particles (Hütsch *et al.* 2002). The release of organic substances from plant roots is crucial in plant-microbe interactions which have strong effects on microbial activity (Lynch and Whipps 1990) and microbial community structure (Marschner *et al.* 2001) and therefore nutrient release.

Rhizosphere respiration can be regarded as an indicator of the rate of root exudation owing to the direct correlation between microbial growth and root exudation. However, it should be noted that CO_2 production in the rhizosphere results from root and microbial respiration (Haller and Stolp 1985).

The quality and quantity of compounds exuded differs with plant species, age (Lynch 1990) and the availability of mineral nutrients (Eldhuset 2005) and external factors. Normally, young plants secrete nearly twice as much of their fixed C as root exudates as older plants. The composition of root exudates usually includes sugars, amino acids, organic acids, hormones, vitamins, and unidentified substances such as microbial growth stimulants and inhibitors (Lynch and Whipps 1990). While sugars provide available sources of carbon for microbial growth, amino acids are a readily available source of nitrogen for microbes (Baldock 2007). Kuzyakov *et al.* (2007) simulated the rhizosphere by adding malate, glucose and glutamate at two different temperatures (15^{o}C and 25^{o}C) to soil with residues and found that the addition of these labile root exudates significantly increased plant residue decomposition, but the increase was regulated by temperature. The present project will address the question of how

microbial community structure, residue respiration as well as nutrient availability will be modified in the presence of living plants and rich and poor N-residues.

2.2.3.2. Rhizosphere priming effects

The rhizosphere priming effect (RPE) is also an important factor affecting the course and rate of plant residue decomposition. As defined by Kuzyakov *et al.* (2000), priming effects (PEs) are short-term changes in the SOM turnover induced by the addition of different organic and mineral substances to the soil. Input of C sources increases microbial growth and may induce PEs. The activity and density of microorganisms determines the SOM turnover. The RPE is defined as a priming effect in the soil surrounding living roots of plants. As reported by Fu and Cheng (2002), soil treatment such as tillage and fertilizer applications has a great influence on plant root exudation and rhizosphere deposition and thereby may induce RPEs. Different plant species and plant development stages have different RPEs. The RPE of low C/N plants is higher than those of high C/N plant species (Fu and Cheng 2004).

Many factors affect RPEs. As reviewed by Kuzyakov (2002), these include root proliferation; plant and soil types; water and nutrient uptake; preferential substrate utilization of soil organisms; soil C and N contents, and rate of photosynthesis. Dijkstra *et al.* (2006) evaluated RPE as affected by plant biomass in two different plant species in two previously differently managed soils, an organically farmed soil and a soil from annual grassland. Their conclusion was that the rhizosphere priming effects of annual plants on SOM decomposition are largely regulated by plant biomass, especially in soils of high fertility that can sustain high plant productivity. Kandeler *et al.* (2002) carried out a study of microbial community composition and functional diversity as affected by the presence of maize roots. Their conclusion was that the microbial community composition within the first 2.2 mm from the root surface was significantly different from that at greater distances, which might consequently influence the decomposition of SOM. However, it is not clear at which distance from the root surface RPE occurs and how residue C/N affects RPE.

2.3 Dynamics of N and P during the decomposition of plant residues

Mineralization and immobilization are the two major processes taking place during the decomposition of plant residues. Jansson and Persson (1982) stated that the availability

of crop residue nitrogen is driven by the balance between mineralization and immobilization and also the stabilization of residue N in SOM pools. Mineralization is defined as the conversion of an organic form of an element to an inorganic form as a result of soil microbial decomposition (Sylvia *et al*. 2005). The products from mineralization consist of available forms of nutrients which can be readily absorbed by plants and living organisms. Conversely, the process of converting inorganic forms in soils into microbial biomass is called immobilization, which helps reduce the loss of nutrients via leaching and erosion.

The mineralization of N from soil plant residues is the major source of N for plants and soil microbes. It is highly dependent on plant residue chemical composition. Nitrogen losses from the soil-plant system via leaching, denitrification and ammonia volatilisation can influence how much N from plant residues is available for subsequent crops. It is therefore crucial to have a better understanding of the dynamics of plant residue N turnover so as to minimise these losses of N.

The main identifiable organic N compounds in soil are amino acids and amino sugars; among them, amino acids are readily mineralized under aerobic conditions (Stevenson 1982). During mineralization, under microbial activity, organic N first deaminates to amino acids (equation 3) which are then converted into NH_4^+. The mineralised NH_4^+ can be immobilized, nitrified to NO_3^-, released from the cells and utilized by other microorganisms as N or energy source (equation 5) (Bolan and Hedley 2003). The continuous process of transferring 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 called "net N mineralization". This portion is a major source of N for plants. Plants can utilise NH_4^+ and NO_3^-. Plants and soil microorganisms compete for the mineralized N.

- Deaminization: Protein or amino acids $\rightarrow$ R-CO-NH_2

$$H_2NCHRCOOH + H_2O + 2H^+ + 2e^- \rightarrow R\text{-}CO\text{-}NH_2 + NH_4^+ + OH^- \quad (3)$$

- Ammonification:

$$R\text{-}CO\text{-}NH_2 + 3H_2O \rightarrow 2NH_4^+ + 2OH^- + CO_2 \quad (4)$$

(R is defined as carbon chain with indefinite length)

- Nitrification: $NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+$ (5)

Net N mineralization is mainly regulated by the C/N ratio of plant residues and soils. Berg and McClaugherty (2003) stated that net N mineralization from plant residues is based on the relationship between (1) gross N mineralization and respiration (C mineralization) and (2) gross immobilization and respiration. Net N mineralization is strongly dependant on the C/N ratio of plant residues added. On average, the C/N ratio of microorganisms is 7:1, thus suggesting 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. Therefore, 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). Thus, 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 dominates at a C/N ratio of residue > 25:1. When low N plant residues are first mixed into the soil, N immobilization will occur because these plant materials do not meet the N nutritional needs of soil microbes. The N immobilized by living organisms will be released when microbes die off. Singh *et al.* (2006) conducted a 2-year field experiment to evaluate the impact of single and mixed plant residues with different C/N ratios in a tropical dry-land area. They 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. Additionally, the C/N ratio in soils affects the N mineralization process. When the N content in soils and N released from SOM decomposition exceed the N required by microbial decomposers, net N mineralization may occur (Carlyle 1986). These processes are governed by the physical and chemical factors that have been reviewed in the previous sections of this review. However, the dynamics of N mineralization in soils, when high and low C/N plant residues are incorporated in the soil individually or mixed repeatedly, remain unknown and are one of the main topics in this project.

Inorganic, organic and microbial P are the major forms present in soils. Organisms need phosphorus for metabolic activity and as a structural component of a large number of biochemical compounds. Only P in the soil solution is available for plant uptake, but its concentration is usually low because of precipitation and immobilization in the soil. Phosphorus contained in crop residues or green manures added to the soil may increase total soil P considerably. The amount of P in the soil solution strongly depends on the P

released from the soluble P fraction in plant residues during decomposition. Inorganic phosphate in soil solution is the P form taken up by plants, but this soluble P is easily absorbed by Al and Fe oxides and hydroxides, clay colloids or immobilized by the microbial biomass. On the other hand, the concentration of P in microorganisms differs depending on its growth stage as well as residue quality. According to Webley and Jones (1971), over 60% of microbial P is usually in the form of nucleic acids, 20% in acid soluble P-esters and 5% in phospholipids, and these proportions may vary based on growth stage of the cells, residue management and cultivation practices.

Mineralization and immobilization of P strongly affect plant-available P in soil and change during plant residue decomposition. During the decomposition, organic P in plant residues is mineralized by phosphatase enzymes which are produced by microorganisms, plants and mycorrhiza (Tarafdar and Jungk 1987). The amount of P in soil solution is dependent on the P released from plant residues. Friesen and Blair (1988) and Kwabiah *et al.* (2003) investigated the relationship between plant residue quality, available (resin extractable) P and microbial biomass P and C. They concluded that the initial increase of available P was a result of the release of soluble P from plant residues. Regarding P immobilization, McLaughlin *et al.* (1988) investigated the role of the microbial mass in P cycling with ^{32}P-labelled fertilizer and ^{33}P-labelled medic residues. They found that the P amount of the fertilizer ^{32}P and ^{33}P in the medic residues recovered in the microbial biomass were 5% and 22-28%, respectively.

2.4. Conclusion

Plant residue decomposition is a continuous and complex process. Several mechanisms are well understood, while others, such as frequent plant residue addition, mixing of residues with different quality, and rhizosphere priming effects are poorly studied. In order to improve soil biological, chemical and physical status, organic matter, crop residues, manure and other sources may have to be added frequently and be (in amounts that are) equal to the rate of decomposition. In most studies conducted to date, plant residues have been added only once. However in natural and agricultural ecosystems, residues may be added frequently and as in the form of mixtures of different plant residues. The decomposition rate of such small amounts of residues when frequently added may differ from that of a large amount added once. Small amounts of residues added may help accelerate the decomposition rate by maintaining an active biomass that decomposes the subsequently added residues more rapidly. Until now, little is

known about the frequent addition of mixed plant residues into soils and their effects on residue decomposability and nutrient accumulation, as well as interactions between plant residues when spatially separated. It is hypothesized that adding plant residues with low and high C/N to soils more frequently will accelerate the decomposability of plant residues and consequently increase nutrient availability. Frequent plant residue addition could be a useful measure to improve soil fertility and soil nutrient status. Since plants are often growing in soils during residue decomposition, another area that needs further research is the effects of roots on high and low C/N residue decomposition. The present project will help answer these questions and aid in management decisions for soil fertility improvement.

The project is designed to further elucidate the role of residue addition frequency in decomposition rates and nutrient release and the response of the soil biomass to different C/N ratio residues. It is focused on studying microbial activity and community composition, N release, microbial C and N and pH, with the aims of determining the effect of:

- Frequent addition of high C/N residues

- Addition of high to low C/N residues at different times during decomposition

- Distance from the interface between low and high C/N residues

- Distance from the interface between roots and residues

Chapter 3

General Materials and Methods

3.1 Experimental System

This project consists of four experiments, three incubation experiments and one glasshouse experiment. The experimental system consisted of a sandy loam soil maintained at 85% of water holding capacity and high and low C/N plant residues (wheat, lupin and lucerne). The air-dry soil was moistened to 85% water holding capacity and pre-incubated for at least 14 days at 25^{o}C to allow the microbial community to recover and stabilize. The residues were added at 2% by weight of soil at different intervals. Autoclaved RO water was added every four days to maintain 85% water holding capacity. The sandy loam soil-residue mixture was incubated in PVC cores with a radius of 1.95 cm, and height of 5cm with a nylon mesh base. Microbial respiration was measured throughout the course of the experiments by measuring carbon dioxide (CO_2) evolution. Other properties such as microbial C and N, pH, available nitrogen and microbial community structure were assessed every sixteen or twenty days depending on the experimental design. The duration of the incubation varied between experiments, ranging from 60 to 80 days. The different treatments used in each experiment will be described in more detail in Chapters 4, 5, 6 and **7**.

3.2. Materials and Methods

3.2.1. Soil and plant residue

A sandy loam soil was obtained from 0-20 cm depth of a natural bushland in Monarto in the semiarid region of South Australia (latitude 35°05´ S, longitude 139°06´ E and elevation 166 m), 60 km south-east from Adelaide and located on the eastern margins of the southern Mount Lofty Ranges. The soil properties are shown in Table 3.1. The soil was air-dried at room temperature and all visible plant and insect debris was removed manually after which it was sieved to <2 mm. For each experiment, the soil was wetted with 144 g of RO water to 1000g of soil to obtain 85% water holding capacity, and incubated at 25^{o}C for at least 2 weeks in the dark before mixing with plant residues. This was done to avoid a flush of microbial activity at the start of the experiment that occurs when air-dried soil is moistened (Fierer *et al.* 2003). The pre-incubation allows recovery and stabilization of microbial biomass and activity after

rewetting of the air-dried soil. Previous studies have shown that microbial activity is stable after 10 days of incubation (Oehl *et al.* 2001).

Table 3.1 Soil properties

Property	
Clay (%)	20
Silt (%)	11
Sand (%)	69
Bulk density (g cm^{-3})	1.61
pH	6.6
Organic C (%)	0.92
Particulate organic carbon (%)	0.16
Total N (g kg^{-1})	0.4
Resin extractable Pi (mg kg^{-1})	1.28
Total P (mg kg^{-1})	90.90
Water holding capacity (%)	18

The mature shoot residues used in this project are important in Australian agriculture: they were wheat (*Triticum aestivum* L.), lupin *(Lupinus albus* L.) and lucerne (*Medicago sativa* L.). The residues were ground and sieved to 0.25-2 mm. This was done to allow thorough mixing and to minimize the physical effects on decomposition that may occur if large pieces of residues with different nutrient ratios are spatially separated from each other. Table 3.2 shows the properties of the residues used in the experiments.

Table 3.2 Residue properties

Property	**Wheat**	**Lupin**	**Lucerne**
Total C (g kg^{-1})	427	425	410
Total N (g kg^{-1})	5	19	22
Water-soluble C (g kg^{-1})	12	157	66
Water-soluble N (g kg^{-1})	0.7	13.1	7
C/N	85	22	18

3.2.2 Analytical methods

3.2.2.1. Respiration measurement

Containers with 40 g or 20 g of soil depending on the experiment were placed individually in Mason jars (volume 1l) together with a vial containing 15 ml of RO water in order to maintain the humidity and minimize water loss from the soil; the jars were incubated at 25ºC in the dark. The Mason jars, which have a septum in the lid to allow gas sampling, were sealed between sampling for CO_2. The CO_2 released from the incubated samples was measured with a Servomex 1450 series foodpack gas analyser every 24 hours for the first four days, after new residue additions, and every 48 hours from day 4 until samples were then harvested. This interval was based on the decomposition rate after residue addition found in earlier studies. For measuring respiration, a needle was pushed through the septum in the lid of the Mason jar and the gas withdrawn by suction. After measurement, the jars were flushed with ambient air and then closed. The CO_2 concentration was measured again immediately after resealing. To maintain soil moisture, the water content was adjusted every four days to the initial weight. Since the samples were incubated in Mason jars or large closed containers with lids to minimise water loss; only small amounts of water had to be added every four days. In Experiment 4, the weight of pots was checked daily and water was added if necessary to maintain the soil moisture.

3.2.2.2. Calculation of respiration parameters

Depending on the experiment, calculation of CO_2-C evolution (mg g residue^{-1} day^{-1}) was based either on the amount of soil or on the amount of residue added in total at a given sampling time.

CO_2-C (mg g residue or soil^{-1} day^{-1}) = CO_2-C (mg)/ amount soil or residue (g)/ time since resealing (h) * 24.

Cumulative respiration was calculated by adding the respiration rates described above over time.

Calculation of the percentage of added residue C respired was based on the amount of C present at a given time with 440 mg C/g of residue = 100-[100-(440-cummulative mg CO_2-C g residue^{-1})/440*100].

3.2.2.3. Available N

Available N was determined based on Page *et al.* (1982). Briefly, 5 g of moist soil was shaken with 50 ml of 2 M KCl for one hour. After a one hour settling time, the supernatant was filtered through a Whatman no. 42 filter paper and stored at 4°C in a refrigerator until analysis took place.

Inorganic N in the extract was measured colorimentrically. NH_4^+-N was measured by the nitroprusside/dichloro-S-triazine modification (Blakemore *et al.* 1987) of the Bertelot indophenol reaction (Searle 1984) at 640 nm. NO_3^-- N was measured by the cadmium reduction method (Henriksen and Selmer-Olsen 1970). NO_3^- is reduced almost quantitatively to nitrite (NO_2^-) in the presence of cadmium (Cd). The NO_2^- produced then is determined by diazotizing with sulfanilamide and coupling with N-(naphthyl)-ethylenediamin to form a highly colored azo dye that is measured colorimetrically at 560 nm.

3.2.2.4. Microbial biomass C and N

Microbial biomass C and N were determined using the method of (Vance *et al.* 1987). Of the two 10 g sub-samples of moist soil, one sub-sample was fumigated with chloroform for 24 hours in a desiccator followed by extraction with 40 ml 0.5 M K_2SO_4 over 1 hour by shaking at 150 rev min^{-1} and then settling down for half an hour. The non-fumigated sample was directly extracted with K_2SO_4. The extracted solutions were then filtered through Whatman no.42 filter paper and were stored at -20ºC until analysis. Before analysis, the samples were defrosted overnight, and then diluted 2:8 with ultra-pure water. The C and N concentrations of diluted extracts were measured with a Slakar DOC analyzer. Microbial biomass C and N were determined by the difference between C and N concentrations of fumigated and non-fumigated samples.

3.2.2.5. PLFA extraction and analyses

Phospholipids fatty acids analysis (PLFA) is one of the most popular methods used to characterize soil microbial community composition. Compared to fatty acid methylester (FAME), PLFA patterns are used to determine active microorganisms in soil only. FAME profiles may also include fatty acids from microorganisms that died recently and plant residues (Jandl *et al.* 2005). Those two methods may underestimate fungal biomass as a limited number of signature fatty acids for fungi have been identified. On

the other hand, DGGE (Denaturing Gradient Gel Electrophoresis) which is used to characterize dead and living organisms relies on separating species of a chosen group of microorganisms (e.g. bacteria, fungi, archaea) according to differences in sequence of target DNA/RNA regions (Wintzigerode *et al*. 1997). This method may not assess all microorganisms in soil as DNA/RNA extraction can be incomplete because microbial cells can be located within soil aggregates and adhere tightly in SOM and minerals. Additionally, as spore walls of fungi and gram-positive bacteria are tough and thick, DNA may only be released after rupturing the spore walls (Wintzigerode *et al.* 1997).

Microbial community composition was analyzed by PLFAs using a modified version described by (Bardgett *et al.* 1996). For PLFA analysis, soil samples were frozen at -80 ^{o}C for at least 5 hours before being freeze-dried for at least 3 days. The extraction of PLFA was carried out in three steps using 2 g of freeze-dried soil sieved to 2 mm. During the first step, lipids are extracted from freeze-dried soil after two hour incubation with a single-phase mixture of chloroform, methanol and aqueous citrate buffer (pH 4.0) by a modification of the method described by (Blight and Dyer 1959). At the second step, the PLFAs were separated from other lipid classes (neutral lipids and glycolipids) by using silica-bonded phase columns (Supelco, Bellefonte, PA, USA). Finally, the polar lipid fraction was methylated by using a mild-alkaline solution which leads to the production of methyl esters of PLFA which were detected by GC-FID. The peak areas expressed in % peak height of the internal standard were subjected to multivariate analyses (see below).

The PLFA was analyzed by gas chromatography (GC) (Agilent Ultra 2 column; temperature ramping 120 to 280°C at a rate of 5ºC per min) using helium as the carrier gas and peaks were detected by a flame ionization detector (Frostegård *et al.* 1996). Identification of methyl fatty acids was based on a comparison of retention times with FAME standards (FAME, C_4-C_{24}, 18919-1, Supelco). The form of A:BωC is the standard nomenclature when referring to different fatty acids (Frostegard *et al.* 1993) in which A designates the total number of carbon atoms, B the number of double bonds, and C is the position of the double bond or the distance of the closest unsaturated C from the aliphatic end of the molecule. A methyl end of the molecule is indicated by ω and a carboxyl end is indicated by Δ. The abbreviations of "-c" and "-t" are for "cis" and "trans", referring to geometric isomers. The prefixes "i-," "a-," and "me-" indicate

iso-, anteisomethyl branching, and mid-chain methyl branching, respectively, with cyclopropyl rings indicated by "cy" (Kates 1986).

The amount of individual PLFA(s) was expressed as the percentage area of the internal standard (C19:0). Signature PLFA(s) were used for indicating specific microorganism groups. According to (Zelles *et al.* 1992), signature fatty acids for bacteria are iC14:0, iC15:0 ($Gram^+$), aC15:0, iC16:0 ($Gram^+$), i17:0, cy17:0, cy19:0, 16:1ω9c, 16:1ω7c ($Gram^-$), 17:1ω8c and 18:1ω7c ($Gram^-$). Actinomycete signature fatty acids are 10Me16:0, 10Me17:0 and 10Me18:0. Other microbial groups identified with PLFA biomarkers are fungi ((18:2ω6c, 18:1ω9c and 18:3ω3c (Frostegard *et al.* 1993), (Zelles *et al.* 1992)), protozoa (20:2ω6c and 20:3ω6c, (White *et al.* 1996)) and algae (20:5ω3c, (Khotimchenco *et al.* 2002)). The ratio of fungal/bacterial PLFAs can be calculated as follows [(18:2ω6c)/i14:0, i15:0, a15:0, 15:0, i16:0, i17:0, 17:0, 16:1ω9c, 16:1ω7c, 17:1ω8c, 18:1ω7c, 18:1ω9c, cy17:0 and cy19:0)]. Microbial biomass was calculated as the sum of the amount of all PLFAs.

3.2.2.6. Soil pH

Five g of freeze-dried soil was mixed with 25 g of RO water. This solution was shaken at 1500 rpm in 60 minutes, allowed to settle for 30 minutes and then the pH was measured by the pH meter-Expandable ion Analyser EA 940 at 25^{o}C.

3.2.3. Data analysis

The data collected were analyzed statistically using the Genstat 10th edition and Microsoft Excel. Analyses of Variance (ANOVA) were performed to examine statistical differences between treatments. Any results declared as statistically different are done so at a 5% level of error ($P < 0.05$ or 95% confidence interval).

The PLFA data were log (x+1) transformed to focus attention on patterns of the whole microbial community by giving rare fatty acids a similar weighting as common fatty acids. Primer E software (Primer-E Ltd, Plymouth Marine Laboratory, Plymouth, UK) was used to analyze log transformed PLFA patterns. The PLFA patterns were analyzed by Non-metric multi-dimensional scaling. Differences between treatments were analyzed by PERMANOVA. DistLM was used to assess the relationship of the different fatty acids or of the environmental variables to the observed patterns.

Chapter 4

Effect of Frequency of Residue Additions

4.1 Introduction

Proper management of plant residues as a source of nutrients may increase plant productivity and reduce dependency on mineral fertilizers (Kachaka *et al.* 1993; Salas *et al.* 2003). Applying mineral fertilizers can have a number of disadvantages, such as limited availability of natural resources, soil and water pollution and high cost. The incorporation of plant residues in agricultural systems is known to influence soil physical properties (Hulugalle *et al.* 1988; Barzegar *et al.* 2002), availability of nutrients (Asghar *et al.* 2006), soil biological activity (Tian *et al.* 1992; Alguacil *et al.* 2008) and soil organic matter content (Spaccini *et al.* 2002). Proper residue management requires a better understanding of plant residue decomposition kinetics. When plant residues are incorporated into the soil, various organic compounds undergo microbial transformation into inorganic and/or organic compounds. The dynamics of crop residue decomposition are complex and driven by three major factors: soil microbial activity, the physical environment such as temperature, water availability and soil texture, as well as plant residue composition (FAO 2003). During plant residue decomposition, different microbial groups will dominate at different stages depending on their capacity to utilize easily decomposable substances or recalcitrant compounds (Paterson *et al.* 2008; Paterson *et al.* 2006; Stemmer *et al.* 2007). Plant residue composition has a particularly strong effect on the decomposition (Moritsuka *et al.* 2004; Trinsoutrot *et al.* 2000). The decomposition rate of plant residues with a C/N ratio <21 is higher than that of residues with high C/N ratio as they satisfy the N requirement of microorganisms and the excess N will become available to plants and microorganisms (Walley and Yates 2002). To date, numerous studies on plant residue decomposition have been carried out in which residues were added once to the soil, but the kinetics of plant residue decomposition when they are added frequently remains unclear. In the field, plant residues are added to soil frequently via dying roots, leaf fall and root exudates. Incorporating crop residues frequently into the soil could result in enhanced decomposition of organic C as it stimulates and maintains a high biological activity, and thus the percentage residue decomposed may increase with the increasing number of additions.

The aim of this experiment was to determine the effect of frequent plant residue addition on the rate of residue decomposition as assessed by respiration and nutrient availability. It is hypothesized that increasing frequency of residue additions will stimulate microbial activity and increase the percentage of residue decomposed, because the microorganisms in the soil remain active due to the constant supply of easily decomposable compounds. A second aim was to investigate if the frequency of residue addition affects soil microbial community structure.

4.2 Materials and Methods

The experiment was carried out using mature wheat residue incorporated into the soil with different portions at different intervals. The treatment structure was 5*4*6 (5 treatments by 4 replicates by 6 sampling times). The total number of experimental units was 120. The experiment was set up as a randomized complete block design. Each repetition was set up as a block so that there were 4 blocks.

The experiment was carried out with the soil from Monarto and wheat straw as described in Chapter 3. The analytical methods used are described in Chapter 3.

4.2.1. Experimental design

Soil moisture was maintained at 85% water holding capacity during the experiment. The total rate of plant residue added was 0.8 g of plant residue in 39.2 g dry soil. After the 2-week pre-incubation of the unamended soil, treatments with different frequency of residue addition were set up as follows (see also Table 4.1):

- 100%W: 100% of the wheat residue was added on the first day of week 0;
- 25%W: 25% of wheat residue was added on day 0; and 25% was added on days 16, 32 and 48;
- 12.5%W: 12.5% of the residue was added every 8 days;
- 6.25%W: 6.25%of the residue was incorporated into the soil every 4 days.
- Control: no residues added (0%)

For all treatments, the soil was mixed thoroughly every 4 days.

Table 4.1 Treatments and frequency of plant residue addition

Day	0	4	8	12	16	20	24	28	32	36	40	44	48	52	56	60
100%W	100															
25%W	25				25				25				25			
12.5%W	12.5		12.5		12.5		12.5		12.5		12.5		12.5		12.5	
6.25%W	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25
0%	0															

Soil samples (40 g soil per container) for each sampling date were kept separately. There were 100 containers (5 treatments×4 container/treatment×5 sampling dates, not including samples for day 0). The samples were incubated in the dark in a constant temperature room (25ºC). Every 4 days, the soil moisture was maintained at 85% water-holding capacity by addition of autoclaved RO water and the soil was mixed. On day 0 and then every 16 days, 4 replicates of each treatment were placed in 1l glass jars for measurement of respiration over 16 days after which microbial community structure (PLFAs), available N, microbial C and N and pH were determined. Respiration rate was measured over 16 days as described in Chapter 3. Then the next set of 4 replicates per treatment was placed in glass jars for respiration measurement and determination of the parameters after 16 days. Respiration rate and cumulative respiration are expressed per g total residue added at a given time.

4.3. Results

4.3.1. Respiration rate and cumulative respiration

The respiration rate of the control was very low compared to the treatments with residue addition throughout the experiment (not shown). In the following discussion, respiration rate and cumulative respiration are expressed per g total residue added at a given time.

