EFFECTS OF INCORPORATED AND MULCHED ORGANIC AMENDMENTS ON SOIL NUTRIENT AVAILABILITY, MICROBIAL ACTIVITY AND PLANT GROWTH

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ABSTRACT

Little is known about the effects of repeated addition of mixes of organic materials differing in C/N ratio and decomposability on nutrient availability, microbial activity in soils and plant growth. Further, there is little information about the effects of mulches differing in C/N ratio and decomposability placed on soil mixed with plant residues. The aim of the thesis is to determine the effects of organic amendments on nutrient availability, microbial activities and plant growth.

The first two incubation experiments were carried out to investigate effects of amendment rate, order and frequency on soil nutrient availability and microbial biomass. In these experiments, residues differing in C/N ratio (high, mature wheat straw C/N 82, H) and (low, young faba bean, C/N 9, L) were added into soil sequentially (H-L or L-H) or as 1:1 mixes (HL). Residues or their mix were added two and four times in the first experiment and two, four and eight times in the second experiment. In both experiments, with repeated addition of H and L, N availability and microbial biomass N were influenced by residue rate and order. From these two experiments it can be concluded that N availability remains stable with frequent addition of residues with different C/N ratio whereas it strongly fluctuates when large amounts of residues with different C/N ratio are added.

The third incubation and first pot experiment were conducted to assess the effect of mixing organic materials differing in C/N ratio and decomposition stage on soil nutrient availability, microbial activity and plant growth. In these experiments, soil was amended with young faba bean shoots (C/N 9), sheep manure (C/N 6) and mature wheat straw (C/N 82) either individually or as 25:75, 50:50 and 75:25 mixes. In the incubation experiment, the effect on N availability, microbial biomass N and respiration was lower with sheep manure than with

faba bean which can be explained by highly decomposed state of sheep manure. Mixing sheep manure or wheat with ≥50% faba bean maintained higher N availability than sheep manure or wheat alone, but prevented the rapid N release that occurred after the addition of sole faba bean. In the pot experiment, in mixes of faba bean with 25% wheat or sheep manure, plant growth and N uptake were similar or higher than the control. It can be concluded that in these mixes, plant roots access organic particles releasing N and thereby compensate low N availability.

To investigate the effects of mulching organic materials differing in C/N ratio and decomposition stage with or without high C/N residues mixed into the soil on soil nutrient availability, microbial activity and plant growth, the fourth incubation and second pot experiment were conducted. In these two experiments, soil either had no amendments, only mature wheat straw incorporated, mulch (mature wheat straw, young faba bean shoot or sheep manure) over wheat straw, or mulch only. In both experiments, wheat straw incorporation under rapidly decomposing low C/N mulch reduced the risk of N leaching from the mulch due to greater microbial N uptake compared to mulch alone.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

It is estimated that food production must double by 2050 to meet the demand of the world's growing population (United Nations, 2009). Inorganic fertilisers have been used to boost food production, but will be increasingly more expensive (Cordell et al., 2009). Further, overuse of inorganic fertilisers contributes to soil degradation, environmental damage, loss of biodiversity (Thangarajan et al., 2013), ground water contamination and therefore threaten human health (Jiang and Yan, 2010). Given those side effects of inorganic fertilisers, application of organic amendments such as crop residues, compost, manures and biosolids is regarded as a promising alternative to restore soil fertility and protect the environment (Thangarajan et al., 2013).

Decomposition and nutrient release from organic amendments are influenced by a number of factors, of which chemical composition is one of the most important factors, including properties such as C/N and C/P ratio, N content, lignin and polyphenol concentration (Vigil and Kissel, 1991; Wang et al., 2004). However, organic materials that have similar C/N ratio may have different decomposition rate and nutrient availability because they differ in decomposability.

This literature review will discuss the following points: i) the general effects of organic amendments on soil properties; ii) factors affecting decomposition rate and nutrient release of organic amendments; iii) effects of organic amendments incorporated or mulched on nutrient availability, microbial activity and plant growth.

1.2 General effects of organic amendments

The application of organic materials can lead to interdependent changes in soil biological, chemical and physical properties (Van-Camp et al., 2004). Regarding biological effects, application of organic amendments increases soil microbial biomass C, respiration and enzyme activity (Diacono and Montemurro, 2010). Organic amendments affect soil microbes by supplying nutrients and changing soil chemical and physical properties. They can have short-term and longer term effects on biological properties (Pankhurst et al., 1997; Crecchio et al., 2001; Murphy et al., 2007). A number of short-term studies showed that organic amendments enhance soil microbial biomass, e.g. within 18 days in Shi and Marschner (2014) and 80 days in Duong et al. (2009). Perucci (1990) found that biomass C and N increased immediately after application of compost for up to 1 month, while biomass P increased gradually over 5 months. Effects of organic amendments on microbial activity have also been observed over long periods of time (e.g. years) after their addition (Diacono and Montemurro, 2010). For example, soil amended with compost and manure had 20 to 40% higher microbial biomass C for 4 years compared to the N fertilizer treatment (Ginting et al., 2003).

Regarding chemical effects, long-term annual addition of organic amendments increased organic carbon stock and thus soil cation exchange capacity (CEC) (Diacono and Montemurro, 2010). Cation exchange capacity of organic materials is due to deprotonated carboxylic acid groups which can bind cations (Mg^{2+}, Ca^{2+}, Fe^{2+}, Fe^{3+}) (Rabeendran et al., 2000). High CEC is crucial for storing nutrients and increasing nutrient availability to plants (Weber et al., 2007; Kaur et al., 2008). Application of compost can increase concentrations of soil N, P and other nutrients and these improvements still remain several years after application (Soumare et al., 2002; Butler et al., 2008). In the first year, only 30-35% of total N content was released from compost (Tittarelli et al., 2007). However, repeated application of compost results in the gradual build-up of available N content and yield increase for 4 – 5 years (Blackshaw et al., 2005; Barbarick and Ippolito, 2007; Leroy et al., 2007). Regular application of organic materials such as composts and manures for more than 10 years increased both organic C and N (Mallory and Griffin, 2007; Sodhi et al., 2009).

Organic amendments also increase P availability (He et al., 2001). For example, soil available P concentration increased by 7-86 mg kg^{-1} soil with compost application rates ranging from 0 to 200 t ha^{-1} (Zhang et al., 2006). Furthermore, a four-year study showed that application of beef manure and composted manure to corn lead to build-up of P in soil as the N/P ratio of manure or compost was lower than the corn N/P uptake ratio (Eghball, 2002). Organic amendments can have varying effect on soil pH with some reports showing increases (Eghball, 2002; Garcıa-Gil et al., 2