Mixing the soil every 4 days resulted in a slight increase in respiration rate in all treatments (Figure 4.1). In the residue treatments, the highest respiration rates occurred immediately after the first residue addition and lasted about one day. In the 100%W treatment, the respiration rate decreased over time until day 48 after which it remained stable. In the treatments with repeated residue addition, the respiration rate increased after each residue addition with the magnitude of the increase decreasing over time. In the first 16 days, there was no significant difference in respiration rate between residue treatments, although the respiration rate in the 6.25%W treatment was slightly higher than in the other residue treatments. From day 32 to 48, the increase in respiration rate after residue addition was similar in all treatments with repeated residue addition. By the end of the incubation, there were no differences in respiration rate among treatments.

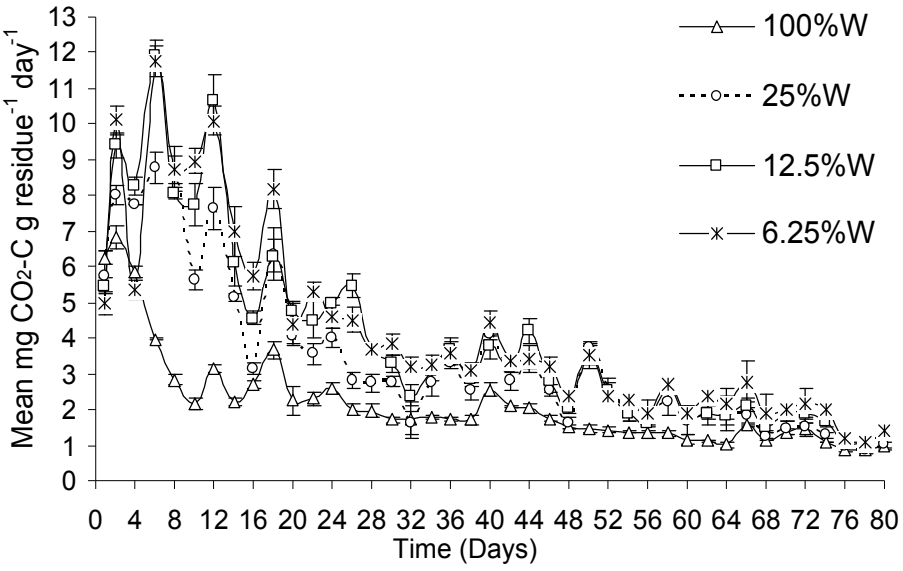


Figure 4.1 Respiration rate (mg CO_2-C g^{-1} residue day^{-1}) of treatments with residue addition over time. Vertical bars = Standard deviation (n=4)

Cumulative respiration increased over time with the strongest increase in the first 32 days (Figure 4.2). In the 100%W treatment, cumulative respiration increased gradually, but was significantly lower than in treatments with repeated residue addition. In the first 32 days, cumulative respiration of the 12.5%W and 6.25%W treatments was similar and significantly higher than the 25%W. From day 64 onwards, cumulative respiration of the 6.25%W was significantly higher than in all other residue treatments. By the end of the incubation, cumulative respiration decreased in the order: 6.25%W>12.5%W>25%W>100%W and cumulative respiration in the 6.25%W was twice as high as than in the 100%W and about 30% higher than in the 25%W and the 12.5%W.

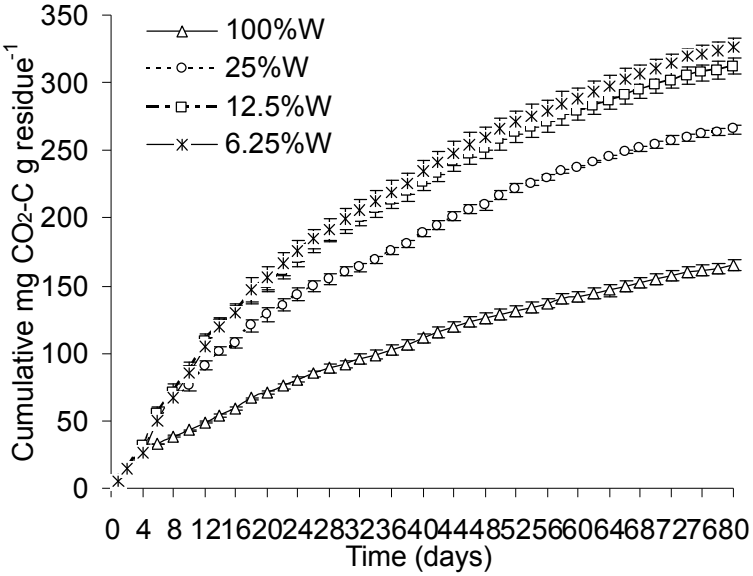


Figure 4.2 Cumulative respiration (mg CO_2-C/g residue) in treatments with residue addition over time. Vertical bars = Standard deviation (n=4)

4.3.2. Microbial community structure

The stress values of the two dimensional MDS plots showing the microbial community composition based on PLFA patterns ranged between 0.05 and 0.08, indicating that the ordination was a good reflection of the overall structure of the microbial communities (Figures 4.3, 4.4). In MDS plots, the distance between symbols is used to evaluate similarities and/or differences in microbial communities of treatments. A large distance between symbols indicates large differences in community structure. Across all sampling times and treatments, 4 fatty acids contributed most to the patterns, namely C20:0 and C16:1ω7c (both explaining 18% of the variability), C18:2 (14%) and C18:1ω9t (8%), together explaining 54% of the variability. The relative abundance of these fatty acids was higher in the treatments with residues than in the control. When all

sampling times and treatments were analyzed together, PERMANOVA showed that there were significant differences among sampling times and treatments, with sampling times contributing slightly more to the variation than treatments, but since the interaction between sampling times and treatment was also significant, the sampling times will be presented separately (Figure 4.3). Overall, more bacterial PLFAs were found in the 12.5%W and 6.25%W treatments than in the 100%W and 25%W treatments. The fungal PLFA 18:2ω6 was found in all treatments with its percentage increasing over time. The percentage of bacterial and fungal fatty acids in the control was significantly lower than in the residue treatments throughout the incubation.

Results from PERMANOVA showed that there were no differences in the microbial community composition between treatments on day 0. On days 16 and 32, the abundance of fatty acids was lowest in the non-amended soil. Among the residue treatments, there was a pronounced difference in microbial community composition between the 100%W and repeated residue treatments (Figure 4.3). The abundance of the gram negative bacterial fatty acids C16:1ω7c, C18:1ω7, and the fungal fatty acids C18:2ω6c, 18:1ω9c and 18:3ω3c was significantly higher in 100%W compared to the other treatments and microbial community composition was correlated with (in decreasing importance) microbial biomass N, extractable N and pH.

On days 48 and 64, the concentrations of bacterial and fungal fatty acids of the control were significantly different from those of residue treatments. There was little difference among residue treatments, although the abundance of fatty acids in the 12.5%W and 6.25%W treatments were higher than in the 100%W and 25%W treatments. There were no significant differences in microbial community composition or abundance of bacterial fatty acids between the 25%W and 12.5%W treatments, but microbial community composition of the 6.25%W treatment differed significantly from 25%W and 12.5%W treatments (Figure 4.3). Microbial community composition was correlated with respiration rate, cumulative CO_2-C evolution and microbial biomass N.

At the end of the experiment (80 days), the community composition of the control differed from the residue treatments (Figure 4.3). Among the residue treatments, the community composition of the 100%W treatment differed from those of the 6.25%W and 12.5%W treatments. The microbial community of the 100%W and 25%W treatments was characterized by a high abundance of the fungal fatty acid C18:2ω6c, 18:1ω9c and 18:3ω3c. The abundance of bacterial fatty acids in the 6.25%W treatment

differed from that of the 12.5%W treatment. Microbial community composition was, in decreasing importance, correlated with extractable N, microbial biomass C, pH, microbial biomass N and respiration rate.

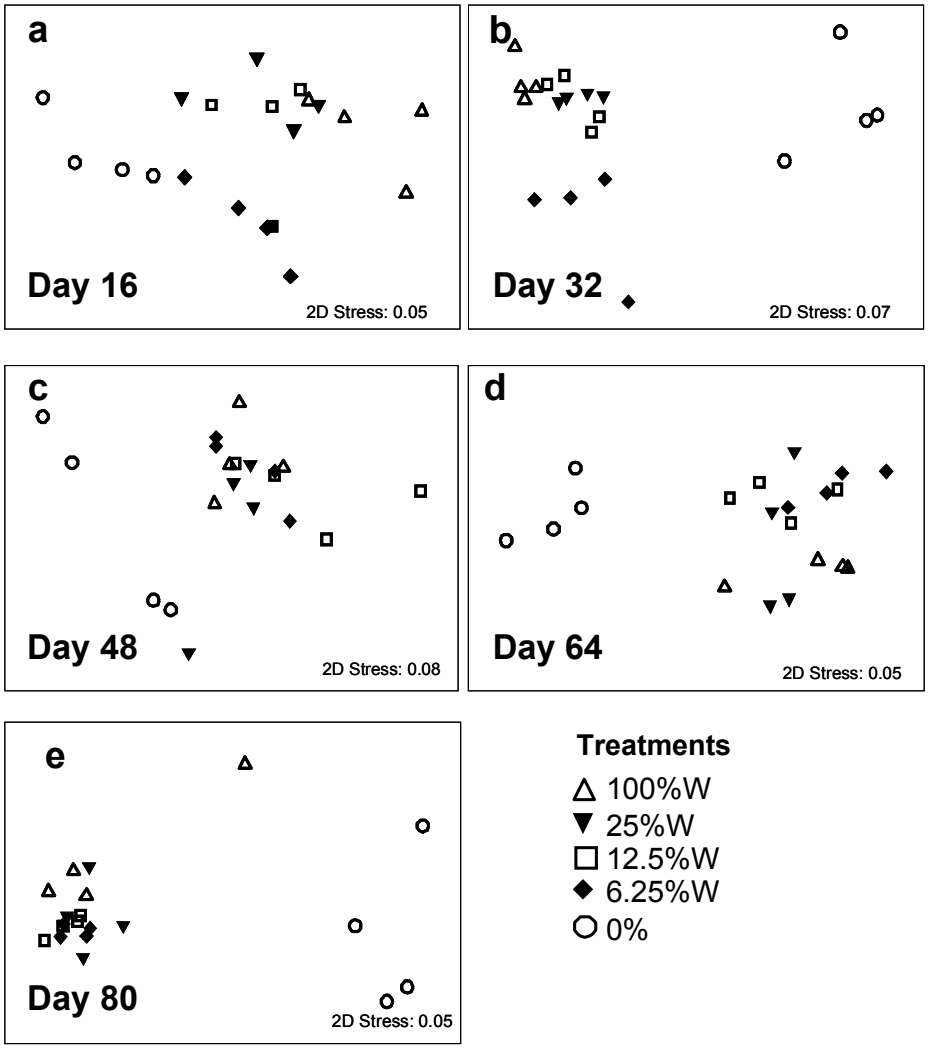


Figure 4.3 Microbial community structure on day 16 (a), day 32 (b), day 48 (c), day 64 (d), day 80 (e).

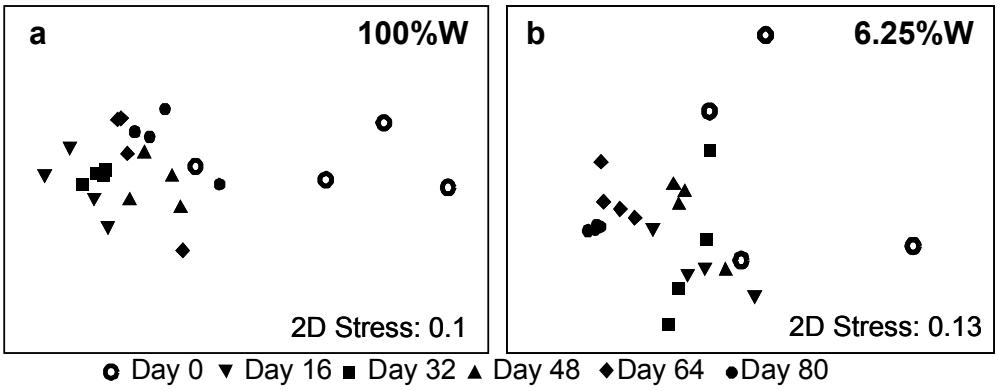


Figure 4.4 Microbial community composition over time in 100%W (a) and 6.25%W (b)

Microbial community composition changed over time in all treatments, with 100%W and 6.25%W treatments being shown in Figure 4.4. In the 100%W treatment, the microbial community on day 0 did not differ from that on day 80 and there were no significant differences between the communities on day 48 and 64 or between those on day 64 to 80. The microbial community in the 6.25%W treatment remained unchanged until day 32, but from then on differed significantly from day 0 with significant differences between communities on days 48, 64 and 80.

4.3.3. Microbial biomass C and N

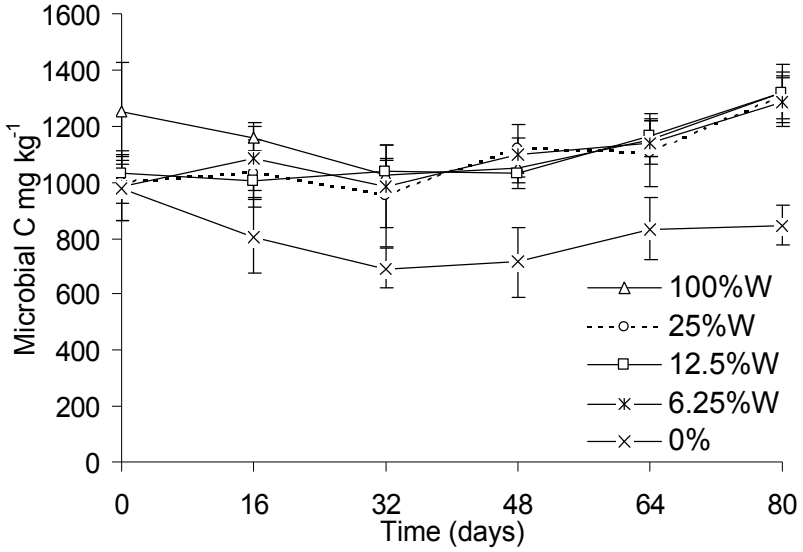


Figure 4.5 Microbial C concentration (mg kg^{-1}) over time.
Vertical bars = Standard deviation (n=4)

Incorporation of wheat straw resulted in changes in microbial biomass as indicated by microbial biomass C (MBC) and N (MBN). Time and residue treatments had significant effects on MBC. There was no significant interaction effect between residue and time on MBC. Microbial biomass C was increased by residue addition, particularly in the later part of the incubation (Figure 4.5). The increasing disparity between control and residue treatments can be explained by the decreasing trend in MBC in the control whereas MBC increased in the residue treatments. There were no significant differences between residue treatments over time. In the 100%W treatment, MBC decreased strongly in the first 32 days and increased afterwards. In the treatments with frequent residue addition, MBC increased from day 32 onwards. Because there were no significant differences between residue treatments, the average value of microbial C in

all treatments (1109.7 mg kg^{-1}) was used to calculate the microbial C/N ratio of the residue treatments.

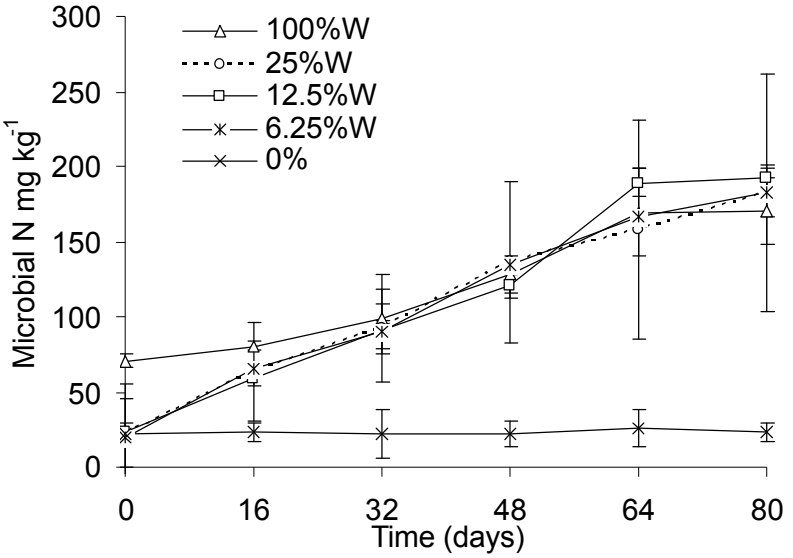


Figure 4.6 Microbial N concentration (mg kg^{-1}) of treatments over time
Vertical bars = Standard deviation (n=4)

The residue treatments increased, MBN significantly between days 0 to 48 (Figure 4.6). There was no significant difference between days 64 and 80. Microbial biomass N remained unchanged in the control. From day 16 onwards, MBN in the residue treatments was higher than in the control. In the first 16 days, MBN in the 100%W treatment was significantly higher than in the other residue treatments. However, from day 32 onwards there were no significant differences among residue treatments.

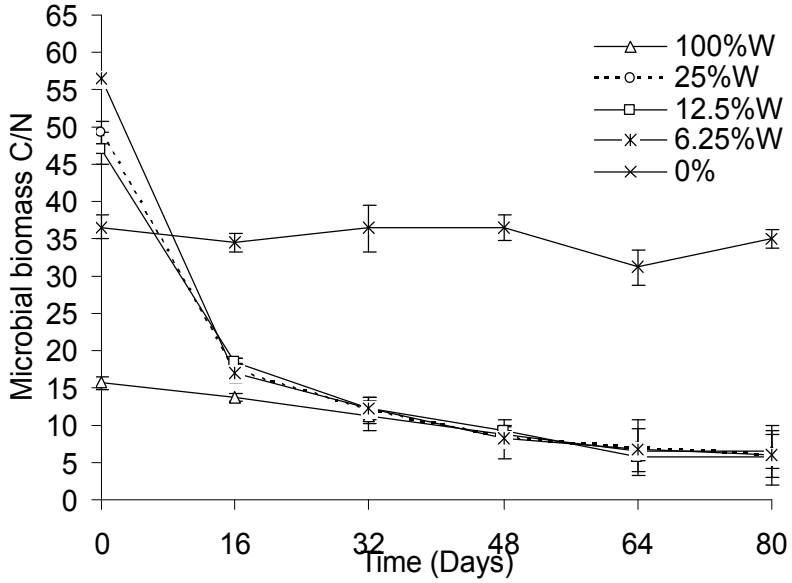


Figure 4.7 Microbial biomass C/N over time
Vertical bars = Standard deviation (n=4)

Residue addition decreased the microbial biomass C/N ratio compared to the control. In the residue treatments, the microbial C/N ratio decreased in the first 16 days and then remained unchanged whereas it did not change over time in the control (Figure 4.7).

4.3.4. Extractable Soil C and N

4.3.4.1. Extractable C

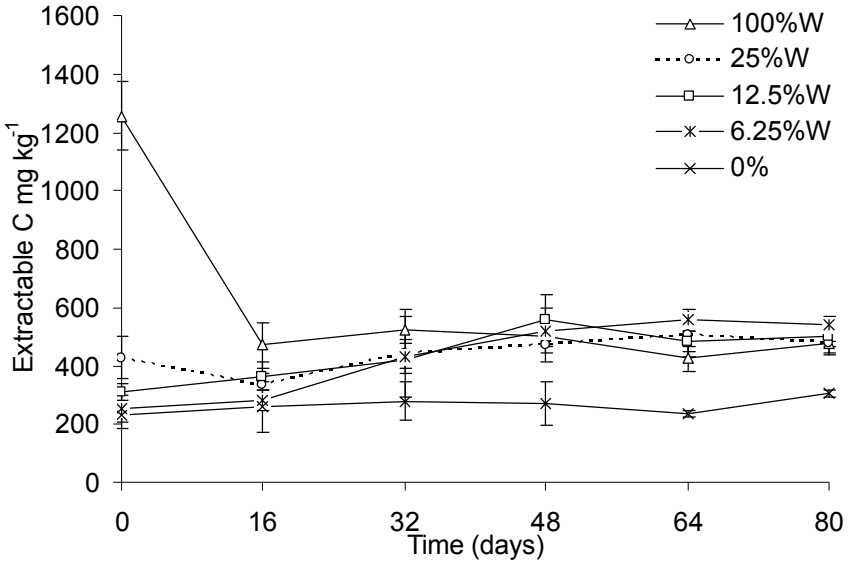


Figure 4.8 Extractable C concentration (mg kg^{-1}) over time

Vertical bars = Standard deviation (n=4)

There was a significant interaction between residue additions and sampling dates on extractable C (C_E). Generally, C_E was lower in the control than in the residue treatments (Figure 4.8). Extractable C remained unchanged in the control but increased in the treatments with repeated residue addition from day 16 to day 48. In the 100%W treatment, C_E was highest immediately after residue addition on day 0 and decreased strongly in the first 16 days, after which it remained unchanged. From day 16 onwards, there were no significant differences between residue treatments.

4.3.4.2. Extractable N

Compared to the control, residue treatments significantly decreased extractable N (N_E). In the residue treatments, N_E decreased in the first 16 days after which it remained stable. In the control, N_E increased over time (Figure 4.9).

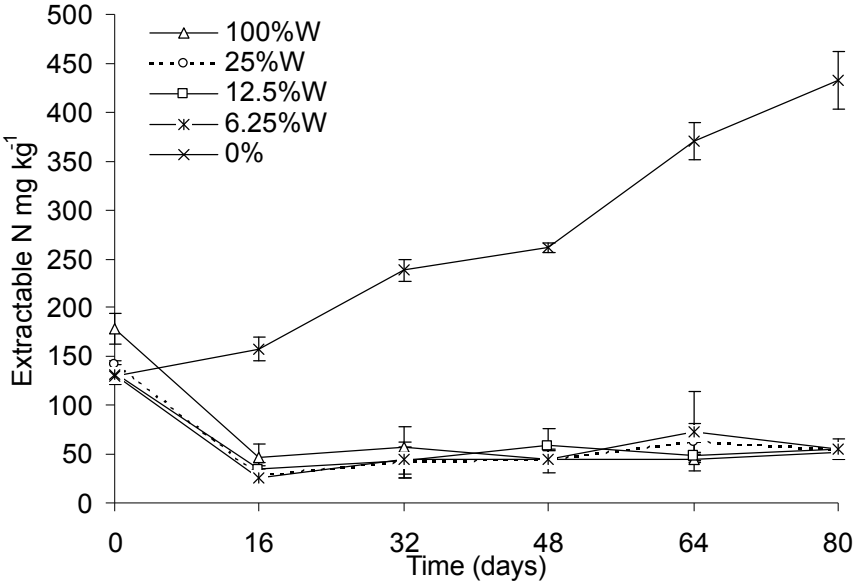


Figure 4.9 Net extractable N concentrations (mg kg^{-1}) over time.

Vertical bars = Standard deviation (n=4)

4.3.5. Inorganic N (NH_4^+ and NO_3^-) concentrations

Sampling times and residue treatments significantly influenced inorganic N (NH_4^+ and NO_3^-) concentrations. Addition of high C/N residue resulted in a significant reduction in inorganic N compared to the control (Figures 4.10, 4.11 and 4.12).

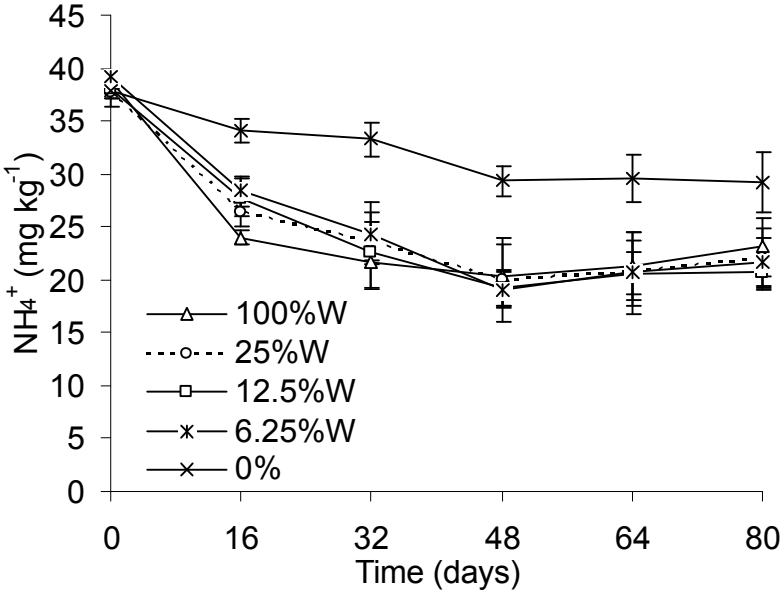


Figure 4.10 NH_4^+ concentration (mg kg^{-1}) over time

Vertical bars = Standard deviation (n=4)

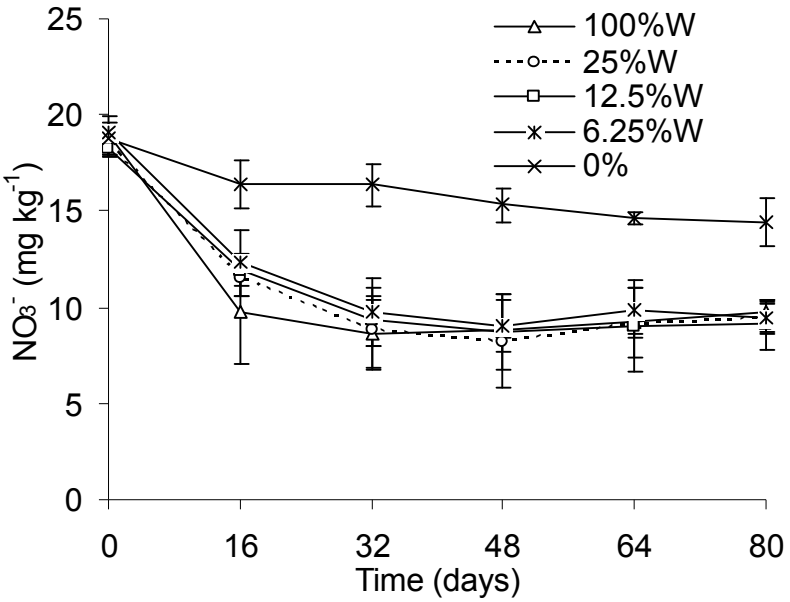


Figure 4.11 NO_3^- concentration (mg kg^{-1}) over time
Vertical bars = Standard deviation (n=4)

The NH_4^+ concentrations of residue treatments were significantly lower than in the control from day 16 onwards (Figure 4.10). On day 16, the 100%W treatment had a significantly lower NH_4^+ concentration than the other residue treatments. From day 32 onwards, the NH_4^+ concentrations of residue treatments were similar and remained unchanged until the end of the experiment.

As with the NH_4^+, the NO_3^- concentrations of the residue treatments were significantly lower than in the control from day 16 onwards (Figure 4.11). Among residue treatments, the NO_3^- concentrations of repeated residue treatments were significantly higher than in the 100%W treatment on day 16. From day 32 onwards, there were no significant differences between residue treatments. In the control, the NH_4^+ concentration changed little over time whereas the NO_3^- concentration decreased.

In general, the percentage of inorganic N as NH_4^+ was 69 ± 2% in all treatments. The inorganic N concentration in the control was significantly higher than in residue treatments, but decreased gradually over time. Among residue treatments, the inorganic N concentration of treatments with frequent residue addition was significantly higher than in the 100%W treatment on day 16 (Figure 4.12). From day 32 onwards, there were no significant differences in inorganic N among the residue treatments.

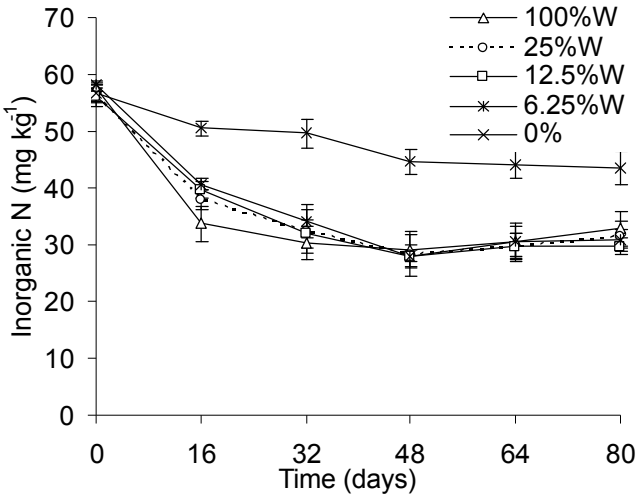


Figure 4.12 Inorganic nitrogen (NH_4^+ and NO_3^-) concentrations (mg kg^{-1}) over time
Vertical bars = Standard deviation (n=4)

4.3.6. Soil pH

Table 4.2 Soil pH over time

Treatments	Day 0	Day 16	Day32	Day 48	Day 64	Day 80
100%W	6.70 [a]	6.98[b]	7.05 [a]	7.13 [a]	7.15 [a]	7.15 [a]
25%W	6.68 [a]	7.10[a]	7.05 [a]	7.18 [a]	7.15 [a]	7.15 [a]
12.5%W	6.68 [a]	7.13[a]	7.05 [a]	7.10 [a]	7.20 [a]	7.13 [a]
6.25%W	6.70 [a]	7.10[a]	7.08 [a]	7.15 [a]	7.20 [a]	7.13 [a]
0%	6.63 [a]	6.65[c]	6.60 [b]	6.55 [b]	6.65 [b]	6.45 [b]

Letters indicate significant differences between treatments at a given day at P<0.05

Soil pH was significantly lower on day 0 compared to days 16, 32, 48, 64 and 80 (Table 4.2). The addition of wheat straw resulted in a gradual increase in soil pH; from day 16 soil pH in all residue treatments was significantly higher than the control. Addition of wheat once or several times increased soil pH by up to 0.6 units (from 6.5 to 7.1). On day 16, the 100%W treatment had a significantly lower soil pH than the other residue treatments. However, from day 32 onwards, the soil pH of all residue treatments was similar, and greater than in the control.

4.4. Discussion

4.4.1. Changes in the respiration rate and cumulative respiration

It is well-known that incorporation of plant residues into the soil results in a rapid increase in microbial activity and biomass followed by gradual decrease (Sparks 2000;

Wang et al. 2004). The results of this study confirm the previous studies but also show that increasing frequency of residue addition increased respiration rates.

After the first addition, the respiration rate of all residue treatments increased immediately and did not differ among residue treatments (Figure 4.1). The high respiration rates in the first few days after residue addition is consistent with previous studies (Trinsoutrot *et al*. 2000; Wang *et al.* 2004) and is due to water-soluble compounds in the residues, which are easily decomposable and readily available, thus enhancing the microbial activity. This assumption is reinforced by several studies, e.g. Cogle *et al*. (1989); Wang *et al*. (2004) and Stemmer et al. (2007). According to Cogle *et al.* (1989), there are two phases involved in crop residue decomposition. The initial phase is more rapid due to the degradation of water-soluble compounds such as amino acids, amino sugars and carbohydrates. In the slower, second stage, recalcitrant and structural components such as lignin, cellulose are decomposed. When residues are added once, respiration rates are initially high due to the decomposition of easily available compounds. Thereafter, respiration rates decrease as easily available compounds are depleted. Respiration rates are low in the later stages of decomposition when only more recalcitrant compounds such as lignin-encrusted cellulose and other macromolecules are left (Wang *et al.* 2004). These changes in residue chemistry are accompanied by changes in microbial community composition over time (Stemmer *et al.* 2007). When residues are added more frequently (in this study every 4 to 8 days), the availability of easily available compounds remains high, hence microbial activity is stimulated which could lead to a priming effect, i.e. increased decomposition of recalcitrant compounds in the residues and, possibly, in the soil organic matter. The respiration rate was similar in the residue treatments from day 1 to day 4.

From day 4 to day 8, the respiration rates declined dramatically in 100%W and 25%W and were lower than in the 12.5%W and 6.25%W. The decline in respiration rates of the 100%W and 25%W treatments resulted in a slower increase in cumulative respiration in those treatments than in the 12.5%W and 6.25%W treatments. A possible explanation is that, as the decomposition process proceeded in treatments 100%W and 25%W, the water-soluble compounds were utilized and degraded and the remaining compounds were resistant and recalcitrant substances. Whereas increasing the frequency of residue addition from once to every 16 days and from every 16 to every 8 days strongly increased decomposition rates, the difference between additions of

residues every 8 or every 4 days was small. This suggests that the addition of residues every 8 days is sufficient to maintain an active microbial community in this soil. From day 32, the respiration rate of treatment 12.5%W was lower than in treatment 6.25%W. Therefore, cumulative respiration of 6.25%W was higher than in 12.5%W. The differences in cumulative respiration between 6.25%W and 12.5%W became clearer from day 56. This was due to the micro-organisms in 12.5%W being less active after 3 weeks stopping the addition of residue, whereas the addition of residue to 6.25% was stopped from day 64. Cumulative respiration and the percentage of added residue C respired were in the following order: 6.25%W>12.5%W>25%W>100%W (Figure 4.2). The decrease in the peak of respiration rate after residue addition in 12.5%W and 6.25%W over time (Figure 4.1) might be explained by an increasing N limitation due to the addition of material with low water soluble N (7.35 g kg residue^{-1}). This is supported by the decrease in inorganic N concentrations observed during incubation.

4.4.2. Changes in microbial community structure

The activity and size of the microbial biomass is an essential mediator of mineralization and immobilization processes of crop residues in the soil and is governed by the availability of substrates (Allison *et al.* 2005; Schomberg *et al.* 1994). Membranes of all living organisms contain phospholipids and the total amount of phospholipids (PLFAs) has been used to assess living organisms in the soil (Zelles *et al.* 1992; Zelles *et al.* 1995). Increasing the number of residue additions could increase the activity as well as size of the microbial community. A greater diversity and larger population of microbial species present will increase the biological capability of the community (Baldock 2007). The term "diversity" is used to indicate the number of different species (richness) and their relative abundance (evenness) (Nannipieri *et al.* 2003).

Four fatty acids, namely C20:0 and C16:1ω7c (marker for gram negative bacteria), C18:1ω7 and C18:1ω9t (marker for fungi) together explained on average 54% of the variability in PLFA patterns, and hence all the other fatty acids were of minor importance in differentiating the treatments. The abundance of all four fatty acids was greater in the treatments with residues than in the control suggesting that residue addition favoured the growth of gram negative bacteria but also fungi. Generally it is assumed that the fast growing gram negative bacteria are more competitive than fungi in the presence of easily decomposable compounds while fungi are considered to be more competitive during decomposition of recalcitrant compounds (Stemmer *et al.*

2007). However, there are also indications that gram negative bacteria and fungi may decompose residues simultaneously and/or that fungi may facilitate the growth of bacteria during the breakdown of complex compounds into simpler ones (Meidute *et al.* 2008).

In 100%W, the abundance of bacterial PLFAs and the fungal fatty acid was significantly higher on day 16 than in 25%W, 12.5%W and 6.25%W, but thereafter the abundance of bacterial and fungal PLFAs decreased. This is in agreement with the findings of Schomberg *et al*. (1994) and Kaur *et al*. (2005). On days 48 and 56, after stopping incorporating residues in 25%W and 12.5%W, the amount of bacterial PLFAs decreased significantly due to the die-off of decomposers utilizing readily decomposable substances that used to be highly available in the 25%W and 12.5%W just after residue addition, whereas the fungal fatty acids such as 18:2ω6c, 18:1ω9c and 18:3ω3c increased. The distinct microbial community composition in 6.25%W can be best explained by the continual addition of soluble compounds from crop residues (Webley and Jones 1971; Landeler *et al.* 2002). In sum, the microbial community composition at very high residue addition frequency (treatment 6.25%W) differed from the other residue treatments at most sampling times (Figure 4.5) indicating that high availability of easily decomposable compounds affects microbial community composition, especially favouring the rapid growth of bacterial decomposers (Waid 1997; Nannipieri *et al.* 2003).

4.4.3. Microbial and extractable C

The results of this study showed that frequent residue incorporation enhanced C mineralization. The concentration of microbial and extractable C was high immediately after incorporation of residues (Figures 4.6 and 4.9); this is due to the presence of water-soluble C in the residues (2.74% of C was water-soluble in the wheat residues). Hence, the extractable C concentration on day 0 was a function of the amount of residues added. The high MBC on day 0 in 100%W (Figure 4.6) could be an artifact. Chloroform may release C and N from the residues, not just from the microbial biomass. This effect would be less pronounced in the treatments with lower amounts of residues added on day 0. Nevertheless, a strong increase in MBC and MBN on day 0, particularly in 100%W cannot be ruled out, since there were around 10 hours between residue incorporation and sampling. The increase in extractable C over time in treatments with frequent residue addition indicates that not all extractable C was

utilized by the microbes, which could be due to N limitation (Figures 4.10, 4.11 and 4.12).

4.4.4. Microbial, inorganic and extractable N

Initial plant residue quality influences residue N dynamics. Results from different incubation studies (Mary *et al.* 1996; Bending and Turner 1999; Wang *et al.* 2004) have indicated that the intensity and dynamics of N immobilization-mineralization turnover depended on the nature of plant residues and densities of decomposers. Khali *et al.* (2005) showed that N mineralization was dominated by ammonification during the first 15 days after incorporating crop residues. Thereafter, NH_4^+ released from the ammonification was nitrified to NO_3^- and this nitrification process was even greater in neutral, light acidic and/or alkaline soils.

In residue treatments inorganic N concentrations decreased and were much lower than in the control throughout the experiment (Figures 4.10, 4.11 and 4.12). This suggests that adding high C/N ratio residue stimulated microbes to grow and immobilize N especially in the first 32 days. Incorporating wheat straw enhanced the immobilization of both nitrate and ammonium because the high C/N ratio did not meet the microbial N requirements (Nishio and Oka 2003). The immobilization of N in the biomass occurred over 64 days as shown by the increasing microbial N (Figure 4.7). The slight increase in inorganic N is mainly due to the decline in the C/N ratio of the decomposing crop residue; once the residue C/N ratio is less than 21:1, the microbial N will be mineralized (Walley and Yates 2002). Mishra *et al*. (2001) also concluded that during the decomposition of wheat straw, residue carbon content decreased, the relative N content increased and the C/N ratio decreased over time. They stated that there was net N immobilization from the wheat straw in the first 10 weeks after the straw incorporation, followed by net N.

Extractable N in treatments with residue addition was significantly lower than the control and decreased over time which suggests that over the course of the incubation period the microbial biomass continues to take up N. As explained above, this is due to adding larger amounts of high C-wheat residue, but low N material. To reinforce this claim, Shindo and Nishio (2005) concluded that following the addition of wheat straw into soils, a decrease of available nitrate and increase of microbial biomass C and N occurred within the first week of the experiment.

4.4.5. Soil pH

Residue addition significantly increased soil pH but there were no significant differences in pH between residue treatments at any time (Table 4.2). The pH was in the range of 6.45-7.20. In general, pH in all residue treatments was 0.6 units higher than in the control. According to Yan *et al*. (1996b) and Xu *et al.* (2006), biochemical decarboxylation of the carboxylic groups of organic acid anions from added crop residue and the production of OH^- from deaminization and ammonification during the mineralization process result in increasing soil pH, whereas nitrification produces protons. Thus, soil pH may initially increase after residue addition until nitrification becomes dominant, resulting in a pH decrease (Paul *et al.* 2001). In the present study, adding high C/N ratio residue led to N immobilization in the early phase and the hydroxyl ions released from the ammonification process and decarboxylation of organic acid anions resulted in an increase in soil pH.

The results from this experiment showed that adding small amounts of crop residues strongly stimulated microbial activity and the decomposition of plant residues compared to a single addition, and thus our hypotheses were strengthened. However, in the experiment described above, only one type of high C/N ratio residue was added repeatedly into the soil. Therefore the following questions need to be further elucidated:

What is the effect of frequently added residues with high C/N ratio into decomposing low C/N ratio residues on microbial activity and hence residue decomposition?

How do the availability of N and soil pH change when high C/N ratio residues are incorporated into the decomposing low C/N ratio residues at different intervals?

A second experiment was carried out in order to gain a better insight and answer these questions.

Chapter 5

Effect of Mixing of Low and High C/N Ratio Residues

5.1 Introduction

The kinetics of plant residue decomposition in soils and their carbon and nitrogen mineralization are largely dependent on the chemical composition of plant residues (Heal *et al.* 1997; Eiland *et al*. 2001). Several studies have found that the C/N ratio or N content of crop residues has a fundamental effect on net N mineralization from residues (Constantinides and Fownes 1994; Soon and Arshad 2002). Results from the experiment described in Chapter 4 showed that, apart from the critical influence of residue chemical composition, frequent addition of plant residue can maintain microbial activity and increase C mineralization (Duong *et al.* 2009). The wheat residue used in the previous experiment had a low N content, and thus, the activity and growth of microbes were limited by the availability of N. An increase in the size of soil microbial biomass is considered essential for soil fertility improvement (Singh *et al.* 2006). Addition of N-rich plant materials enhances N in the soil and the microbial biomass (Kara 2000). According to Melillo (1982) and Walley and Yates (2002), a high concentration of N in residues results in a high decomposability as it favours soil microbial activity. Results from Joffre and Ågren (2001) showed that different N concentrations of a given residue type (high or low C/N ratio) strongly affected the rate of respiration and net N mineralization, especially in the early stage of the decomposition. Singh *et al.* (2006) suggested that the application of a mixture of high and low C/N residue can modulate N release, which can help minimize N loss from ecosystems. However, little is known about the effects of mixing high C/N ratio residues into decomposing low C/N plant materials on soil microbial biomass, soil pH and N availability. Incorporating high C/N ratio residues into the soil will affect soil microbial activity and nutrient availability as it provides lower concentrations of N than low C/N residues.

The aim of this experiment was to determine the effects of adding high C/N residues (wheat) at different times to decomposing low C/N (lupin) residues on (1) respiration rate and availability of N, (2) microbial community composition and (3) soil pH. It is hypothesized that the addition of high C/N residue to low C/N residue will (1) decrease

respiration rate and cumulative respiration and reduce N release, and (2) change soil pH and microbial community structure compared to low C/N residue alone.

5.2 Materials and Experimental Design

The second incubation experiment was carried out in the same soil and the water holding capacity as described for the previous experiment described in Chapter 4. The total amount of residues added was 0.8 g of plant residue in 39.2 g dry soil; 25% of this amount was added over 4 times intervals of 16 days (0.2 g residue for each addition). The unamended soil was mixed as thoroughly as the amended soils every 16 days. The C/N ratios of wheat and lupin were 82 and 22.4, respectively (Table 3.2). The residues were ground and sieved to 0.25-2 mm. After two weeks' pre-incubating the soil, the six treatments were set up as shown in Table 5.1.

Table 5.1 Treatments and frequency of plant residue addition

Treatment	Day 0	Day 16	Day 32	Day 48
100%L	Lupin	Lupin	Lupin	Lupin
LW-16	Lupin	**Wheat**	Lupin	Lupin
LW-32	Lupin	Lupin	**Wheat**	Lupin
LW-48	Lupin	Lupin	Lupin	**Wheat**
100%W	**Wheat**	**Wheat**	**Wheat**	**Wheat**
0%	no residues	-	-	-

All measurements were carried out as described for the previous experiment.

5.3. Results

5.3.1. Respiration rate and cumulative respiration

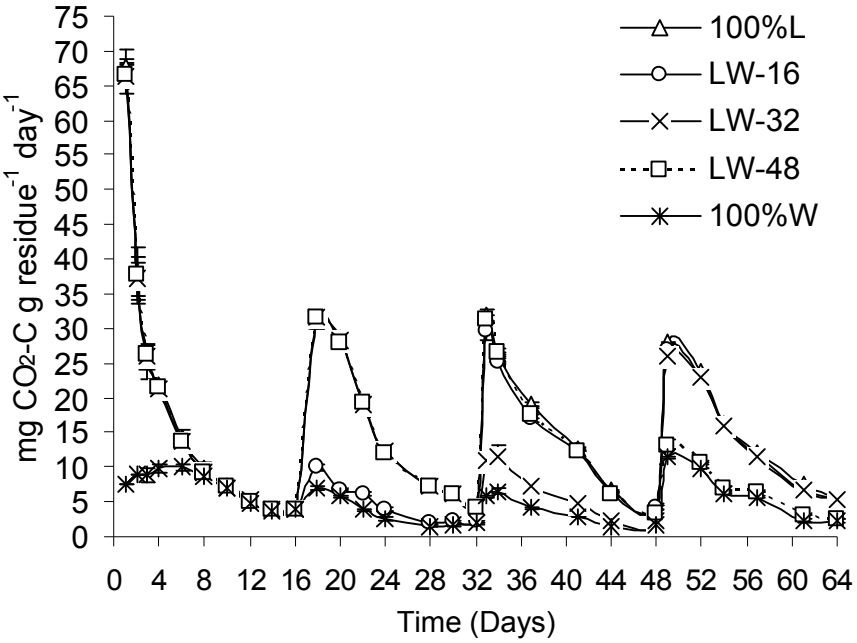


Figure 5.1 Respiration rate (mg CO_2-C g residue^{-1} day^{-1}) of treatments with addition of the same or different residues over time. Vertical bars=standard deviation (n=4)

There were significant differences in respiration rate between days 16, 32, 48 and 64. There was a significant interaction between residue and sampling time on respiration rate. The respiration rate of the non-amended soil was significantly lower than in the residue treatments and is not presented. In all residue treatments, the addition of wheat straw resulted in a decrease in respiration rate compared to the addition of lupin (Figure 5.1). Among the residue treatments, the respiration rate in all lupin treatments was highest in the first 16 days and significantly higher than in 100%W. After wheat residue addition on day 16 (LW-16), the respiration rate was much lower compared to that on day 0 when lupin residue was added and was significantly lower than in 100%L, LW-32 and LW-48. Compared with 100%W, the respiration rate of LW-16 was slightly higher.

Addition of wheat residues on day 32 (LW-32) or 48 (LW-48) decreased respiration rates compared to 100%L and LW-16, but there was no significant difference between LW-32 and 100%W.

By the end of the incubation period (64 days), although the respiration rates of lupin residue treatments were slightly higher than in 100%W, there were no differences in the respiration rates between residue treatments.

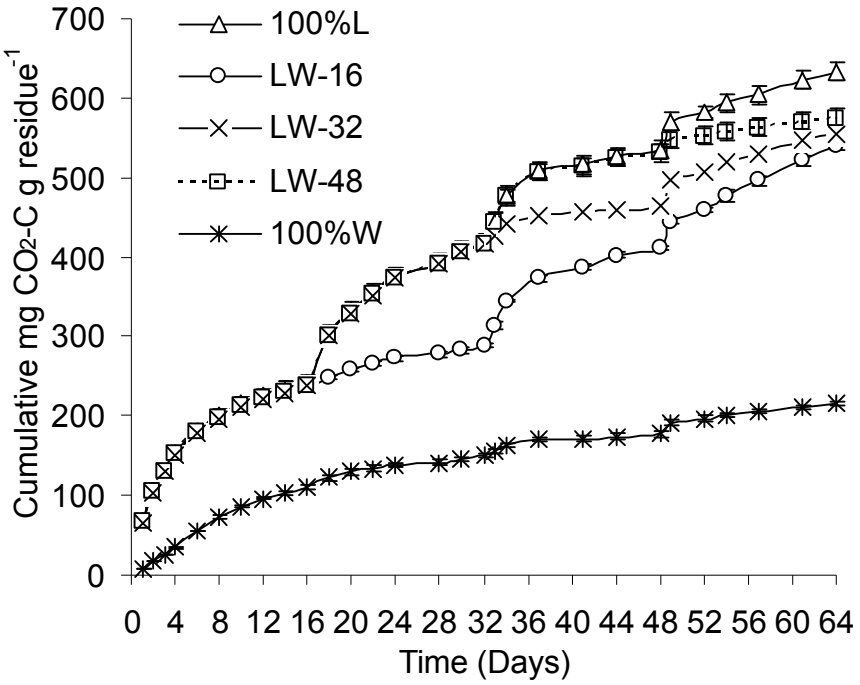


Figure 5.2 Cumulative respiration (mg CO_2-C g residue^{-1}) of treatments with residue addition over time. Vertical bars=standard deviation (n=4)

Cumulative respiration increased over time and the strongest increase occurred in the treatment with lupin residue only (Figure 5.2). Adding high C/N ratio residue into the decomposing lupin residues induced a significantly smaller increase in cumulative respiration compared to lupin residue addition, but a greater increase than in 100%W. By the end of the incubation, the cumulative respiration of LW-16, LW-32 and LW-48 were similar (483 mg, 515 mg and 530 mg CO_2-C g residue^{-1}, respectively). They were significantly lower than in the 100%L (588 mg CO_2-C g residue^{-1}*)*. The cumulative respiration of 100%W increased gradually, but was significantly lower than in the other residue treatments over time.

5.3.2. Microbial community composition

The abundance of PLFAs was lowest in the control across all sampling times. Microbial community composition changed over time in all treatments.

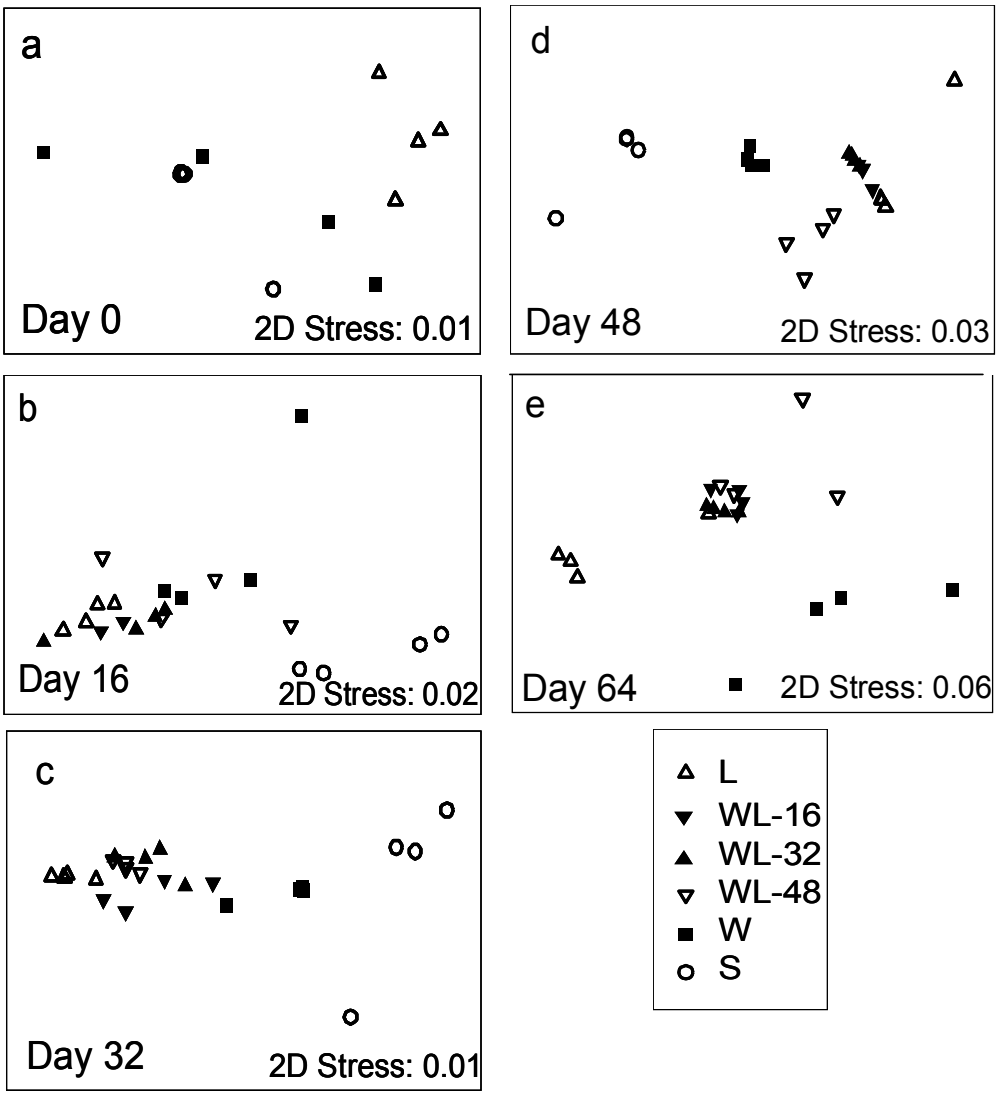


Figure 5.3 Microbial community structure at different sampling times: day 0 (a), day 16 (b), day 32 (c), day 48 (d), day 64 (e).

The stress values of the two dimensional MDS plots showing the microbial community composition based on PLFA patterns ranged between 0.01 and 0.06, indicating that the ordination was a good reflection of the overall structure of the microbial communities (Figure 5.3). Across all sampling times and treatments, four fatty acids contributed most to the patterns, namely C16:1ω7c, C20:0, C18:2ω6t and C18:1ω9t. The relative abundance of these fatty acids was much higher in the residue treatments than in the control. The PERMANOVA showed that there were significant differences among

sampling times and treatments and significant interactions between treatments and sampling times.

Results from the PERMANOVA showed that there were significant differences in microbial community composition of 100%L compared to 100%W from day 16 onwards. The abundance of fatty acids characteristic for gram negative bacteria was significantly higher in 100%L than in 100%W.

On day 16, there was a significant difference between 100%W and the other residue treatments (Figure 5.3). The concentration of bacterial and fungal fatty acids in 100%W was significantly lower than in treatments with lupin residue (100%L, LW-16, LW-32 and LW-48). Among treatments with lupin residue addition, the abundance of signature fatty acids for gram negative bacteria (C16:1ω7c) was relatively similar and significantly higher than that of signature fatty acids for fungi (C18:2ω6c, C18:1ω9c and C18:3ω3c).

Results from the remaining sampling times showed that although microbial community composition of 100%W changed after each addition of 25% wheat straw, it differed significantly from that of the other residue treatments (Figure 5.3). On day 32, among lupin residue treatments, the concentration of total fatty acids in LW-16 was significantly lower than in 100%L, LW-32 and LW-48 (data not shown). On day 48, 100%L and LW-48 were significantly different in microbial community composition compared to LW-16, and LW-32 and the microbial community composition of LW-16 differed from that of LW32. By the end of the experiment (day 64), among lupin residue treatments, the microbial community composition of 100%L differed significantly from those of LW-32 and LW-48, and LW-32 differed from LW-48, whilst there was no significant difference between 100%L and LW-16.

5.3.3. Inorganic N (NH_4^+ and NO_3^-)

There was a significant difference in NO_3^- and NH_4^+ concentrations between harvest times and between residue treatments. There was a significant interaction between harvest time and treatments. During the experiment, the addition of lupin residue significantly increased the availability of NO_3^- and NH_4^+ concentrations compared to 100%W and the non-amended soil, whereas the incorporation of wheat straw resulted in a significant decrease in available nitrogen concentration in comparison with the control. There were significant decreases in NO_3^- and NH_4^+ concentrations when wheat

straw was incorporated into the decomposing low C/N ratio treatments (LW-16, LW-32, LW-48), but, the nitrogen concentrations (NO_3^- and NH_4^+) of those treatments were still significantly higher than in 100%W and the control (Figures 5.4 and 5.5). Overall, the NH_4^+ concentrations of lupin and wheat-lupin treatments decreased over time, whereas NO_3^- concentrations increased.

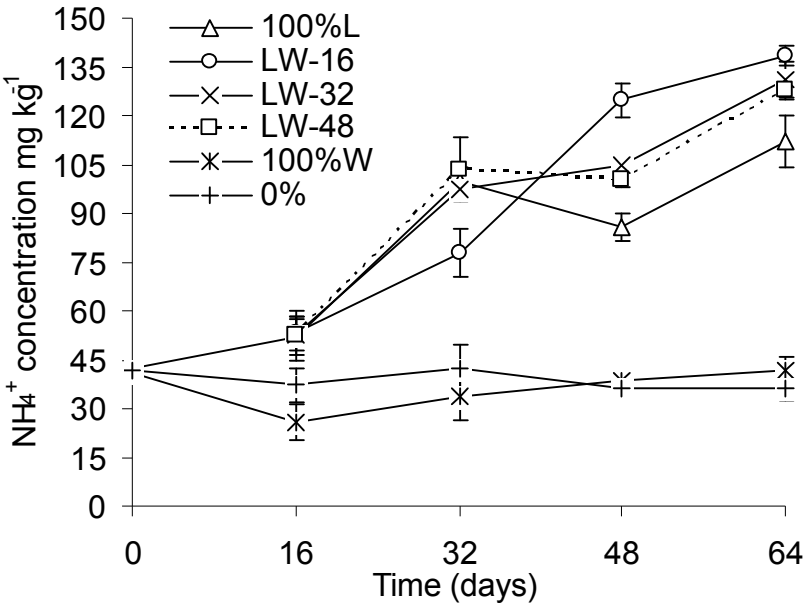


Figure 5.4 NH_4^+ concentration (mg kg^{-1}) of treatments over time
Vertical bars=standard deviation (n=4).

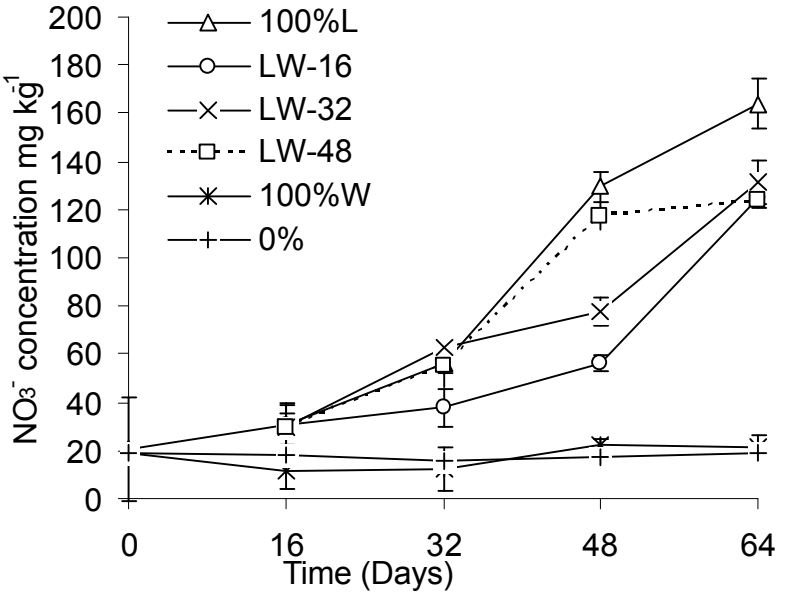


Figure 5.5 NO_3^- concentration (mg kg^{-1}) of treatments over time
Vertical bars=standard deviation (n=4).

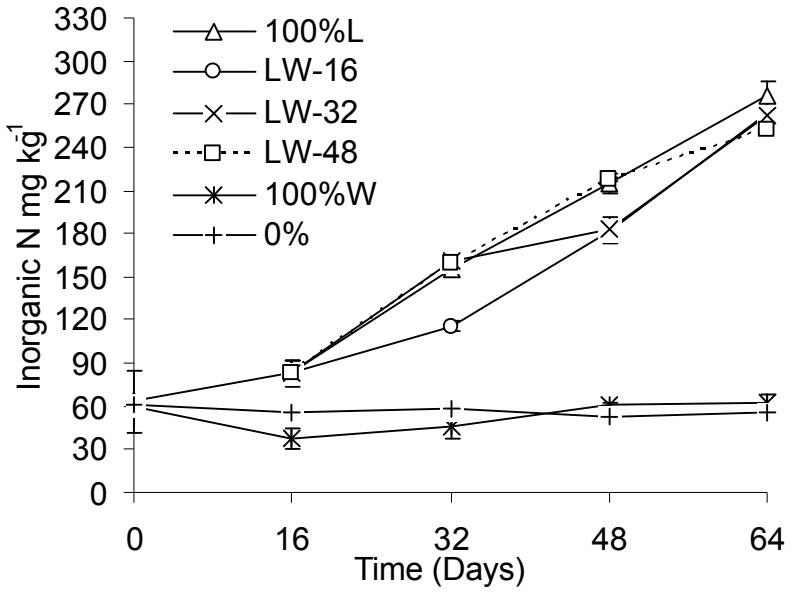


Figure 5.6 Inorganic nitrogen (NH_4^+ and NO_3^-) concentration (mg kg-^{1}) of treatments over time. Vertical bars=standard deviation (n=4)

In 100%L in the first 32 days, NH_4^+ concentration was on average 63% of the total available N and the NH_4^+ and NO_3^- concentrations increased significantly compared to 100%W, LW-16 and the control (Figures 5.4, 5.5). However, from day 48, although the amount of available N concentrations increased significantly compared to other residue treatments, the percentage of NH_4^+ of total available N in 100%L was significantly lower (40%) than on day 0.

After addition of wheat straw into the decomposing lupin residue on day 16 (LW-16), the concentrations of NH_4^+ and NO_3^- on the following sampling (day 32) were significantly lower than in 100%L, LW-32 and LW-48. From day 48 onwards, the incorporation of lupin residue on days 32 and 48 resulted in a significant increase in NH_4^+ concentration compared to other residue treatments, except for LW-16. The percentage of total inorganic N as NH_4^+ in LW-16 ranged from 69% (day 48) to 53% (day 64).

The addition of wheat straw on day 32 (LW-32) or on day 48 (LW-48) led to a significant decrease in NH_4^+ and NO_3^- concentration compared to 100%L and other wheat-lupin treatments. In LW-32, the percentage of total inorganic N as NH_4^+ decreased over time, from 64% (day 16) to 50 % (day 64).

100%W, in the first 32 days, demonstrated a strong decrease in both NH_4^+ and NO_3^- concentrations compared to the control. From day 48, a slight recovery occurred. The percentage of total inorganic N as NH_4^+ slightly decreased from 69% (day 0) to 66% (day 64). The inorganic nitrogen concentration of the control (0%) did not change over time.

5.3.4. Soil pH

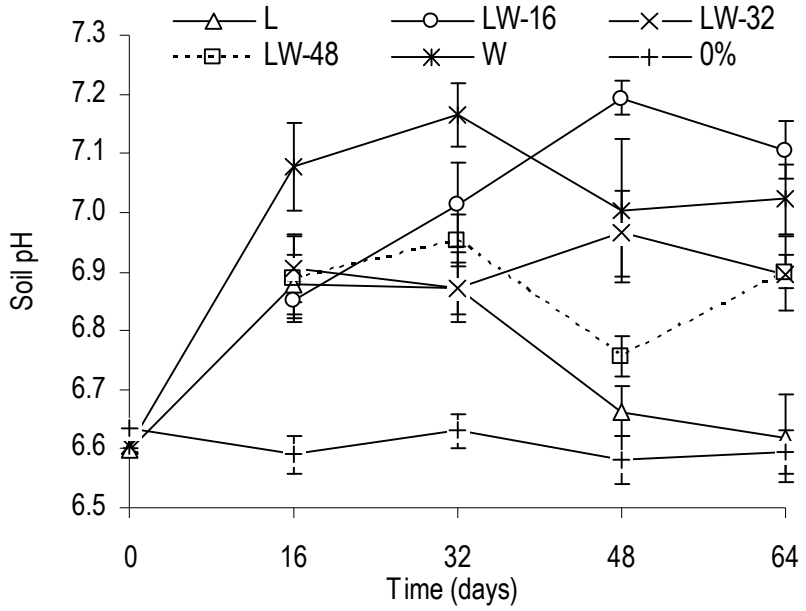


Figure 5.6 Soil pH over time
Vertical bars=standard deviation (n=4)

There was a significant difference in soil pH between day 0 and the other sampling dates (Figure 5.7). There was a significant interaction between sampling time and treatment. In contrast to the treatments with residue additions, soil pH of the non-amended treatment remained unchanged over time. Adding high C/N residues to lupin residues resulted in a significant increase in soil pH compared to the initial pH and addition of lupin residues alone. For example, after the first residue addition, the pH of residue treatments increased by 0.5 units in lupin residue treatments and by 0.3 units in 100%W compared to the non-amended treatment, respectively. In 100%L, the pH increased significantly in the first 16 days and then decreased strongly from day 32. By the end of the incubation, there was no significant difference in pH between 100%L and the control.

The incorporation of lupin into the soil in LW-16, LW-32 and LW-48 resulted in a pH decrease whereas addition of wheat straw increased pH. Soil pH of the lupin-wheat

residue treatments was higher than the control, but significantly lower than in 100%W. The soil pH increased after each wheat addition and decreased gradually later over time.

In 100%W the soil pH in the first 32 days was significantly greater compared to the other residue treatments and it decreased significantly from day 48. By the end of the experiment, the soil pH of 100%W was lower than in LW-16, but it was significantly higher than in 100%L, LW-32 and LW-48.

5.4. Discussion

5.4.1. Changes in respiration rate and cumulative respiration

This study showed that, in agreement with our hypothesis, the respiration rates decreased significantly when wheat straw was added compared to adding lupin (Figure 5.1). In 100%L, the respiration rate increased immediately after each addition of lupin residue and was higher than in the other residue treatments. Moreover, after each addition of lupin residues, the respiration rates were nearly 10-fold higher than when wheat residues were added. This is due to the lower C/N ratio and the greater supply of easily decomposable C and N in lupin compared to wheat residues (Table 3.2), (Ocio *et al.* 1991; Singh *et al.* 2006).

In the first 8 days after residue addition, the decomposition rate from the residue treatments decreased significantly and was similar in both low and high C/N residue treatments, which suggested that after day 8 only more recalcitrant materials remained (Gaillard *et al*. 1999; Meidute *et al*. 2008).

However, by the end of the incubation, there were no significant differences between residue treatments in respiration rate, probably because water soluble compounds were utilized. The lower respiration rates immediately after each wheat straw addition, lead to lower cumulative respiration of LW-16, LW-32 and LW-48 than 100%L (Figure 5.2).

5.4.2. Changes in microbial community composition

The difference in microbial communities composition during decomposition of high and low C/N plant residue may be attributed to the difference in residue chemical composition. According to Meidute *et al*. (2008), Gram negative bacteria are more competitive than fungi in the presence of easily decomposable compounds while fungi

are considered to be more competitive during decomposition of recalcitrant compounds. In general, a reduction in any microbial group at different phases of decomposition process in soil is due to other microorganisms taking on its function in order to utilize the nutrient sources (Nannipieri *et al.* 2003).

The incorporation of wheat straw into decomposing lupin residue provided lower amounts of readily decomposable compounds compared to lupin addition and changed the microbial community composition compared to 100%L and 100%W at most sampling times. Additionally, adding low C/N residue changed the soil pH and N availability which may also affect microbial community composition (Marschner *et al.* 2005; Osono *et al.* 2003; Meidute *et al.* 2008).

In 100%L the concentration of bacterial fatty acids that was significantly lower than fungal fatty acids from day 48 onwards. This may be due to the depletion of easily available compounds in the lupin residues, which reduces the competitiveness of bacteria in the later stages of decomposition.

5.4.3. Mineralization and immobilization of nitrogen

As elucidated in section 4.4.4, crop residues with C/N ratios of <21 are decomposed rapidly, often with a release of NH_4^+, which can be transformed into NO_3^- in the presence of oxygen (Walley and Yates 2002). Generally, residues rich in N, but with low lignin and polyphenol concentration decompose rapidly and release a large amount of N during the early stages of decomposition, but may not contribute much to the maintenance of SOM (Wang *et al.* 2004). This explains why the concentrations of NH_4^+ and NO_3^- were higher when lupin residues were added compared to wheat residues. During the 64 day incubation, net N mineralization occurred in lupin and lupin-wheat residue treatment with different NH_4^+/NO_3^- ratios over time, whereas N immobilization was dominant in 100%W in the first 48 days and slight net N mineralization only started afterwards (Figures 5.4, 5.5 and 5.6). The strong net N immobilization after addition of high C/N residues and a strong net N mineralization in plant materials with low C/N ratios has been shown in many previous studies (Bending *et al.* 1998; Trinsoutrot *et al.* 2000) and are explained in sections 2.3 and 4.4.4.

In accordance with our hypothesis, NH_4^+ and NO_3^- concentrations were significantly lower when wheat residues were added. This is due to the high C/N ratio and the low N content in the wheat straw used in this experiment. The NH_4^+ concentration in all lupin-

wheat treatments was significantly higher than the NO_3^- concentration in the first 32 days, accounting for 64% of total inorganic N. This is in agreement with Jansson and Persson (1982), Kara (2000), and Bolan and Hedley (2003), as they claimed that proteins and amino acids from crop residues are first converted to NH_4^+ before being nitrified to NO_3^- in the presence of oxygen. The high concentration of NH_4^+ in the first 32 days together with decarboxylation of organic acid anions may explain the strong increase in soil pH of the residue treatments (Figure 5.7). From day 48, the lower percentage of total inorganic N as NH_4^+ as well as the increase in NO_3^- concentrations (Figures 5.4, 5.5 and 5.6) suggests that nitrification became more important.

The strong increase of nitrate concentrations of lupin-wheat residue treatments from day 48 onwards resulted in significant decreases in soil pH, which can be attributed to the proton release during nitrification (Bolan and Hedley 2003). With regard to LW-32 and LW-48, after the incorporation of wheat straw into the decomposing lupin residue, a slight increase in NH_4^+ concentration resulted in a significant increase in soil pH compared to treatments with continuous lupin residue addition.

5.4.4. Changes in soil pH

It is well-known that the composition of plant residues affects their decomposition rate as well as their release of nutrients and alkalinity/acidity when incorporated into the soil (Haydes 1986; Tang and Yu 1999; Marschner and Noble 2000; Xu *et al.* 2006). The C/N ratio in crop residues is a decisive factor regulating the rate of mineralization and immobilization which strongly affects soil pH. The increase in soil pH with the addition of wheat residue compared to lupin residue is puzzling because decomposition rates and NH_4^+ concentrations were lower with wheat addition than with lupin addition. A possible explanation would be a lower nitrification, and thus lower H^+ production. The continual incorporation of lupin residue significantly increased the soil pH in the first 32 days and lowered the soil pH significantly afterwards (Figure 5.7). The 100%L treatment resulted in soil pH ranging from 6.6 on day 0 to 6.9 on day 32. However, from day 48 onwards, pH decreased significantly to 6.7 (Day 48) and 6.6 (Day 64). This can be attributed to the proton release during nitrification (Yan *et al.* 1996). This explanation is supported by the higher percentage of NO_3^- in total mineral N from the day 48 onwards (Figure 5.5).

In conclusion, the results from this experiment are in agreement with our hypotheses as they showed that adding high C/N crop residues into decomposing low C/N ratio residue strongly decreased the decomposition rates and that inorganic N release initially increased soil pH and changed microbial community composition. However, in the experiment described above, high and low C/N ratio residues were mixed together into the soil. Therefore there are several questions that need to be further elaborated:

(i) How does the spatial separation of high and low C/N ratio affect microbial activity and hence residue decomposition?

(ii) How does the availability of N and soil pH change when high and low C/N ratio residues are spatially separated at different distances from an interface?

(iii) A third experiment was carried out in order to provide a better explanation and deal with this question.

Chapter 6

Effects of Spatial Separation of High and Low C/N Residues on Decomposition

6.1 Introduction

In the management of crop systems, incorporation of residues into the soil has come to the attention of the scientific community as well as crop growers because of its critical role in sustaining soil fertility and crop production (Kwabiah *et al.* 2003). Chapter 5 showed that incorporating high C/N ratio residue into the decomposing low C/N residue decreased soil respiration, reduced N availability, increased soil pH and changed microbial community structure. To date, research has focused on the effects of plant chemical composition on rates of decomposition, N mineralization and soil pH of different plant materials as they are mixed together. However, fewer studies have been carried out to evaluate how high and low C/N crop residues interact with one another when they are spatially separated during decomposition in soil.

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. Therefore, the spatial contact between residues with contrasting biochemical qualities is likely to influence soil respiration and nutrient release of both residues, especially within the first 4-5 mm of the interface. Here the term "interface" refers to the area of contact between C/N residues with different biochemical properties, e.g. high and low C/N ratios. Residues with a different C/N ratio may occur in adjacent patches in natural ecosystems as well as in agricultural systems with crop rotation. However, there is a need to know at what distance a substrate is accessible to soil microorganisms and hence how it can modify the surrounding microbial population. Therefore the aims of this study were to assess carbon mineralization; nutrient release soil pH and microbial community structure in spatially separated high and low C/N residues at different distances from the interface.

It was hypothesized that, compared to the residues by themselves, the spatial contact of high and low C/N residues would increase the decomposition rate at the interface of the high C/N residues, whereas it would be decreased in the low C/N residues.

6.2 Materials and experimental design

The experiment was carried out in the same soil and water holding capacity as described for the experiments described in Chapters 4 and 5. The total amount of plant residues was 2% (w/w) in all treatments added once at the start. The C/N ratios of wheat and lupin were 82 and 19, respectively (Table 3.2). The residues were ground and sieved to 0.25-2 mm. After a two week pre-incubation of the soil (as described in section 3.2), the five treatments were set up as shown in Figure 6.1.

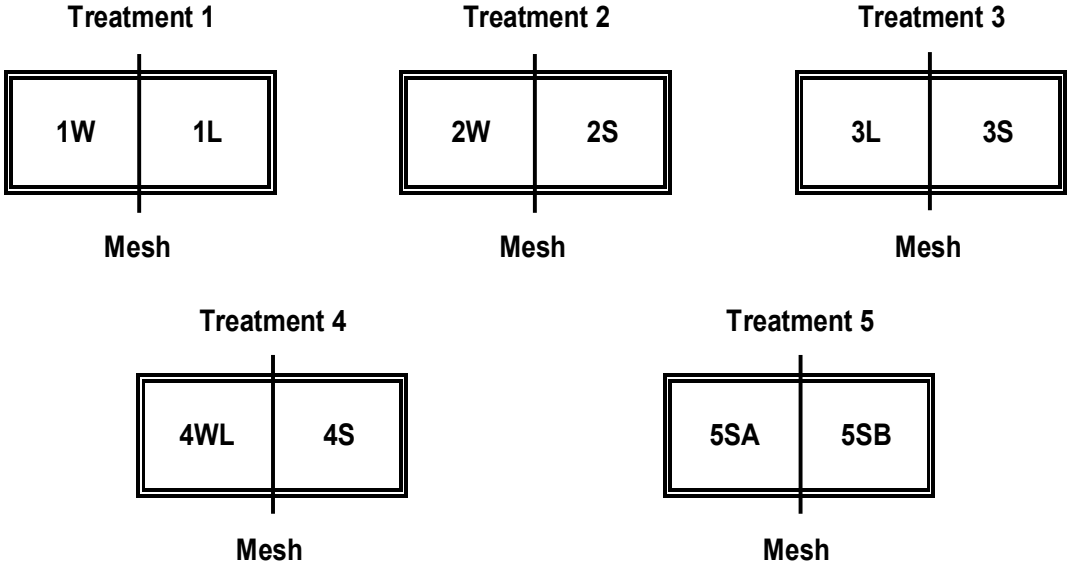


Figure 6.1 Treatments in Experiment 3

(W): wheat; (L): lupin; (S): soil, numbers indicate treatment number in order to be able to differentiate between e.g. wheat adjacent to lupin (1W) and wheat adjacent to soil (2W).

Each microcosm consisted of two PVC caps of 70 mm diameter and 20 mm height with the open end facing each other, separated by a 30 μm mesh. Each cap was filled with 110 g pre-incubated soil and 2.25 g residues (homogenously mixed into the soil). For WL, equal amounts of wheat straw and lupin residue were mixed thoroughly and then added to the soil. Fine nylon mesh (30 μm) was cut into circles with a diameter of 85 mm and placed between the two caps. The two caps were held together with rubber bands. The microcosms were incubated at 25^{o}C in the dark. RO water was added through small holes at the back of each of the two caps every 4 days maintaining soil moisture by weight. Soil sampling was carried out after 20, 40 and 60 days of

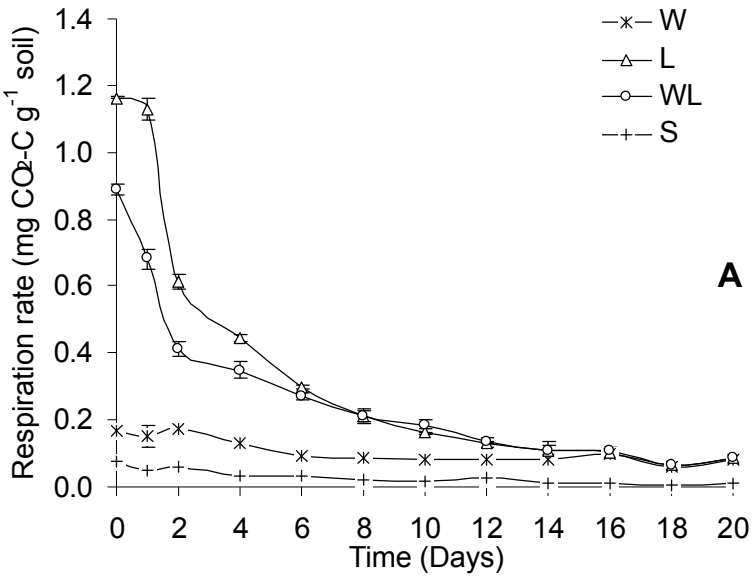
incubation on both sides of the mesh from different layers (0-5 mm and 5-10 mm from mesh). All measurements were carried out as described for the first experiment.

The 60 day incubation consisted of two periods. On day 0, 20 g of soil were collected from 4 treatments (W, L, WL and S with three replicates for measuring microbial respiration over 20 days, and determination of pH, PLFA and available N on day 0. This sampling represents the conditions before the joining of the two sides. Hence, it was a bulk soil sampling without differentiation between two layers. On days 20 and 40, samples were taken from the jointly incubated caps for analyzing the above mentioned variables in the two different layers. In each 5 mm slice, 20 g of soil were used for respiration measurement and another 20 g for measuring PLFA, pH and available N. At the last sampling on day 60, soils were only analyzed for PLFA, N and pH. Respiration was not measured after day 60.

6.3. Results

6.3.1. Respiration rate and cumulative respiration

In order to assess the effect of adjacent residues on respiration of non-amended soil, respiration is expressed per g of soil. The highest respiration rates occurred immediately after residue addition and then decreased with at different rates depending on residue treatment (Figure 6.2A).



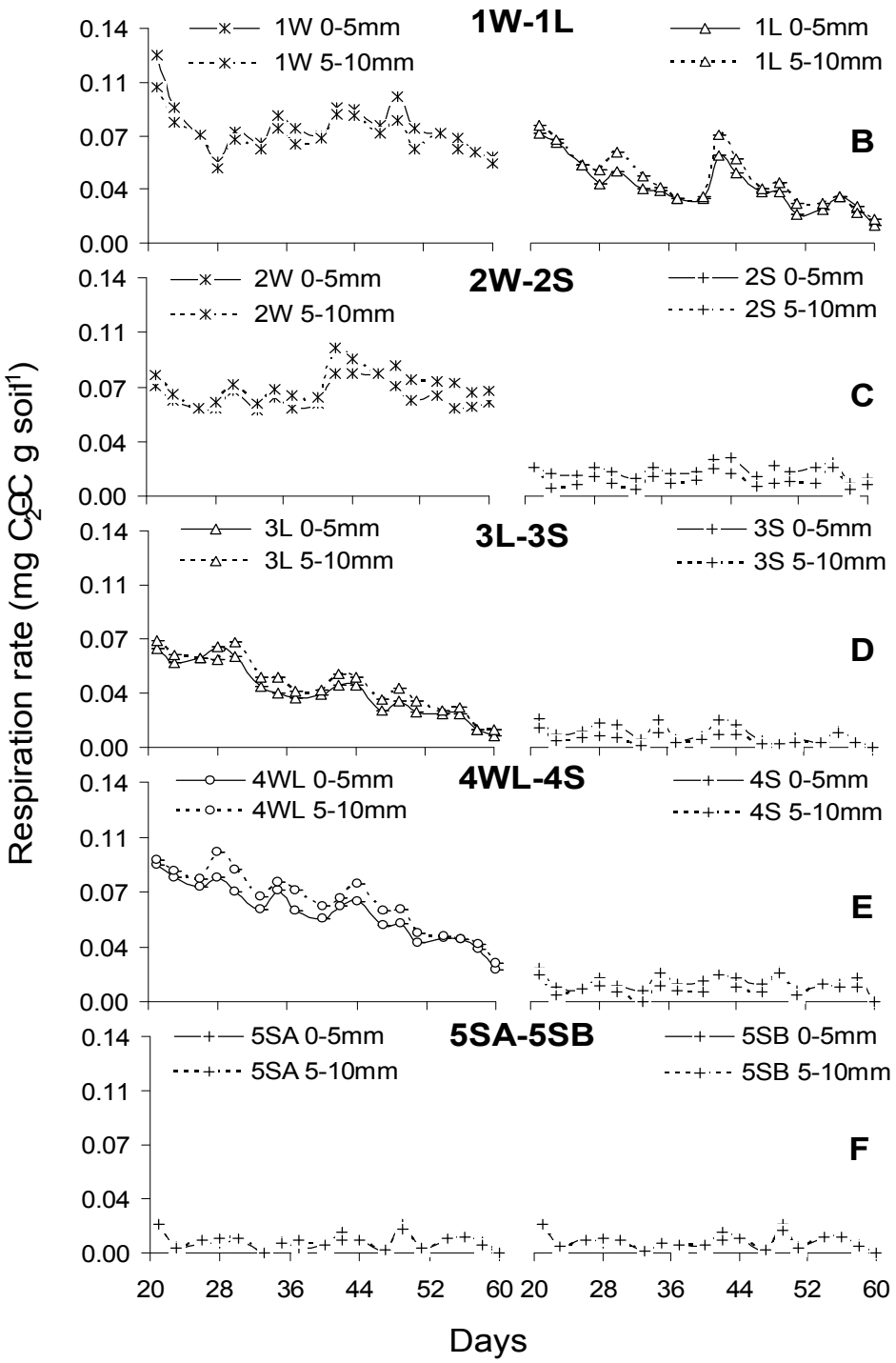


Figure 6.2 Respiration rate (mg CO_2-C g $soil^{-1}$ day^{-1}) over time for soil amended with wheat, lupin, wheat+lupin and control from Day 0 to 20(A); and for day 20 to 60 for treatments 1(B); 2(C); 3(D); Treatment 4(E); 5(F).

Vertical bars = Standard deviation (n=3)

In the first few days after adding the residues, lupin residue (L) (C/N 22) had the highest respiration rate and wheat (W) (C/N 82) the lowest. The respiration rate of L and the wheat-lupin mixture (WL) decreased rapidly from day 2. In the first 4 days, the

respiration rate of residue treatments was in the following order: L>WL>W. From day 6 to 20, the decrease in respiration rates of L and WL was similar. With regard to W, the respiration rate gradually decreased and was significantly lower than in L and WL in the first 14 days. From day 14 to day 20, there were no differences in respiration rate among residue treatments. The respiration rates in the second period of the 5 treatments in different layers are shown in Figures 6.2 (B), (C), (D), (E) and (F).

From day 40 to 60, the respiration rate of the wheat treatments (1W and 2W) was significantly higher than the lupin treatments (1L and 2L). The respiration rate of 1W was significantly higher than 2W until day 56, but there were no significant differences between the two wheat treatments by the end of the experiment (Figures 6.2B and C). On average, the respiration rate in the 0-5 mm layer of 1W was significantly higher than that in the 5-10 mm layer, whereas the respiration rate in the 0-5 mm layer of 2W was lower than in the 5-10 mm layer.

When considering lupin residue, the respiration rate of 1L and 3L decreased rapidly from day 20 onwards and was significantly lower than in 1W, 2W and 4WL. The respiration rate of 1L was generally lower than in 3L, except for the first few days after each sampling (Figures 6.2B and 6.2D). The respiration rates of the 0-5 mm layer of 1L and 3L were significantly higher than in the 5-10 mm layer.

After day 40, the WL treatment had a lower respiration rate than 1W and 2W, but a higher respiration rate than lupin residue only (1L and 3L). Until day 48, the respiration rate of the 0-5 mm layer of 4WL was significantly higher than that of the 5-10 mm layer. From day 48, there was no difference between the two layers.

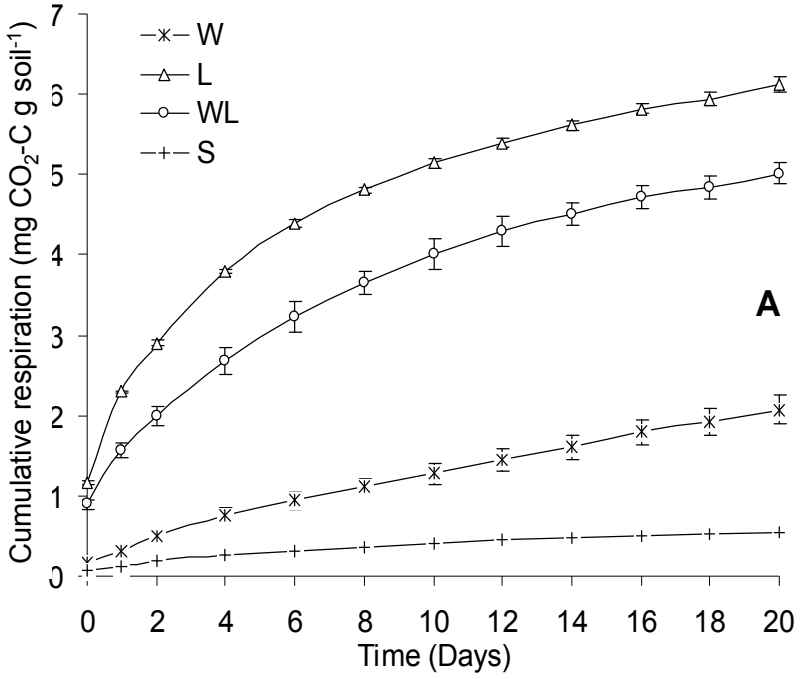
From day 20 onwards, the respiration rate of the non-amended soil was in the following order: 2S>4S>3S>5S (Figures 6.2C, D, E and F). With regard to the soil which was in contact with residues (treatments 2S, 3S, 4S), the respiration rates of the layer 0-5 mm were slightly (but not significantly) higher than the 5-10 mm layer.

The increase in cumulative respiration of residue treatments was strongest in the first 20 days (Figure 6.3A). Cumulative respiration of 1L and 3L was significantly higher than in 4WL, 1W and 2W. Cumulative respiration of 4WL on day 60 was twice as high as in treatments with wheat residue only (1W and 2W) (Table 6.1).

Table 6.1 Cumulative respiration as average of both layers (mg CO_2-C g $soil^{-1}$) of treatments on day 60

Treatments	Day 60
1W	2.60 [c]
1L	5.22 [a]
2W	2.50 [c]
2S	0.60 [d]
3L	5.21 [a]
3S	0.49 [f]
4WL	4.59 [b]
4S	0.55 [e]
5SA	0.49 [f]
5SB	0.49 [f]

Letters indicate significant differences between treatments at P<0.05



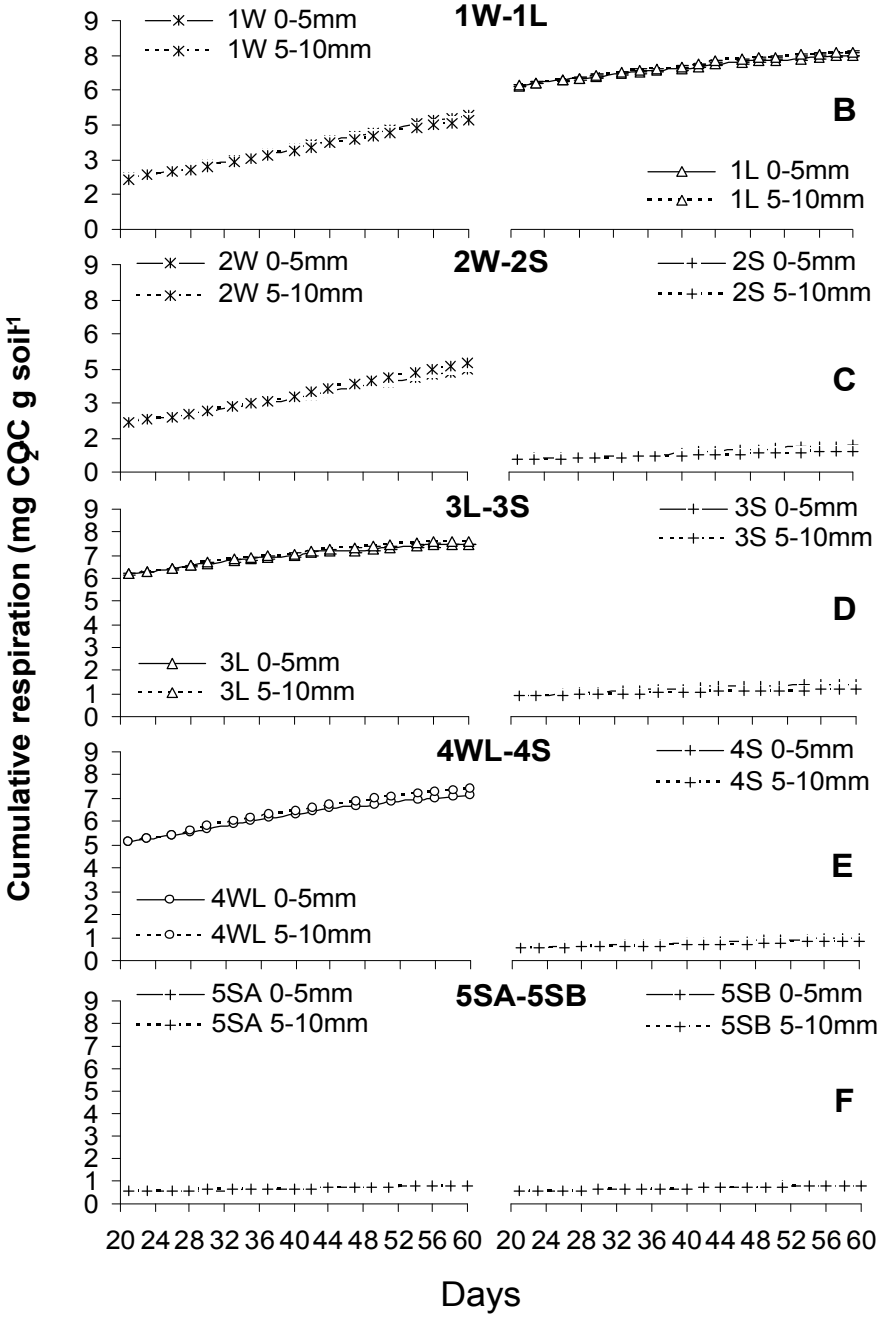


Figure 6.3 Cumulative respiration (mg CO_2-C g $soil^{-1}$) over time for soil amended with wheat, lupin, wheat+lupin and control from Day 0 to 20(A); and for day 20 to 60 for treatments 1(B); 2(C); 3(D); 4(E); 5(F).Vertical bars = Standard deviation (n=3)

From day 20 onwards, the cumulative respiration of 1L was similar to that of 3L (Figure 6.3D). Cumulative respiration in the 0-5 mm layer of 1W was on average slightly higher than that of the 5-10 mm layer; whereas the reverse was true for lupin (1L and 3L). From day 40 to 60, cumulative respiration in 1L only slowly increased but it was still significantly higher than in 1W. Cumulative respiration of 1W was slightly higher than 2W (Figures 6.3B and C).

Cumulative respiration of 4WL was significantly lower compared to 1L and 3L (Figures 6.3B, B and E; Table 6.1) but significantly higher than in wheat residue treatments (1W and 2W).

From day 20 onwards, cumulative respiration of the non-amended soil in different treatments was in the following order: 2S>4S>3S>5SA = SB (Figures 6.3C, D, E and F). On day 60, cumulative respiration of 2S and 4S was significantly higher than in 3S, 5SA and 5SB (Table 6.1).

6.3.2. Microbial community composition

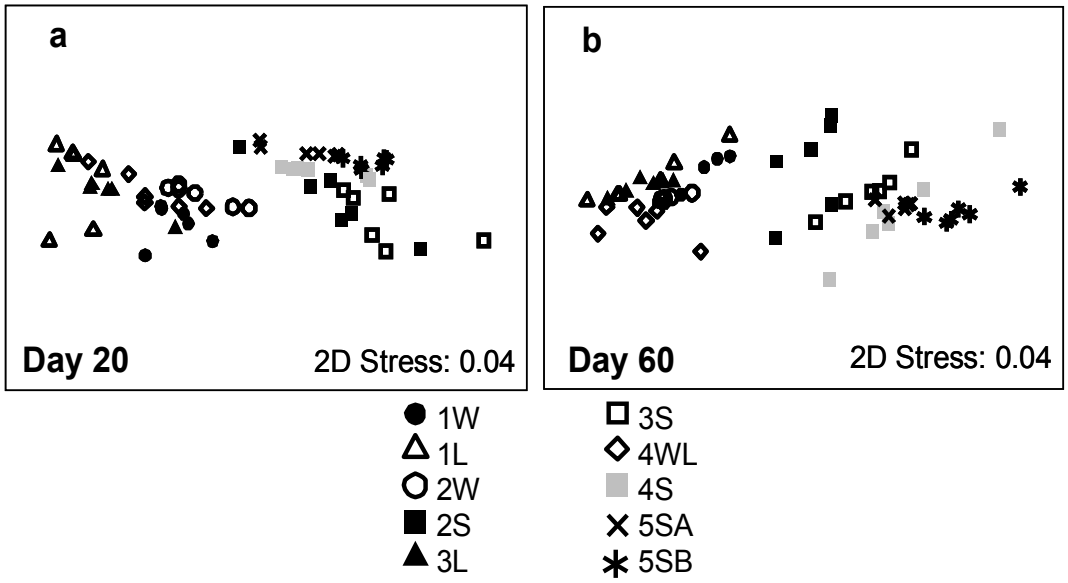


Figure 6.4 Microbial community structure of different treatments at different sampling times (a) day 20, (b) day 60.

Generally, the addition of plant residues had a significant influence on the PLFA pattern compared to the non-amended soil across all sampling times. The microbial community composition also differed between the different types of residues. However, there were no significant differences in microbial community composition between the different distances from the interface. Therefore no distinction is made in the following figures between the two layers. The stress values of the two dimensional MDS plots ranged between 0.04 and 0.05 indicating that the ordination was a good reflection of the overall structure of the microbial communities based on PLFA patterns. Results from the PERMANOVA showed that there were significant differences among sampling times and treatments and significant interactions between treatments and sampling times.

On day 20, across all treatments, five fatty acids contributed more than 50% of the pattern in microbial community composition, namely cy-C18:0 2-octyl; C16:1 isomer; C18:3w3c; C18:1w9c; C18:3w6c. The concentrations of bacterial and fungal fatty acids were higher in residue treatments than in the control (data not shown). There were significant differences in microbial community composition between residue treatments and the non-amended soils in contact with different residues (Figure 6.4a). The microbial community composition of 1L differed significantly from 3L and 4WL and 1W differed significantly from 2W. In the non-amended soil, the contact with different residues led to significant differences in microbial community composition between 2S, 3S, and 4S. The contact with soil only resulted in significant differences in the microbial community composition of 2W and 3L compared to 1W and 2L, and there was a significant difference between 2W and 4WL, but not between 3L and 4WL.

On day 60, the 6 fatty acids cyC18:0 2-octyl, i-C15:0, C16:0, C18:3w6c, C18:2w6, C16:1 isomer explained more than 50% of the pattern in microbial community composition across all treatments. As with day 20, the abundance of fungal fatty acids (C18:2ω6, C18:1ω9 and C18:3ω6) and fatty acids characteristic of gram positive bacteria (iC15:0, iC16:0) was significantly higher in residue treatments and unamended soil in contact with residues than in Treatment 5 (S-S). There were significant differences in microbial community composition between 1L, 1W, 2W, 3L and 4WL (Figure 6.4b). For lupin, the contact with wheat residue (1L) resulted in significantly different microbial community composition compared to lupin in contact with soil (3L). Similarly, there was a significant difference in microbial community composition between 1W and 2W. The microbial community composition of the non-amended treatments was significantly affected by the soil amendment on the other side of the mesh; there were significant differences between 2S, 3S, 4S and 5SA and 5SB (Figure 6.4b).

PLFA evenness was significantly higher in treatment 5 (5SA and 5SB) than in the other treatments throughout the incubation.

6.3.3. Inorganic N

The concentrations of NO_3^- and NH_4^+ in different treatments and layers changed over time, after the addition of residues with different C/N ratios. Generally, the addition of lupin residue significantly increased the concentrations of NO_3^- and NH_4^+ in lupin

residue treatments themselves, as well as in soils adjacent to that with lupin residue. On the other hand inorganic N concentration was low in the treatments where wheat residue was incorporated (Figures 6.5A and B; 6.6A and B).

6.3.3.1. NH_4^+ concentration

In 1W the NH_4^+ concentration increased significantly, whereas there was a significant decrease in 2W in the first 20 days (Figures 6.5A and B). From day 40 onwards, the NH_4^+ concentration of 1W decreased significantly. In 2W the NH_4^+ concentration decreased significantly in the first 40 days but then it tended to increase until the end of the experiment. The 0-5 mm layer of 1W had a significantly higher NH_4^+ concentration than the layer 5-10 mm in the first 40 days whereas the reverse was true on day 60. In 2W, the NH_4^+ concentration of layer 0-5 mm was generally lower than in layer 5-10 mm, but the difference was not statistically significant.

The NH_4^+ concentration of 1L and 3L increased significantly in the first 40 days; 1L had a significantly higher NH_4^+ concentration than 3L. On day 60, there was no significant difference in NH_4^+ concentration between 1L and 3L. The NH_4^+ concentration of 0-5 mm layer of 1L and 3L was significantly higher than in the 5-10 mm layer.

The NH_4^+ concentration was lower in WL than in 1L or 3L but higher than in soil with wheat residues. The NH_4^+ concentration of layer 0-5 mm of WL was significantly higher than in layer 5-10 mm.

The NH_4^+ concentration of the non-amended treatments was: 3S>4S>2S (Figures 6.5B-E). Generally, the 0-5 mm layer had higher NH_4^+ concentrations than layer 5-10 mm, and significant differences between different distances occurred in 3S and 4S.

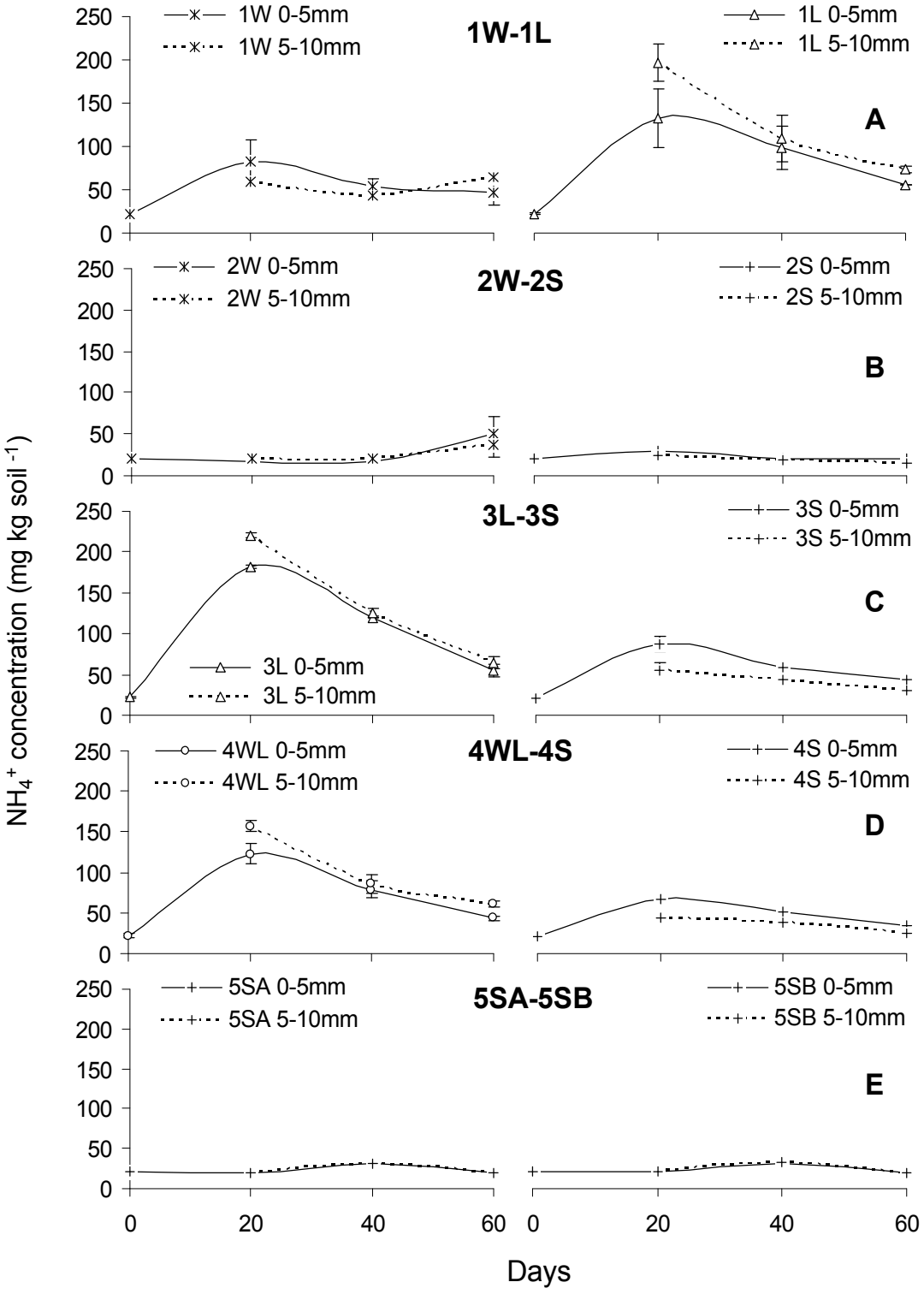


Figure 6.5 NH_4^+ concentration (mg kg^{-1}) over time of treatments 1(A); 2(B); 3(C); 4 (D); 5(E). Vertical bars = Standard deviation (n=3)

6.3.3.2. NO_3^- concentration

The NO_3^- concentration in 1W increased significantly over time as a result of contact with lupin residue (Figures 6.6A and 6.6B). On the other hand, the NO_3^- concentration

of 2W was lower than in 1W at day 20 and remained unchanged until the end of the experiment. In 1W, the NO_3^- concentration of layer 0-5 mm was slightly higher than in layer 5-10 mm, whereas in 2W the reverse was true, but the difference in NO_3^- concentration between layers was not statistically significant.

The lupin residue treatments increased the NO_3^-concentration of 1L and 3L significantly over time; 1L had a significant higher NO_3^- concentration than 3L (Figures 6.6A and 6.6C). On day 60, there was no significant difference in the NO_3^- concentration between 1L and 3L. The NO_3^- concentration of the 0-5 mm layer of 1L was significantly lower than in the 5-10 mm layer. On the other hand, the 0-5 mm layer of 3L had a slightly higher NO_3^- concentration than layer 5-10 mm in the first 40 days, whereas the reverse was true on day 60.

As with NH_4^+, the NO_3^- concentration of the mixture of wheat and lupin was lower than in soil with lupin only and higher than in soil with wheat only. The NO_3^- concentration of layer 0-5 mm was significantly higher than layer 5-10 mm in the first 20 days but thereafter, the NO_3^- concentration of layer 0-5 mm decreased and was significantly lower than in layer 5-10 mm at the end of the experiment.

The NO_3^- concentrations of the non-amended soil showed the same treatment differences as the NH_4^+ concentrations (Figures 6.6B, C, D and E). Generally, the 0-5 mm layer of 2S and 4S had higher NO_3^- concentrations than in the 5-10 mm layer. For 5SA and 5SB, there were no significant differences between distances.

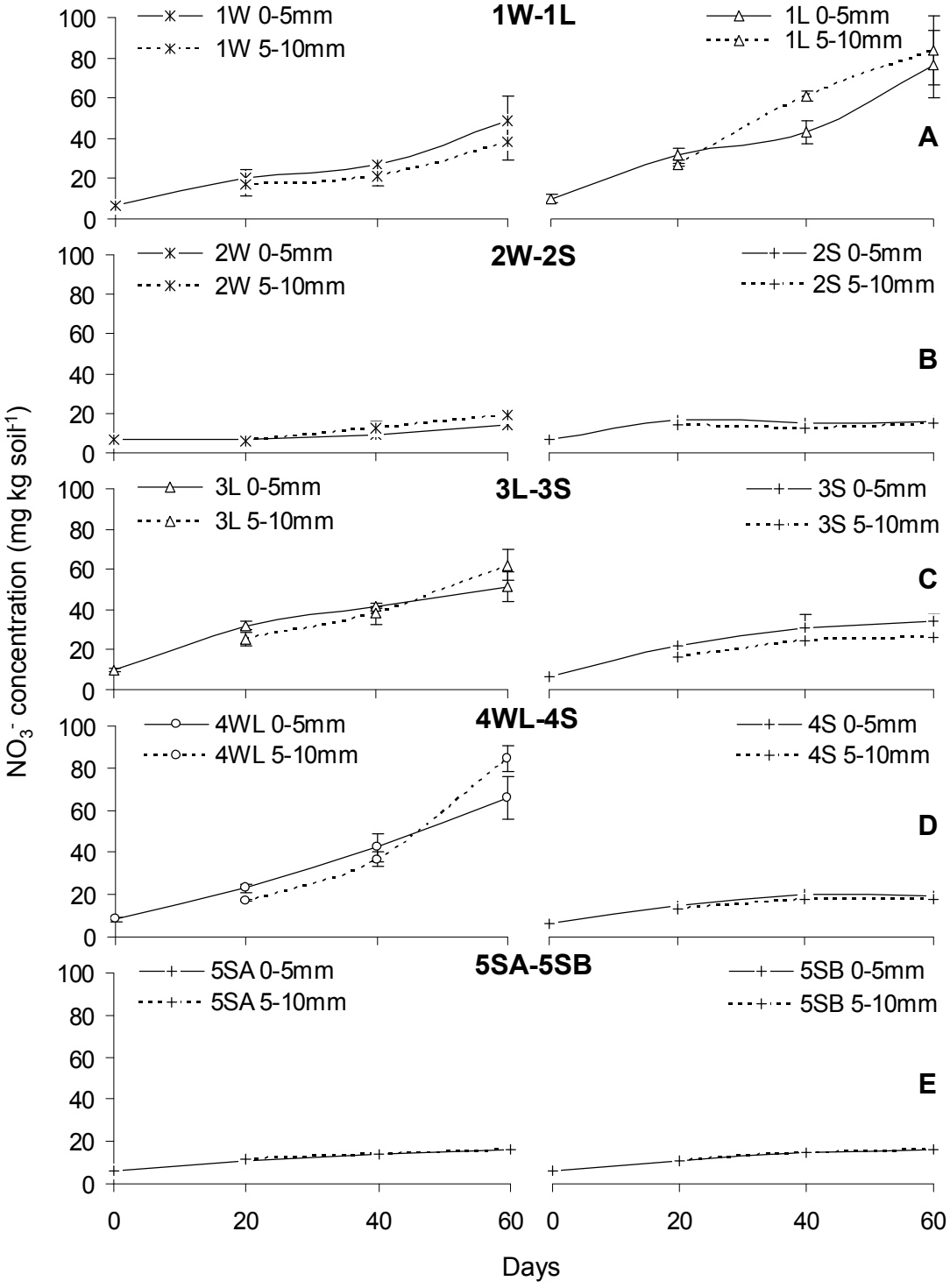


Figure 6.6 NO_3^- concentration (mg kg^{-1}) over time of treatments 1(A); 2(B); 3(C); 4 (D); 5(E). Vertical bars = Standard deviation (n=3)

6.3.4. Soil pH

Compared to the initial soil pH, the addition of crop residues significantly increased soil pH, but in 1L and 3L the pH significantly decreased from day 40 onwards.

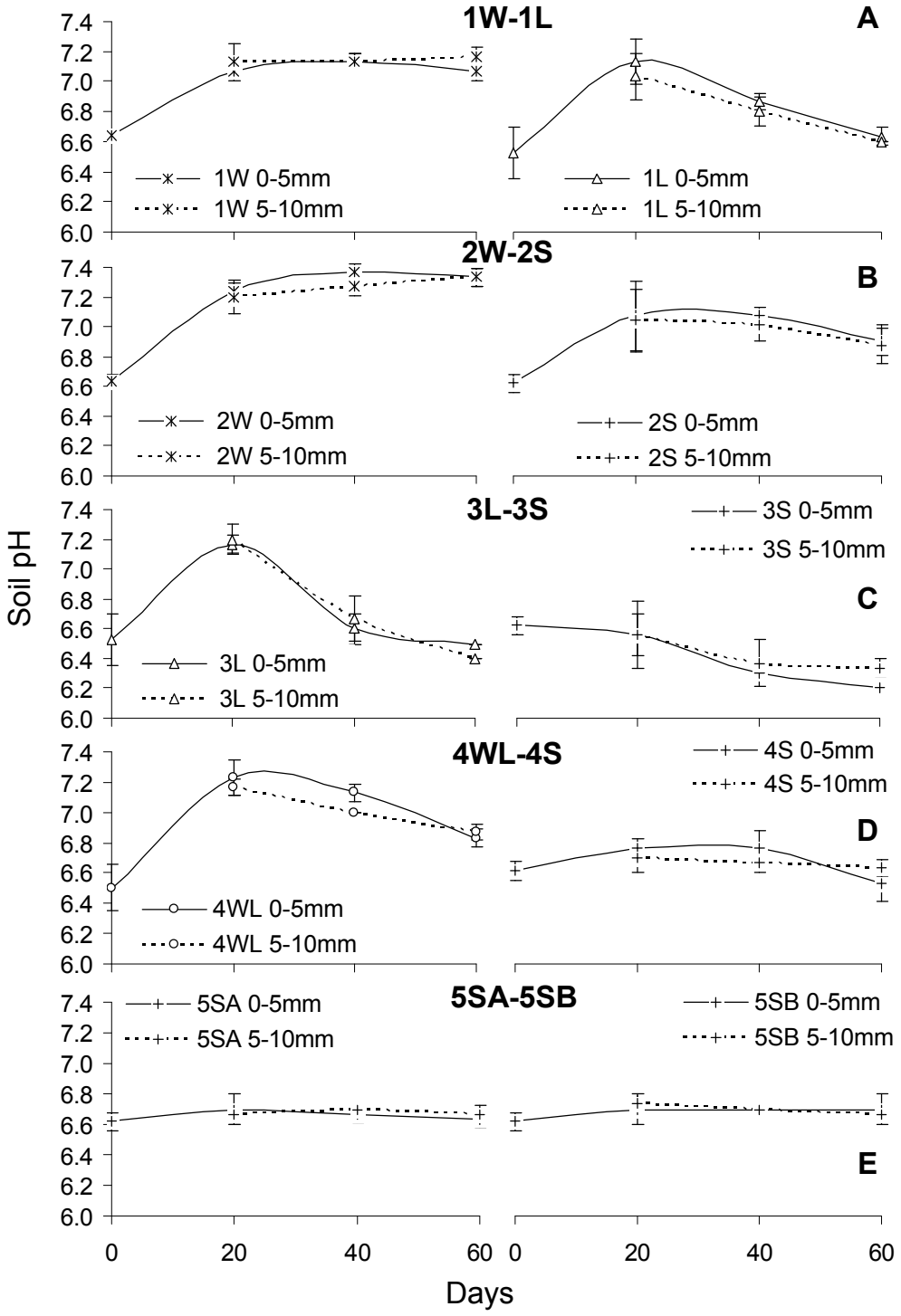


Figure 6.7 Soil pH over time of treatments 1(A); 2(B); 3(C); 4 (D); 5(E). Vertical bars = Standard deviation (n=3)

The soil pH in 1W and 2W was significantly higher than in the unamended soil. During the incubation, the soil pH in 1W and 2W increased significantly until day 40 and then remained unchanged (Figures 6.7A and 6.7B). As the result of contact with lupin residue, the soil pH of 1W was significantly higher than in 2W. The soil pH of the 0-5 mm layer of 1W was similar to the 5-10 mm layer in the first 40 days and significantly

lower than in the 5-10 mm layer at day 60. Conversely, in 2W the soil pH of the 0-5 mm layer was significantly higher than in the 5-10 mm layer at day 40 and there was no significant difference at day 60.

The soil pH of 1L and 3L increased strongly in the first 20 days but decreased after day 20; the pH decrease in 3L was significantly greater than in 1L (Figures 6.7A and 6.7C). The soil pH in the 0-5 mm layer of 1L was significantly higher than in 5-10 mm layer in the first 20 days, but from day 40 onwards, there were no significant differences between the two distances. With regard to 3L, the soil pH of the two layers was similar in the first 40 days, but at day 60 the pH of the 0-5 mm layer was significantly higher in the 5-10 mm layer.

The soil pH on day 60 was in the following order: 2W>4WL>3L (7.33, 6.85 and 6.45, respectively). The addition of the mixture of lupin and wheat residues resulted in a significant increase in soil pH in the first 20 days and then a gradual decrease over time. The soil pH in the 0-5 mm layer of 4WL increased significantly in the first 20 days, but then decreased. Thus on day 40, the pH of the 0-5 mm layer was lower than in the 5-10 mm layer. There was no significant difference in soil pH between different distances on day 60 (Figure 6.7D).

In the non-amended soil, on day 60, soil pH decreased in the following order: 2S>5S>4S>3S (6.88, 6.68, 6.58 and 6.27, respectively). When in contact with wheat residue, the soil pH of the 0-5 mm layer was slightly higher than in the 5-10 mm layer (Figure 6.7E). In 3S there were no significant differences in soil pH between layers in the first 40 days, but on day 60, the soil pH of the 0-5 mm layer was significantly lower than in the 5-10 mm layer. In 4S, in the first 40 days, soil pH of the 0-5 mm layer was significantly higher than in the 5-10 mm layer; however the reverse was true on day 60.

6.4. Discussion

6.4.1. Effect of residue interaction on respiration rate and cumulative CO_2-C

It is of interest to elucidate the interactions between high and low C/N residue on when they are spatially separated. 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.

The differences in respiration rates of lupin and wheat residue treatments from day 0 to day 20 in the current study (Figure 6.2A) have been explained in detail in Chapters 4 and 5. The higher respiration rate of WL compared to L from day 20 can be attributed to the higher C content remaining in wheat residue of the WL mix in the form of recalcitrant compounds (cellulose and hemicelluloses) after the depletion of easily utilizable sugars (Van Soest and Wine 1967; Bending and Turner 2004). A limitation of this experiment was that respiration rate was not measured *in situ* and was separated into two periods. This may explain why there were only small differences in respiration rate of different residues at the different distances from day 20 onwards.

In the second period of the experiment (days 20 to 60, Figures 6.2 B-F), the hypothesis that the presence of low C/N residue would increase respiration in the adjacent soil was confirmed as the respiration rates at the interface (0-5 mm) of 1W and 3S were higher than in the 5-10 mm layers, whereas the opposite was true of lupin and the mixture of lupin and wheat residue treatments in contact with non-amended soil or wheat residues. These results imply that in contact with lupin residues, microbial communities in wheat or non-amended soil were stimulated by substrates released from the nearby low C/N residues. According to Petersen *et al.* (1993), Kandeler *et al.* (1999), Gaillard *et al.* (1999) and Gaillard *et al.* (2003), there are two mechanisms for transporting molecules released from crop residues into the surrounding soil: 1) transport through fungal hyphae and 2) diffusion in the soil solution. Filamentous fungi are unique amongst soil microbes in their ability to transport/translocate nutrients, an ability which enables them to extend hyphal networks in a heterogeneous environment and to utilize spatially separated C and N released from residues through their hyphal networks (Tester 1988; Lindahl *et al.* 2001). Diffusion of nutrients occurs predominantly in solution and is, therefore, related to water availability. Since in this experiment the soil was moist, both diffusion and fungal hyphae may have contributed to transfer of substances from one side of the interface to the other. 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 loss of these compounds to the adjacent soil layers resulted in a decrease in respiration rate of the soil with lupin residues. Gaillard *et al.* (1999) found that the addition of plant residues induced strong gradients of microbial activity in soil at the millimeter scale, especially within 4 mm of the surfaces of plant residues. This is in agreement with the higher respiration rate in layer 0-5 mm than in 5-10 mm in treatments in association with low C/N residue or their mixture with

wheat residue, as uni-cellular soil microbes are only able to utilize and access substrates in close vicinity of rich-nutrient residues. Although lupin (1L, 3L) and the mixture of lupin and wheat (4WL) had lower respiration rates in the second period, their cumulative respiration rates in the end were still higher than in wheat residue (1W and 2W) because their respiration rates in the first period were significantly higher.

6.4.2. Changes in microbial community composition

Gupta and Germida (1988), Kandeler *et al*. (1999) and Poll *et al.* (2006) found that soils are extremely heterogeneous at a millimeter scale in terms of distribution of nutrients and microorganisms. The present study investigated how residues with different C/N ratios, that are separated spatially, affect each other in terms of microbial community composition.

During the first 20 days, water soluble compounds were utilized by microbes and this led to changes in microbial community composition. The hypothesis of depletion of readily decomposable compounds is supported by a strong decrease in respiration rate in the residue treatments from day 1 to day 8 (Figure 6.2A). Therefore, it can be assumed that after day 20, mainly recalcitrant compounds were present. This is also supported by the fact that, from day 20 to day 60, gram positive bacterial and fungal fatty acids dominated. Gram positive bacteria and fungi are known to be able to decompose recalcitrant compounds better than gram negative bacteria (Nannipieri *et al*. 2003; Rantalainen *et al*. 2004). The lack of differences in microbial community composition between different layers from the interface suggests that the conditions in the first 10 mm from the mesh were quite similar. Compounds diffusing from one residue to the other appear to move at least 10 mm in the soil that was kept moist throughout the incubation. Additionally, fungal hyphae may have translocated nutrients over this distance from one residue to the other. This explanation is supported by the fact that respiration, N availability and pH differed little between the layers. In 1W, which was adjacent to lupin residues, N availability was higher than in 2W which was adjacent to soil and the two wheat treatments differed in microbial community composition. On the other hand, N availability and cumulative respiration differed little between 1L (in contact with wheat) and 3L (in contact with soil). Nevertheless, microbial community composition differed between 1L and 3L (Figures 6.4a and b). This could be due to differences in nutrient availability not assessed, e.g. P, or to exchange of microbial species between the two sides or changes in community

composition that are not directly related to nutrient availability. Moreover, the differences in microbial community composition of non-amended soil in contact with different crop residues strengthens the suggestion that nutrients diffuse from the residue amended soil into the adjacent non-amended soil.

6.4.3. Nitrogen transformations and soil pH

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). It has been shown that soluble N can move up to 5 mm in soil, which can stimulate the growth of microbes (Gaillard *et al.* 1999).

The results from the present study showed that in the first 20 days, contact with lupin residue led to a significant increase in NH_4^+ concentration in 1W at 0-5 mm compared to 5-10 mm, whereas a strong immobilization occurred at both distances in wheat residue in contact with soil (2W; Figures 6.5A and B). This can be attributed to N transfer from the high N availability in the soil with lupin residues (1L) into the adjacent soil with low N availability; this resulted in a significant decrease in NH_4^+ concentration in the 0-5 mm layer of 1L compared to 5-10 mm. In contact with soil, net N immobilization occurred in wheat residue (2W) during the incubation. Nitrogen transfer into the adjacent non-amended soil also occurred in treatments 3 and 4 where contact with lupin and or the wheat-lupin mixture resulted in a significant increase in NH_4^+. Schwenndener *et al.* (2005) and Schimel and Hättenschwiler (2007) showed that there is movement of N from high-N residues to low-N residues in a heterogeneous environment regardless of whether the residues are considered as N-rich or N-poor in the classical sense.

From day 40 onwards, except for 1W, the NH_4^+ concentrations of most treatments decreased, whereas NO_3^- concentrations increased significantly (Figure 6.6). Treatments with high NO_3^- concentrations such as 1L and 4WL showed a strong soil pH decrease in the same period (Figures 6.7A, C and D) as a result of protons produced during nitrification (Yan *et al*. 1996a; Yan *et al*. 1996b; Xu *et al*. 2006). The wheat straw or non-amended soil in contact with N-rich residue had a higher concentration of NO_3^- in layer 0-5 mm than 5-10 mm that coincided with a lower soil pH of the 0-5 mm layer than the 5-10 mm layer (Figure 6.7).

In conclusion, soil is an ecosystem characterized by various spatially separated niches at different scales. The current experimental set-up allowed us to assess how plant residues affected one another, at the millimeter distances, in terms of microbial respiration and community structure, soil pH as well as N release. The results showed that N and soluble C compounds move from easily decomposable residues into the adjacent soil, thereby enhancing microbial activity. Thus our hypotheses were confirmed. However, in agriculture, plants are often growing in soils after residue application. Therefore, another area that needs further research is the effect of plant roots on high and low C/N residue decomposition and the following question arise and need to be further elaborated:

How do roots of cereals affect microbial activity and hence residue decomposition of high and low C/N residues, N availability and pH at different distances from the root mat?

The experiment, which is described below, was carried out to answer these questions.

Chapter 7

Effect of Plant Roots on the Decomposition of Residues

7.1 Introduction

The interaction of plant roots with residues and microorganisms occurs in the rhizosphere, which is defined as the soil surrounding the root, being influenced by the roots. Compared to the bulk soil, the rhizosphere has distinct biological, chemical and physical properties (Dakora and Phillips 2002). The main factor determining the interaction between roots and the soil is rhizodeposition which consists of low and high molecular weight C and N compounds released by the roots (Kuzyakov 2002). This rhizodeposition has diverse functions in improving nutrient availability for plants, e.g. carboxylic acids for phosphate and micronutrients (Hinsinger 2001; Keller and Römer 2001), phytosiderophores and malate for Fe (Fan *et al.* 2001), and other compounds which can relieve Al-toxicity by chelation (Heim *et al.* 2001). Cheng and Kuzyakov (2005) concluded that there are three main groups of organic substances exuded by roots into the rhizosphere, namely sugars (50-70% of total exudates); carboxylic acids (20-30% of total exudates) and amino acids (10-20% of total exudates). It is widely recognized that photosynthates released by roots are an important C source for microorganisms living in the vicinity of growing roots and a key factor in plant-microbe interaction (Paterson *et al.* 2006). In turn, decomposition of organic material by the microbial community in the rhizosphere releases available nutrients for plants. According to Marschner *et al.* (2005), rhizosphere microbial communities play an important role in fundamental processes that contribute to nutrient cycling, plant growth and root health. The microbial community composition in the rhizosphere is influenced by root exudate amount and composition, which vary with plant species (Bais *et al.* 2006); plant development stage/age (Van Veen *et al.* 1991; Marschner *et al.* 2001); and nutritional status (Marschner *et al.* 2001; Fan *et al.* 2001). Root exudates are mainly water-soluble, including sugars, amino acids, organic acids, hormones, vitamins and unidentified substances, such as microbial growth stimulants and inhibitors (Lynch and Whipps 1990; Kuzyakov 2005). Sugars provide available sources of carbon for the growth of microorganisms, whereas amino acids are a readily available source of nitrogen for microbes (Baldock 2007). The release of root exudates may induce a rhizosphere priming effect leading to increased decomposition of soil organic matter (Lynch and Whipps 1990; Fu and Cheng 2002).

Despite their involvement in many soil ecological functions, little is known about how root-borne compounds affect residue decomposition and nutrient release during decomposition. Therefore, the aim of the present study was to investigate the decomposition rate of high and low C/N residues, as well as soil pH, inorganic N and microbial community composition, at different distances from roots.

It is hypothesized that the presence of plant roots will increase the decomposition rate of residues, alter N transformation, soil pH and microbial community composition and that the effect will be greatest in the immediate vicinity of the roots. In the present experiment, the effect of wheat roots on decomposition of wheat and lucerne residues was investigated. These two residues differ in C/N ratio which could modify the effect of plant roots on decomposition and N dynamics.

7.2. Materials and Experimental Design

The experiment was carried out in a glasshouse, using the same soil and water holding capacity (WHC) as described for the first three experiments. The experiment was a 2 x 3 factorial design of plant (presence/absence) and wheat or lucerne residues or no residue in a randomized complete block design with 4 replicates.

Wheat seeds (Krichauff) were pre-germinated and sown in 450 ml pots with 350 g soil at 85 % WHC to which nutrient solution was added in order to avoid nutrient deficiency and to maximize plant growth. The composition of the nutrient solution was based on Hoagland, but the nutrient concentration was only 50% (Table 7.2). A preliminary experiment was carried out with soil without added nutrients, or amended with half-strength or full strength nutrient solution in which the dry matter (g) of the plants in the half-strength treatment was 11% higher in the full strength and 140% higher than in un-amended soil. Since the preliminary experiment showed that the half-strength solution resulted in the greatest plant growth and therefore supplied an adequate amount of nutrients for wheat growth over the duration of the experiment, it was used for the experiment described here. The half-strength Hoagland solution for macronutrients and a modified Long Ashton solution for micronutrients (Cavagnaro and Smith 2001) (Table 7.2) were added once, at the start of the experiment. One solution was added for all micronutrients except for Fe EDTA which was added separately. The amount of each nutrient solution added per pot of 350 g of soil was 1.75 ml.

Throughout the experiment, soil moisture was maintained at 85% WHC by adding RO water to weight. It should be noted that nutrient movement from the pots to the soil in the caps can not be ruled out.

The bases of the pots were cut off and covered with 30µm mesh which prevented root penetration and allowed the formation of a root mat on the mesh. After three weeks in the glasshouse, a root mat had formed (>50% mesh coverage). Then caps (PVC with a diameter of 70 mm and 20 mm height) with 110g of two-week pre-incubated soil amended with 2% w/w residues (2.25 g of residues in 107.75 g dry soil) were placed against the mesh as shown in Figures 7.1 and 7.2. There were 6 treatments: PW, PL, PS, W, L and S (Table 7.1). The C/N ratios of wheat and lucerne are 82 and 18, respectively and the residues were ground and sieved to 0.25-2 mm. Day 0 samples were taken immediately after amendment of the soil with residues.

Table 7.1 Treatments of Experiment 4

Pot	Cap	Treatment
Plant	High C/N residue	PW
Plant	Low C/N residue	PL
Plant	Soil no residues	PS
No plant	High C/N residue	W
No plant	Low C/N residue	L
No plant	Soil no residues	S

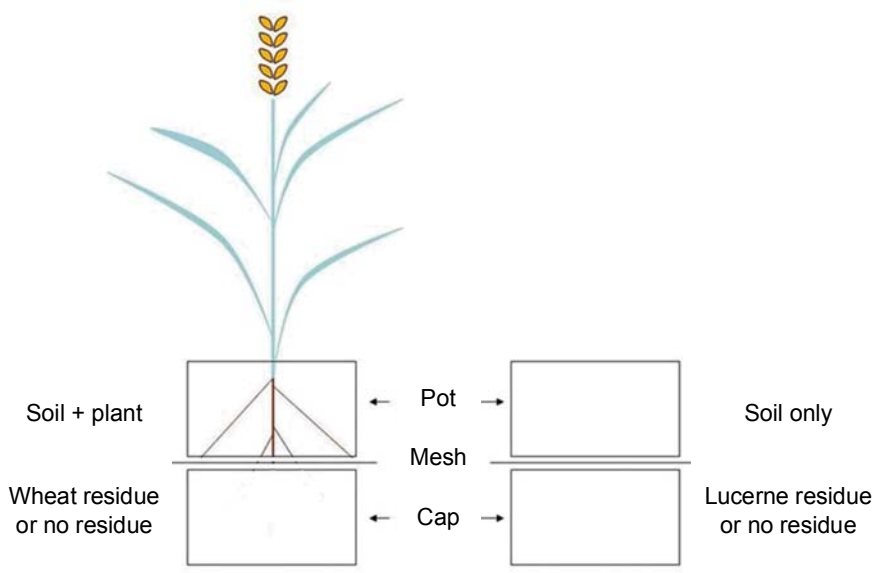


Figure 7.1 Experimental design of Experiment 4

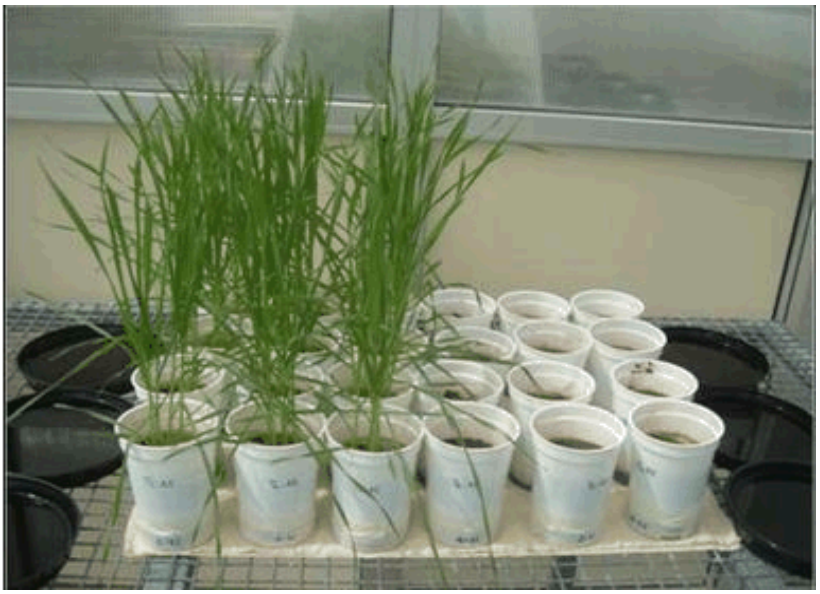


Figure 7.2 Plant growth after 25 days

Table 7.2 Composition of nutrient solutions (Half-strength)

Concentration of nutrient solutions in treatment of		Amount of nutrients
Hoagland solutions g l^{-1}		mg kg $soil^{-1}$
NH_4NO_3	21.45	107.25
$Ca(NO_3)_2$ $4H_2O$	21.1	105.5
KH_2PO_4	10.95	54.75
K_2SO_4	8.7	43.5
$MgSO_4$ $7H_2O$	18.95	94.75
Fe EDTA	20	0.10
Long Ashton solution µg l^{-1}		
$CuSO_4$ $5H_2O$	100	0.50
$MnSO_4$ $4H_2O$	30	0.15
$CoCl_2$ $6H_2O$	17	0.085
H_3BO_3	25	0.125
Na_2MoO_4 $2H_2O$	35	0.175
$ZnSO_4$ $7H_2O$	110	0.55
$CuSO_4$ $5H_2O$	100	0.50

The plants were watered daily with RO water and RO water was added to the caps every 4 days to the initial weight. The caps were removed 16 days after attachment to the bottom of the pots and sliced in different layers (0-5 mm and 5-10 mm from mesh). From each 5 mm slice, 20 g of soil was used for respiration measurement over 14 days and 20g for immediate analysis of PLFA, pH and available N (day 16). At the end of the respiration measurements (day 30), the soil was analysed for pH and inorganic N analyses as described in Chapter 3. Thus sampling was carried out on day 0 (placement of the caps on the bottom of the pots), day 16 (removal of caps from the pots) and day 30 (after 14 days incubation of the soil in absence of the roots). Sampling times and analyses were presented in Table 7.3.

Table 7.3 Sampling dates and analyses

Experimental stage	Sampling dates	Samples analysed for
Plant growth	3 weeks	
Attachment of caps with amended and unamended soil	Day 0	PLFA, inorganic N and pH
Removal of caps from mesh	Day 16	PLFA, inorganic N and pH
Incubation of soil after removal	Day 16-30	Respiration
Final harvest	Day 30	Inorganic N and pH

7.3 Results

7.3.1. Dry matter

Shoot and root dry matter was similar in all treatments. The dry matter of shoots and roots were 2.02 ± 0.12 and 0.75 ± 0.07 (g pot^{-1}), respectively.

7.3.2. Respiration rate and cumulative respiration

In order to assess the effect of roots on respiration in amended and non-amended soil, respiration is expressed per g soil rather than per g residue. In the treatments with plants, respiration of soil with wheat and lucerne residues significantly increased in the first 4 days after removal from the root mat, compared to treatments without plants (Figure 7.3 A, B). Among residue treatments, although the respiration rate of PL was higher than in PW in the first 4 days, the difference was not statistically significant.

During the 14 days of measurement of respiration, the respiration rate of PS was significantly higher than in S, but the respiration rate of PS was significantly lower than residue treatments with or without plants (Figure 7.3 C).

From day 4 onwards, there were no significant differences in respiration rates between residue treatments with plants or without plants. The presence of plants led to a significantly higher respiration rate in layer 0-5 mm than 5-10 mm, while the reverse was true in treatments without plants (Figures 7.3A-C).

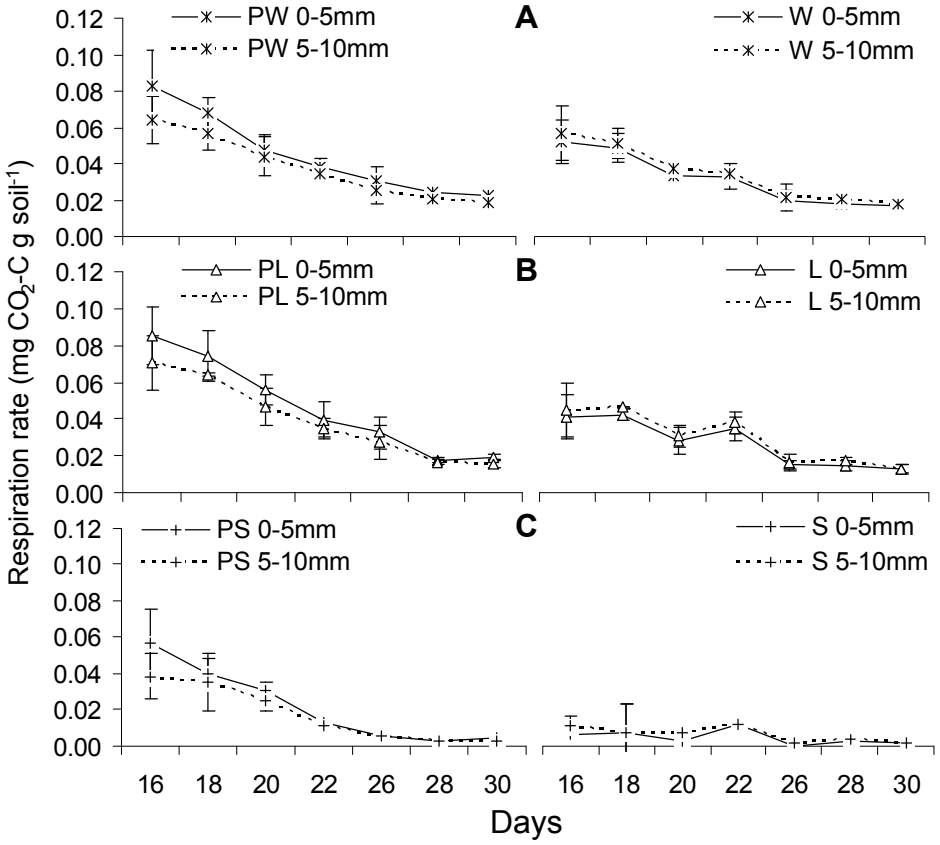


Figure 7.3 Respiration rate (mg CO_2-C g^{-1} soil day^{-1}) of planted (indicated by P) and unplanted soil that was unamended (S) or amended with wheat straw (W) or lucerne residues (L): PW and W (A); PL and L (B) and PS and S (C) over time.
Vertical bars = Standard deviation (n=4)

Although cumulative respiration of PS was significantly higher than in S, both were significantly lower than cumulative respiration of residue treatments with and without plants (Figure 7.4). Among residue treatments, cumulative respiration was higher with plants than without plants, with no significant differences between wheat and lucerne amended soil. Among the residue treatments without plants, cumulative respiration of L was slightly lower than in W. The presence of plants resulted in a significantly higher

cumulative respiration of layer 0-5 mm than of the 5-10 mm layer, whereas in the absence of plants there was no significant difference between the two layers (Figures 7.4A and B).

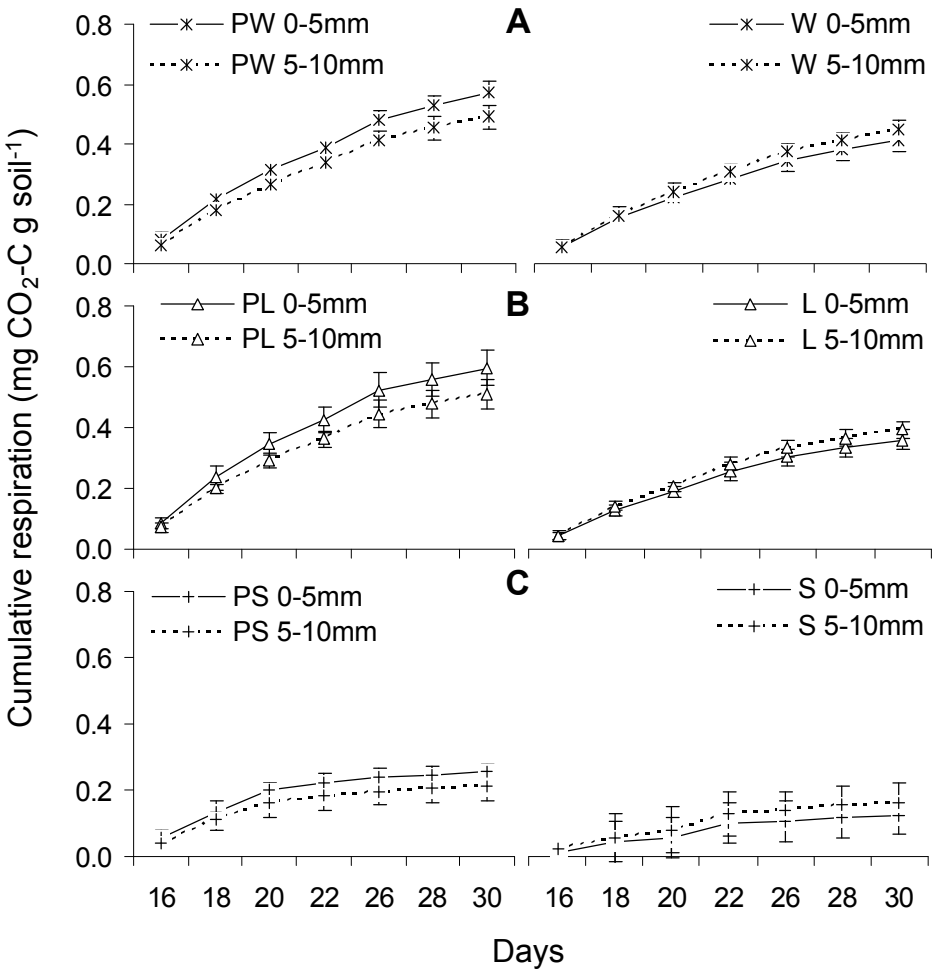


Figure 7.4 Cumulative respiration mg CO_2-C g $soil^{-1}$ of planted (indicated by P) and unplanted soil that was unamended (S) or amended with wheat straw (W) or lucerne residues (L): PW and W (A); PL and L (B) and PS and S (C) over time.
Vertical bars = Standard deviation (n=4)

7.3.3. Microbial community composition

Multivariate analyses of the PLFA data generally showed that, on day 16, microbial community composition differed significantly between treatments with plants or without plants, as well as between layers (Figure 7.5). The stress values of the two dimensional MDS plots ranged between 0.01 and 0.03, indicating that the ordination was a good reflection of the overall composition of the microbial communities based on PLFA patterns. The concentrations of total PLFAs in treatments with plants (PW, PL

and PS) and the concentration of gram positive bacterial fatty acids was significantly higher than in W, L and S (Table 7.3).

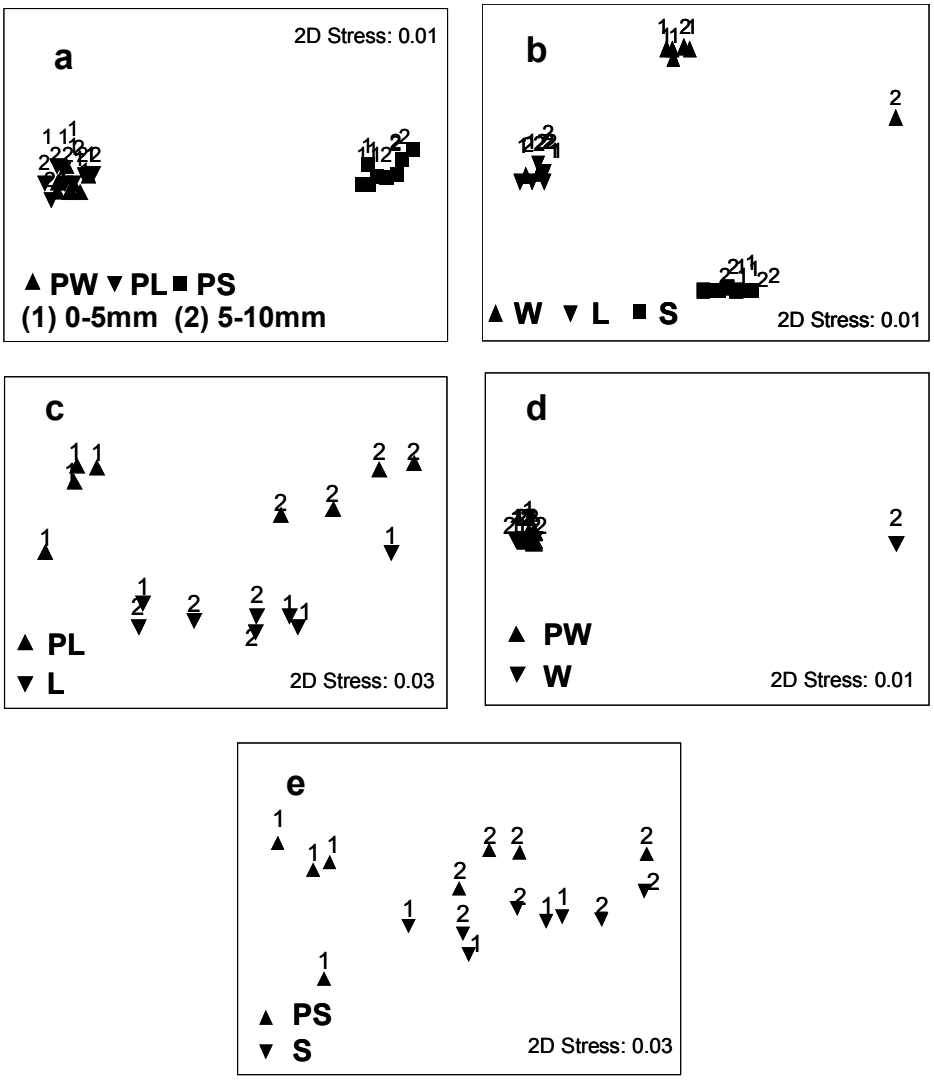


Figure 7.5 Microbial community compositions of planted (indicated by P) and unplanted soil that was unamended (S) or amended with wheat straw (W) or lucerne residues (L): PW and W (A); PL and L (B) and PS and S (C) over time: PW, PL and PS (a), W, L and S (b), PL and L (c), PW and W (d) and PS and S (e) over time in (1) layer 0-5 mm and (2) layer 5-10 mm

PERMANOVA showed that on day 0, immediately after residue addition and before placing the soil in contact with the root mat, the microbial community composition of the non-amended soil differed from that of the amended soil (data not shown). After 16 days, PLFA evenness was significantly higher in S than in the other treatments. Results from the PERMANOVA showed that there were significant differences between residue treatments and significant interactions between residue addition and the plant

presence at different distances from the root mat on day 16 (Figure 7.5). Across all treatments, three fatty acids, cy-C18:0 2-octyl; C16:0 and C17:0, were the main contributors to the differences in microbial community composition.

Table 7.4 Total microbial biomass and gram positive signature PLFA in different treatments, expressed as % area of internal standard (n=4, ± standard deviation)

Treatments	Total microbial biomass	Gram positive bacterial PLFA
PW 0-5 mm	20.3 ± 2.3	1.9 ± 0.3
PW 5-10 mm	17.4 ± 2.2	1.5 ± 0.2
PL 0-5 cm	26.8 ± 0.8	2.2 ± 0.7
PL 5-10 cm	19.6 ± 2.7	1.6 ± 0.6
PS 0-5 cm	8.8 ± 0.9	1.0 ± 0.5
PS 5-10 cm	5.8 ± 0.9	0.7 ± 0.4
W 0-5 cm	10.8 ± 1.9	1.7 ± 0.3
W 5-10 cm	14.8 ± 3.9	1.9 ± 1.8
L 0-5 cm	20.1 ± 2.5	2.4 ± 0.5
L 5-10 cm	18.6 ± 2.3	2.2 ± 0.2
S 0-5 cm	6.3 ± 0.9	0. 8 ± 0.2
S 5-10 cm	5.5 ± 0.9	0.6 ± 0.1

There were significant differences in microbial community composition between treatments and between layers (Figure 7.5). The microbial community composition of residue treatments without plants differed significantly from one another and differences between layers occurred in L and S (Figure 7.5b). The concentrations of the gram-positive bacterial fatty acid iC16:0 was higher in plant treatments than in treatments without plants, which had higher concentration of fungal fatty acids, (C18:2ω6c, C18:1ω9c and C18:3ω6c) in all residue treatments (Table 7.3).

The presence of plants significantly affected microbial community composition in soil with residues. In the soil with lucerne residues, the concentrations of bacterial and fungal fatty acids were significantly higher in PL than in L and the community composition in the layer 0-5 mm of PL and L was significantly different from the layer 5-10 mm (Figure 7.5c, Table 7.3). In the soil with wheat residue, significant differences in microbial community composition were found between PW and W in the distance of 0-5 mm, but not in the 5-10 mm layer (Figure 7.5d). In the non-amended treatments, the

concentrations of bacterial and fungal fatty acids were higher in the layer 0-5 mm of PS than in S but there were no significant differences between PS and S in layer 5-10 mm (Figure 7.5e, Table 7.3).

7.3.4. Inorganic nitrogen

The concentrations of NH_4^+ and NO_3^- in different treatments and layers changed over time after the addition of residues with different C/N ratios**.** Generally, the presence of plants (PW, PL and PS) significantly decreased the concentrations of NH_4^+ and NO_3^- compared to treatments without plants. Inorganic N concentrations were lower in treatments with wheat residue (W) compared to soil with lucerne residues and non-amended soil. On the other hand, inorganic N concentrations increased rapidly in soil with lucerne residue (L) in the absence of plants (Figures 7.6 and 7.7).

7.3.4.1. NH_4^+ concentration

The presence of plants (PW, PL and PS) led to a significant decrease in NH_4^+ concentrations compared to treatments without plants (Figures 7.6A, B and C).

Among plant treatments, despite a significant decline compared to the treatments without plants (L), the NH_4^+ concentration in PL was significantly higher than in PW and PS. On day 16, the NH_4^+ concentration of PW was slightly lower than in W, whereas the NH_4^+ concentration was significantly lower in PL and PS in comparison with L and S, with a decrease of 83% and 71%, respectively. The NH_4^+ concentration in the 0-5 mm layer of PL and PW was lower than in the layer 5-10 mm in the first 16 days; however, this decrease was significant only for PL. On day 30, after 14 days incubation in the absence of plants, the NH_4^+ concentrations were significantly higher than on day 16, except in PW. The NH_4^+ concentration increased more strongly in treatments without plants (W, L and S) than in PL and PS. Compared to the layer 0-5 mm, the NH_4^+ concentration of the 5-10 mm layer was significantly higher in PL and PS.

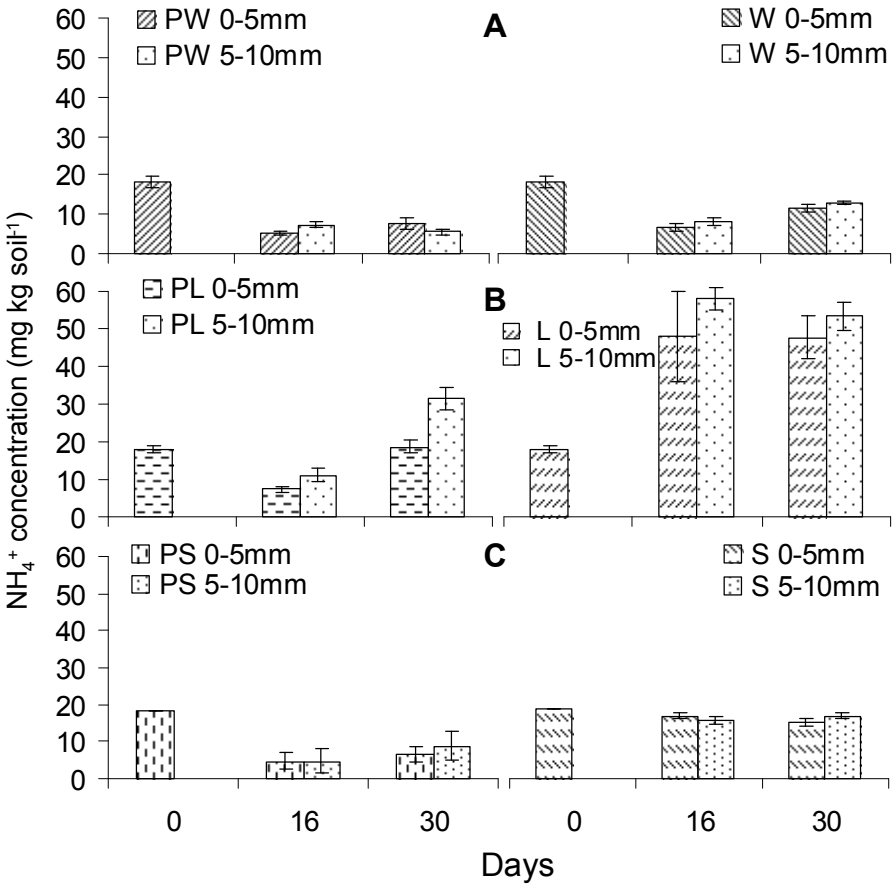


Figure 7.6 NH_4^+ concentrations of treatments (mg kg^{-1}) of planted (indicated by P) and unplanted soil that was unamended (S) or amended with wheat straw (W) or lucerne residues (L): PW and W (A); PL and L (B) and PS and S (C) over time.
Vertical bars = standard deviation (n=4)

7.3.4.2. NO_3^- concentration

The reduction of the NO_3^- concentration in treatments with plants was generally less pronounced than the decrease in NH_4^+ concentration. On day 16, the NO_3^- concentration was significantly lower in PL and PS than in L and S (Figures 7.7B and C). The NO_3^- concentration of PL was twice as high as in PW and PS. In the presence of plants, the NO_3^- concentration of the layer 0-5 mm of PL and PS was significantly lower than the layer 5-10 mm, whereas the reverse was true in L and S. For soil with wheat residues, the presence of plants had no significant effect on NO_3^- concentrations and there was no significant difference between layers (Figure 7.7A). On day 30, the differences between treatments and layers were similar to those on day 16.

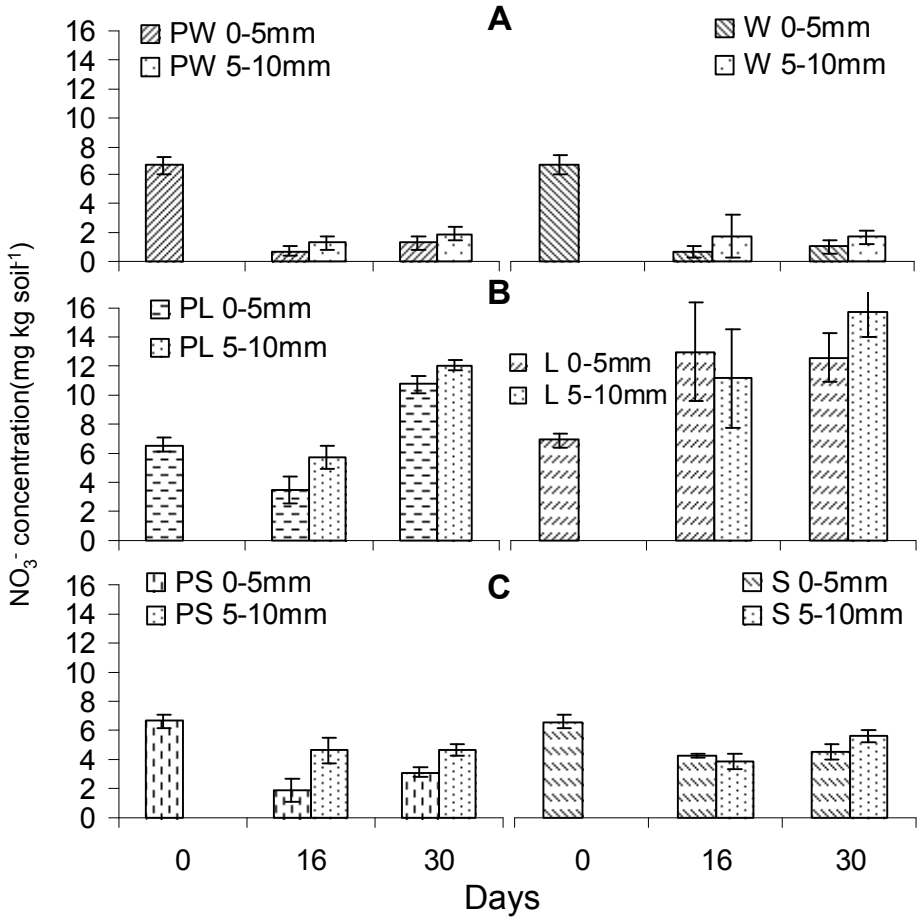


Figure 7.7 NO_3^- concentrations of treatments (mg kg^{-1}) of planted (indicated by P) and unplanted soil that was unamended (S) or amended with wheat straw (W) or lucerne residues (L): PW and W (A); PL and L (B) and PS and S (C) over time

Vertical bars = standard deviation (n=4)

7.3.5. Soil pH

The addition of crop residues as well as the presence of plants led to a significant increase in soil pH compared to initial soil pH (Figures 7.8A, B and C).

On day 16, the pH of PL was significantly higher than in PW and PS and there were no significant differences between PW and PS. Compared to treatments without plants, PL and PS had significantly higher pH (0.45 and 0.65 on average, respectively), whereas the pH of PW was only 0.1 unit higher than W. In PL and PS, the pH of the layer 0-5 mm was significantly higher than in layer 5-10 mm, whereas there were no differences between layers in PW.

On day 30, after a 14 day-incubation in the absence of plant roots, soil pH of treatments with plants or without plants had decreased slightly in the two layers and there were no significant differences between layers.

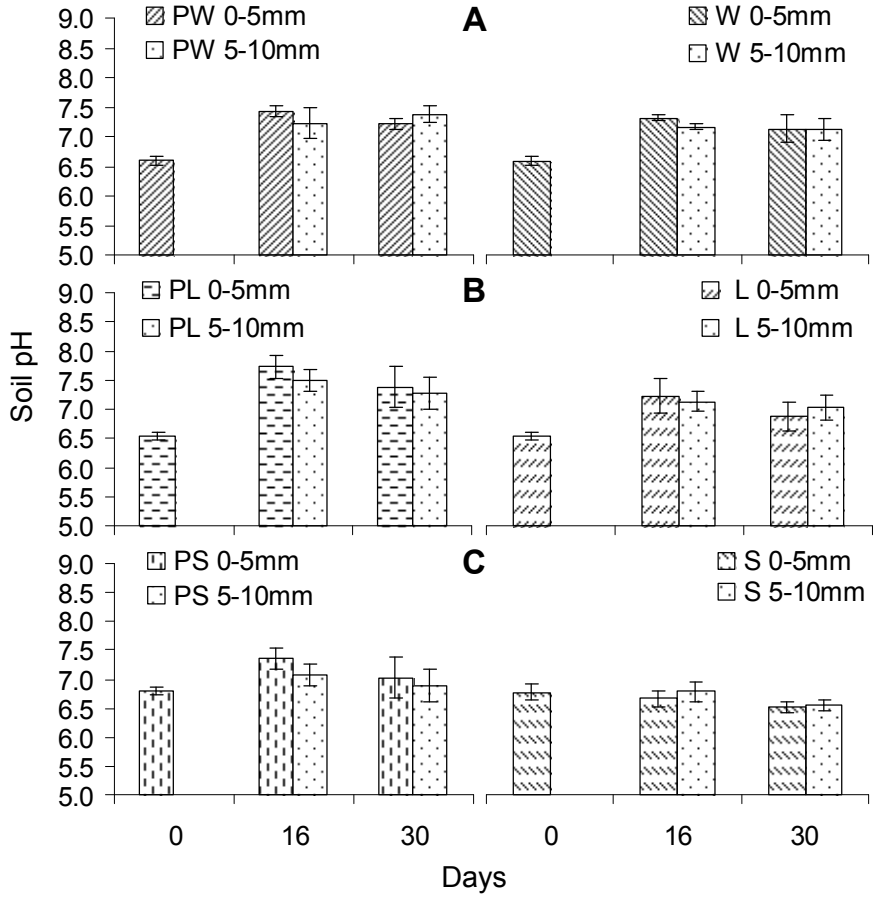


Figure 7.8 Soil pH of planted (indicated by P) and unplanted soil that was unamended (S) or amended with wheat straw (W) or lucerne residues (L): PW and W (A); PL and L (B) and PS and S (C) over time.

Vertical bars = standard deviation (n=4)

7.4 Discussion

7.4.1. Effect of plant roots on respiration rate and cumulative respiration

In agricultural ecosystems, together with residue addition, the presence of living plants may result in changes in microbial community structure and residue decomposition rate, via root exudation. Hence, there is a need to have a better insight into the way plant roots affect decomposition of high and low C/N residue, soil pH, N transformation and soil microbial communities.

In the present experiment, respiration was measured over 14 days after removal of the soil from the roots. After the first 4 days, respiration rates were similar in PW and PL, which suggests that by then (more than 20 days after mixing the residues into the soil), easily available compounds, which had presumably been initially higher in lucerne residues than in wheat, had been depleted and the remaining recalcitrant compounds (cellulose and hemicelluloses) were decomposed at similar rates in both residues. In the first 4 days after removing the soil from the root mat (from day 16 to day 20), the higher respiration rate in PW, PL and PS compared to treatments without plants can be attributed to decomposition of root exudates (Figure 7.3) (Kuzyakov 2002; Hütsch *et al*. 2002). The increased respiration in the first 4 days is in agreement with Hamilton and Frank (2001), Morgan *et al.* (2005) and Bais *et al.* (2006), who reported that plants can stimulate soil microbes and their nutrient supply via root exudation. Compared to PS, the higher respiration rates in PW and PL may be due to the decomposition of remaining residues or soil organic matter, which is in agreement with Eldhuset (2005) and suggests a priming effect (Kuzyakov 2002). From day 4 onwards, the respiration rate of all treatments decreased similarly, which can be explained by the depletion of root-borne substances and the dominance of recalcitrant compounds in the residue and native soil organic matter.

The significantly higher respiration rate in the layer 0-5 mm, compared to the layer 5-10 mm in the presence of plants especially in the first 4 days, implies that root exudates do not move up more than 5 mm from the root surface into the surrounding soil.

7.4.2. Microbial community composition

In the previous chapters, the addition of residues with different C/N ratios led to significant shifts in microbial community composition, due to the differential chemical properties of the residues. The present study was set up to test the effect of roots and root exudates on changes in microbial community composition after the incorporation of residues with different C/N ratios.

The significant differences in microbial community composition induced by the addition of residues with different C/N ratios were discussed in Chapters 4, 5 and 6, and can be attributed to the presence of easily available compounds in low C/N residues favouring rapidly growing r-strategists. The presence of root-borne substances in the vicinity of plant roots was hypothesized to induce significant changes in microbial

community composition at different distances from the root mat. This hypothesis was confirmed as the results from the current study showed that the composition of microbial communities the residue treatments not only significantly differed from one another, but also differed between planted and unplanted pots and between layers after 16 days in contact with the roots (Figures 7.5 a-e).

The concentrations of bacterial and fungal fatty acids in PW, PL and PS were 89%, 33% and 41% higher than in W, L and S respectively. The concentrations of PLFAs in PW, PL and PS in the 0-5 mm layer were 17%-53% higher than in the layer 5-10 mm (Table 7.3). The higher concentration of bacterial and fungal fatty acids in treatments with plants, particularly in the 0-5 mm layer, indicates that the microbial biomass was enhanced by root exudates (Marschner *et al.* 2001; Diab El Arab and Vilich Sikora 2004). For example, compared to the layers 5-10 mm, the concentration of gram positive bacterial fatty acids (iC15:0 and iC16:0) in PW, PL and PS in the layer 0-5 mm was 26%; 43% and 53% higher, respectively (Table 7.3). Brimecombe *et al*. (2001), Nunan *et al*. (2005), Nannipieri *et al*. (2007) and Allison *et al*. (2005) concluded that rhizodeposition will lead to a more diverse bacterial community in the rhizosphere compared to the bulk soil. Kandeler *et al*. (2002) stated that the changes in bacterial community composition and functional diversity induced by roots may extend several millimetres into the soil, with the steepest gradients between 0 and 5 mm from the root surface. Among plant treatments, the concentration of PLFAs in PL was 32% and 103% higher than in PW and PS, respectively. The higher microbial biomass in PL than in PW and PS may be attributed to the stimulating effect of low C/N residue on microbial growth (Cheng and Kuzyakov 2005). Cheng (2008) argued that gradients in bacterial and fungal abundance are strongly correlated with changes in labile organic C concentrations.

7.4.3. N mineralization and immobilization

Changes in N transformations after application of high and low C/N residues into the soil are relatively well-understood and are discussed in Chapters 4 and 5. However, it is of interest to learn how the presence of plants modifies N availability. In principle, there is an inherent relationship between plant, soil and microbes which regulates nutrient cycling and loss in ecosystems (Patrick *et al.* 2001); it is well-known that plants and microbes compete for nutrients, especially N (Nicolardot *et al.* 2001; Neergaard and Magid 2001).

The significant decrease in NH_4^+ concentration in the first 16 days in treatments with plants compared to the initial NH_4^+ concentrations and treatments without plants can be attributed to N uptake by the plants, and possibly N immobilization by the microbial biomass, which may have had a greater N demand due to its greater size (Table 7.3) and higher respiration (Figure 7.7). Marschner *et al*. (1986) and Bohlen *et al*. (2001) stated that the release of low-molecular-weight root exudates may enhance the activity of rhizosphere microbes which can lead to greater immobilization of N in the plant-soil interface. For example, the presence of plants in the low C/N residue treatments (PL) resulted in a lower NH_4^+ concentration than in L, with NH_4^+ concentrations 6.5-fold lower in the layer 0-5 mm and 5-fold lower in the layer 5-10 mm. This decrease was less pronounced in PW and PS compared to W and S. The presence of roots induced stronger effects closer to the root-soil interface (0-5 mm) than the layer 5-10 mm, indicating a limited effect of root exudates at distances greater than 5 mm from the root surface. Among plant treatments, although the inorganic N concentrations decreased significantly in the first 16 days, the N concentration of PL was significantly higher than in PW and PS. This is likely to be due to the greater N release from low C/N residue, which can also be seen in the unplanted treatments.

From day 16 to day 30, in the absence of plants, the concentration of inorganic N increased in all treatments due to the absence of N uptake by the plants. However, this increase was significant only in PL and L due to greater net N mineralization from the low C/N residues compared to wheat or non-amended soil, a result which is in agreement with the results described in the previous chapters and the findings of Walley and Yates (2002), Corbels *et al.* (2003) and Coppens *et al.* (2006).

7.4.4. Soil pH

In agreement with the previous studies reported in this thesis, the addition of plant residues resulted in an increase in soil pH. The changes in soil pH after the incorporation of residues with different C/N ratio residues have been discussed in Chapters 4 and 5. However, little is known about the effect of plant residue decomposition in association with living plant roots on soil pH changes. In order to maintain electrical neutrality, plants have the ability to modify the rhizosphere pH by extruding OH^- and HCO_3^- when anion uptake exceeds cation uptake to facilitate growth in low pH soils or conversely release H^+ when cation uptake exceeds anion uptake (Dakora and Phillips 2002). 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. The presence of plants lead to a strong increase in soil pH in PW, PL and PS compared to the initial soil pH (from 0.4-1.1 units) and treatments without plants. This can be explained by a greater anion uptake compared to cation uptake, suggesting that the plants took up mainly NO_3^- (Dakora and Phillips 2002).

The decrease in soil pH from day 16 to 30 is probably due to nitrification. Although there was no consistent decrease in NH_4^+ concentration or increase in NO_3^- concentration that would support this explanation, it should be noted that here only net NH_4^+ and NO_3^- concentrations were measured. Therefore, enhanced nitrification cannot be ruled out. The fact that NH_4^+ concentrations remained low, even after the soil was no longer in contract with roots (no more N uptake), suggests stronger nitrification.

In conclusion, the results of the current study are in agreement with our hypothesis; they showed that the presence of plant roots increased respiration rate, decreased N availability and changed pH and microbial community composition. Respiration was significantly increased only in the first 4 days after removal of the soil from the root surface as a consequence of the greater availability of easily available compounds in root exudates, which enhanced microbial activity and N immobilization and increased soil pH. The results clearly demonstrate that, for a better understanding of residue decomposition in the field, the effects of plant roots need to be taken into consideration.

Chapter 8

General Discussion

8.1 Introduction

In agriculture, plant residues or SOM have been used for soil fertility improvement for thousands of years. Incorporation of plant residues in agricultural systems has been shown to significantly improve soil fertility (Spaccini *et al.* 2002; Asghar *et al*. 2006, Alguacil *et al.* 2008). Plant residue decomposition is a complex process strongly affected by environmental factors, plant residue composition and soil native organisms (FAO 2003). Several mechanisms are well understood while others, such as the effect of frequent plant residue addition, mixing of residues of different quality, spatial interactions between plant residues and rhizosphere priming effects, are poorly understood, although plant residues can be added to the soil continuously, often in mixtures of residues from different plant species in the presence of growing plants. These knowledge gaps were addressed in this thesis.

8.2 Effect of frequency of residue additions

The present study found that frequent residue addition increased C mineralization by up to 90% compared to a single addition; this is probably due to the more constant supply of water soluble compounds by frequent addition of residues which enhanced microbial activity. Increasing the frequency of residue additions from once only to every 16 days and from every 16 to every 8 days strongly increased C mineralization rates (57% and 84%), whereas the difference between additions of residues every 8 or every 4 days was small (84% and 92%). This suggests that the addition of residues every 8 days is sufficient to maintain an active microbial community in the soil used in the present study. The frequency of residue addition for maximal respiration will depend on soil properties (e.g., soil organic matter content, size of the microbial biomass and nutrient availability) and residue properties (e.g., C/N ratio and concentration of water-soluble compounds). The source of the increased CO_2 release could not be determined in this experiment. It could be either residue C or SOC. Increased decomposition of SOC or recalcitrant compounds in the residues would indicate a priming effect (Kuzyakov 2002).

The increased decomposition rate with frequent residue additions was more pronounced in the first 30 days than later, which could be due to low N availability in the later stages of the incubation. Thus it can be hypothesized that in soils where N is not limiting, frequent addition of high C/N residues increase C mineralisation even more than in the soil used in this study..

Despite increased C mineralization, frequent residue addition did not affect N dynamics or the size of the microbial biomass and had little effect on microbial community composition. This suggests that microbes respire more of the C per unit biomass, but that this does not change their N requirement and does not require a change in microbial community composition.

8.3. Effect of mixing of low and high C/N ratio residues

Addition of high C/N residues to decomposing low C/N residues, which may occur in mixed species pastures, rotations or natural ecosystems, induced a significant decrease in respiration compared to the addition of low C/N residues, with cumulative respiration at the end of the experiment being reduced by about 27%. Microbial community composition was significantly affected by the mixing of residues with different C/N ratios. The ten-fold decrease in respiration rate can be explained not only by the higher C/N ratio, but also by the lower amount of water-soluble, and thus readily decomposable, compounds contained in wheat straw compared to lupin residues. The timing of the addition of high C/N residues did not affect respiration rate. By the end of the 64-day incubation, cumulative respiration was reduced by 27% to 32% compared to 100% lupin residue. This may be due to the experimental design, where the microbial community was maintained active by residue addition every 16 days. If the low C/N residues had been added once at the start and high or low C/N residues added only in the later stages of decomposition, the decrease in respiration compared to the addition of low C/N residues may not have been as pronounced, because the microbial community would have been better able to decompose recalcitrant compounds.

The decreased respiration after adding high C/N residues compared to adding low C/N residues alone, as found in this experiment, is in agreement with other studies where high and low C/N residues were mixed (Bending and Turner 1999; Dresboll and Magid 2006; Loranger-Merciris *et al*. 2006). However, in contrast to these previous studies, residues were added several times during the incubation period, as would be the case in

mixed species pastures or native ecosystems. Apart from the decrease in C mineralization rate, the incorporation of high C/N residue into soils prevented a decrease in soil pH, which could affect microbial community composition and reduced N release, due to greater immobilization, which may help prevent N loss compared to adding residues with low C/N ratio.

Changes in microbial community composition are likely to be due to altered substrate availability (C and N). However, since changes in microbial community composition may or may not be associated with changes in function (Osono *et al*. 2003; Marschner *et al*. 2005; Meidute *et al*. 2008), it is not clear whether the observed differences would have an impact on ecosystem function.

8.4. Effect of spatial separation of high and low C/N residues on decomposition

The use of two crop residues with contrasting biochemical composition was expected to provide a better insight into how far soluble organic compounds can diffuse from the residue interface into the adjacent soil and how this nutrient movement contributes to changes of respiration rate, microbial community and nutrient release on both sides of the interface. Studies on the interactions of residues with different biochemical quality have been carried out; however the unique aspect of the present experiment was the use of a mesh as an interface between two soil compartments with different C/N ratios, which allowed a clear separation of the two soil compartments with different C/N ratios. The respiration rate of soil with wheat residue treatment in contact with lupin residue was 4% higher than in soil with wheat residue in contact with soil only. Additionally, the inorganic N concentration in soil adjacent to the low C/N lupin residues was increased, whereas it was decreased in soil adjacent to high C/N wheat residues. The results showed that compounds diffusing from low C/N residues stimulated respiration and N availability in unamended soils and soil with high C/N residues, whereas contact with high C/N residues reduced respiration and N availability in unamended soil and soil with low C/N residues. The contact with soil with different C and N availability also altered the microbial community composition. Transport of nutrients via fungal hyphae and diffusion in the soil solution are the two well-known mechanisms explaining this movement of nutrients (Tester 1988; Lindahl *et al*. 2001). The lack of differences between the two layers (0-5 and 5-10 mm) may be due to the experimental design. Since the strongest effect is likely to occur within a few millimeters from the interface (see e.g. Gaillard *et al*. 1999; Kandeler *et al*. 1999;

Lindahl *et al*. 2001). Thus, it is possible that this strongly affected zone was diluted by less affected soil in the 0-5 mm layer. Despite the limitations of this study, it clearly demonstrates the interactions between soils with high and low C/N, which could play an important role in ecosystems where residues with different C/N ratio are in contact with each other.

8.5. Effect of plant roots on the decomposition of residues

In the fourth experiment, the effect of plant roots on decomposition of residues and nutrient availability was studied. This is an important research gap since roots are often present in soil with decomposing residues. Generally, the presence of plant roots increased respiration rates, decreased N availability and changed the pH and microbial community composition which can be explained by a stimulation of microbial activity and growth by root exudates.. Respiration was significantly increased only in the first 4 days after removal of the soil from the interface, suggesting rapid decomposition of root exudates. On day 1, the respiration rate in the 0-5 mm layer of treatments with plants was 36-88% higher than in treatments without plants, indicating a strong impact of plant roots on microbial activity. The source of CO_2 could not be determined; it may have been root exudates, but an increased decomposition of residues or SOM (priming effect) cannot be ruled out. The plant effects on N availability were longer-lasting because the previous contact with roots decreased N availability up to 30 days, particularly in the 0-5 mm layer. This decrease can be explained by plant N uptake and increased N immobilization by a larger microbial biomass as a consequence of the greater availability of easily available compounds in root exudates. The longer lasting effect of plants on N availability suggests that this soil had a relatively low N mineralization potential, otherwise one would have expected a more rapid recovery of available N after plant N uptake had ceased and microbial N uptake was probably also lower due to the absence of root exudates. As a result of differential substrate availability, the microbial community composition of treatments with plants or without plants, at different distances from the root mat, was significantly different. Additionally, an increase in soil pH adjacent to the roots was observed, suggesting that anion uptake exceeded cation uptake. The changes in pH and microbial community composition in the rhizosphere are in accordance with previous studies (Marschner *et al*. 1986; Dakora and Phillips 2002), but here we have been able to demonstrate the strong effect of roots even after direct contact with roots had ceased. The results clearly

demonstrate that, for a better understanding of residue decomposition in the field, the effects of plant roots need to be taken into account.

8.6. Suggestion for future research

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

The microbial community composition was assessed by PLFA. Although this method is used by many other researchers to study microbial community composition, it has several limitations. Limitations include (i) only broad groups can be distinguished, detailed community analyses of particular groups, e.g. bacteria, Archaea or fungi is not possible, (ii) only abundant fatty acids are measured and (iii) microbial function can not be assessed. Other methods such as PCR followed by denaturing gradient gel electrophoresis or terminal restriction length polymorphism (TRFLP) could be used to study community composition of certain groups. Quantitative PCR could be used to study the abundance of certain groups or functional genes. However, these methods also have limitations such as (i) bias towards amplification of certain gene sequences in the PCR, and (ii) specificity or lack thereof of the primers.

In some of the experiments described here, the pH changed quite strongly and, based on the literature, these pH changes were used to explain the observed effects on microbial community composition etc. However, no direct proof could be provided that this is really the case. An experiment could be conducted where the pH of the soil is adjusted by addition of acid or alkaline solutions and the effect on microbial community composition measured. Determination of the pH buffer capacity would also be desirable because then the net OH- release (cation/anion balance) during residue decomposition could be calculated.

The soil moisture was maintained by adjusting the weight of the containers every 4 days for experiments without plants and daily for the experiment with plants. Water loss was quite small between watering times, thus soil drying was minimal. However, some soil drying in the top few centimeters can not be ruled out. It would be useful to conduct an experiment in which the soil moisture is carefully maintained by daily watering or allowed to dry slightly and then determining the properties measured in the work described here.

The sampling interval for all properties except respiration ranged between 14 and 20 days. Particularly in the first 20 days, large changes in inorganic N, pH and microbial community composition occurred. It would be useful to conduct similar experiments with short sampling intervals (e.g. every 2 days) in the first 20 days to better monitor the short-term changes occurring in this period.

In the studies described here, it was not possible to determine the source of CO_2-C. Addition of ^{13}C or ^{14}C labeled residues to soil either once or frequently could be used to determine the source of evolved CO_2 and to determine which microbial groups decompose the residues by assessing the ^{13}C or ^{14}C in PLFAs.

In the study described in Chapter 6, it is hypothesized that N moves from low C/N to high C/N residues, but this could not be proven. Determination of the movement of residue-released N at different distances from the interface could be done by labeling of residues with ^{15}N and determining ^{15}N in inorganic N forms and the microbial biomass.

In the experiment in Chapter 7, it was not possible to measure soil respiration in situ because of the lack of appropriate equipment. Therefore, the direct effect of roots on residue decomposition was not possible. In situ measurement of soil respiration, possibly in combination with ^{13}C-labeled residues or exposing plants to $^{13}CO_2$ or $^{14}CO_2$ to follow plant-derived C into the soil, respiration and soil microorganisms would provide information that is more relevant for the situation in the field.

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Appendix

Appendix 1: Analysis of variance for soil properties measured in experiment 1

1. Cumulated respiration rate

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Treatment	4	84817.713	46204.428	7355.82	<.001
Time	5	177164.357	35432.871	5640.97	<.001
Treatment x Time	20	52081.711	2604.086	414.57	<.001
Residual	90	565.321	6.281		
Total	119	14629.102			

2. Available nitrogen

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Treatment	4	1883.383	470.846	29.81	<.001
Time	5	4878.553	75.711	268.99	<.001
Treatment x Time	20	538.131	26.907	7.42	<.001
Residual	90	326.457	3.627		
Total	119	7626.524			

3. Extractable carbon

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Treatment	4	1425271	356318	92.05	<.001
Time	5	285193	57039	14.74	<.001
Treatment x Time	20	2377489	118874	30.71	<.001
Residual	90	348374	3871		
Total	119	4436327			

4. Extractable nitrogen

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Treatment	4	785489.1	196372.3	971.81	<.001
Time	5	96049.9	19210.0	95.07	<.001
Treatment x Time	20	314866.1	15743.3	77.91	<.001
Residual	90	18186.2	202.1		
Total	119	1214591.2			

5. Microbial carbon

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Treatment	4	1798099	449525	45.97	<.001
Time	5	878928	175786	17.98	<.001
Treatment x Time	20	460312	23016	2.35	0.003
Residual	90	880011	9778		
Total	119	4017351			

6. Microbial nitrogen

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Treatment	4	160724.2	40181.1	134.19	<.001
Time	5	223488.1	44697.6	149.27	<.001
Treatment x Time	20	60598.5	3029.9	10.12	<.001
Residual	90	26949.3	299.4		
Total	119	471760.1			

7. pH

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Treatment	4	3.956667	0.989167	238.99	<.001
Time	5	1.993417	0.398683	96.33	<.001
Treatment x Time	20	0.775333	0.038767	9.37	<.001
Residual	90	0.372500	0.004139		
Total	119	7.097917			

Appendix 2: Analysis of variance for soil properties measured in experiment 2

1. Available nitrogen

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Treatment	5	35815.50	47163.10	2464.85	<.001
Time	3	154234.19	51411.40	2686.88	<.001
Treatment x Time	15	66974.18	4464.95	233.35	<.001
Residual	72	1377.66	19.13		
Total	95	458401.53			

2. Cumulated respiration rate

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Treatment	5	148827.594	29765.519	12438.98	<.001
Time	3	83461.798	27820.599	11626.20	<.001
Treatment x Time	15	29478.326	1965.222	821.26	<.001
Residual	72	172.290	2.393		
Total	95	261940.008			

3. Ammonium

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Treatment	5	54452.07	10890.41	994.75	<.001
Time	3	25715.65	8571.88	782.97	<.001
Treatment x Time	15	13304.76	886.98	81.02	<.001
Residual	72	788.25	10.95		
Total	95	94260.73			

4. Nitrate

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Treatment	5	69722.996	13944.599	2728.86	<.001
Time	3	57483.145	19161.048	3749.69	<.001
Treatment x Time	15	31586.105	2105.740	412.08	<.001
Residual	72	367.923	5.110		
Total	95	159160.168			

5. Respiration rate

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Treatment	5	47.90737	9.58147	143.17	<.001
Time	3	83.19792	27.73264	414.38	<.001
Treatment x Time	15	18.27913	1.21861	18.21	<.001
Residual	72	4.81866	0.06693		
Total	95	154.20309			

6. pH

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Treatment	5	2.485621	.497124	152.93	<.001
Time	3	0.058808	0.019603	6.03	0.001
Treatment x Time	15	0.587304	0.039154	12.04	<.001
Residual	72	0.234050	0.003251		
Total	95	3.365783			

6. Microbial nitrogen

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Treatment	4	160724.2	40181.1	134.19	<.001
Time	5	223488.1	44697.6	149.27	<.001
Treatment x Time	20	60598.5	3029.9	10.12	<.001
Residual	90	26949.3	299.4		
Total	119	471760.1			

7. pH

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Treatment	4	3.956667	0.989167	238.99	<.001
Time	5	1.993417	0.398683	96.33	<.001
Treatment x Time	20	0.775333	0.038767	9.37	<.001
Residual	90	0.372500	0.004139		
Total	119	7.097917			

Appendix 3: Analysis of variance for soil properties measured in experiment 3

1. pH on day 20

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Block	2	0.12416	0.06208	4.73	
Core_combination	4	1.47563	0.36891	28.10	<.001
Layer	3	0.93835	0.31278	23.83	<.001
Core_combination x Layer	12	1.03024	0.08585	6.54	<.001
Residual	38	0.49884	0.01313		
Total	59	4.06722			

2. pH on day 40

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Block	2	0.005333	0.002667	0.46	
Core_combination	4	3.736667	0.934167	160.38	<.001
Layer	3	1.093333	0.364444	62.57	<.001
Core_combination x Layer	12	0.376667	0.031389	5.39	<.001
Residual	38	0.221333	0.005825		
Total	59	5.433333			

3. pH on day 60

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Block	2	0.013000	0.006500	1.16	
Core_combination	4	3.932333	0.983083	174.84	<.001
Layer	3	1.333833	0.444611	79.07	<.001
Core_combination x Layer	12	0.583667	0.048639	8.65	<.001
Residual	38	0.213667	0.005623		
Total	59	6.076500			

4. Cumulated respiration rate on day 40

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Block	2	0.009139	0.004569	3.26	
Core_combination	9	205.551136	22.839015	16270.97	<.001
Layer	1	0.000001	0.000001	0.00	0.981
Core_combination x Layer	9	0.031811	0.003535	2.52	0.023
Residual	38	0.053339	0.001404		

5. Cumulated respiration rate on day 60

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Block	2	0.005136	0.002568	0.54	
Core_combination	9	229.057296	25.450811	5309.50	<.001
Layer	1	0.000465	0.000465	0.10	0.757
Core_combination x Layer	9	0.125650	0.013961	2.91	0.010
Residual	38	0.182151	0.004793		

6. Ammonium on day 20

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Block	2	256.17	128.09	1.55	
Core_combination	4	204551.66	51137.91	620.63	<.001
Layer	3	2102.41	700.80	8.51	<.001
Core_combination x Layer	12	59311.72	4942.64	59.99	<.001
Residual	38	3131.05	82.40		

7. Ammonium on day 40

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Block	2	46.069	23.034	2.92	
Core_combination	4	67510.449	16877.612	2140.70	<.001
Layer	3	485.539	161.846	20.53	<.001
Core_combination x Layer	12	8146.628	1512.219	191.81	<.001
Residual	38	299.597	7.884		

8. Ammonium on day 60

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Block	2	18.181	9.090	1.01	
Core_combination	4	42756.570	10689.143	1191.18	<.001
Layer	3	1277.224	425.741	47.44	<.001
Core_combination x Layer	12	13418.724	1118.227	124.61	<.001
Residual	38	340.996	8.974		

9. Nitrate on day 20

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Block	2	725.56	362.78	9.33	
Core_combination	4	53177.67	13294.42	341.99	<.001
Layer	3	207.36	69.12	1.78	0.168
Core_combination x Layer	12	10518.07	876.51	22.55	<.001
Residual	38	1477.20	38.87		

10. Nitrate on day 40

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Block	2	115.68	57.84	3.69	
Core_combination	4	43489.55	10872.39	693.76	<.001
Layer	3	237.95	79.32	5.06	0.005
Core_combination x Layer	12	8163.98	680.33	43.41	<.001
Residual	38	595.53	15.67		

10. Nitrate on day 60

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Block	2	793.49	396.75	13.20	
Core_combination	4	33942.18	8485.55	282.41	<.001
Layer	3	944.73	314.91	10.48	<.001
Core_combination x Layer	12	10746.66	895.55	29.81	<.001
Residual	38	1141.77	30.05		

Appendix 4: Analysis of variance for soil properties measured in experiment 4

1. pH on day 16

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Block	3	0.49396	0.16465	7.51	
Treatment	11	3.63563	0.33051	15.07	<.001
Residual	33	0.72354	0.02193		
Total	47	4.85312			

2. pH on day 30

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Block	3	0.16202	0.05401	1.29	
Treatment	11	3.46382	0.31489	7.54	<.001
Residual	33	1.37903	0.04179		
Total	47	5.00487			

3. Respiration rate on the first day

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Block	3	3.5314	1.1771	3.24	
Treatment	11	90.8262	8.2569	22.71	<.001
Residual	33	11.9973	0.3636		
Total	47	106.3549			

4. Respiration rate on the third day

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Block	3	0.2693	0.0898	0.67	
Treatment	11	70.4354	6.4032	48.08	<.001
Residual	33	4.3953	0.1332		
Total	47	75.1001			

5. Cumulated respiration rate on day 14

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Block	3	11.7743	3.9248	7.44	
Treatment	11	1462.2896	132.9354	251.83	<.001
Residual	33	17.4198	0.5279		
Total	47	1491.4836			

6. Ammonium on day 16

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Block	3	0.00003	0.00001	1.16	
Treatment	11	0.00870	0.00079	83.53	<.001
Residual	33	0.00031	0.00001		
Total	47	0.00905			

7. Ammonium on day 30

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Block	3	0.00001	0.00000	1.07	
Treatment	11	0.00371	0.00034	150.10	<.001
Residual	33	0.00007	0.00000		
Total	47	0.00379			

8. Nitrate on day 16

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Block	3	0.00000	0.00000	1.09	
Treatment	11	0.00059	0.00005	172.03	<.001
Residual	33	0.00001	0.00000		
Total	47	0.00060			

9. Nitrate on day 30

Source of variation	Degree of Freedom	Sum of Square	Mean of Square	Variation Ratio	F Probability
Block	3	0.00000	0.00000	1.17	
Treatment	11	0.00068	0.00006	165.79	<.001
Residual	33	0.00001	0.00000		
Total	47	0.00070			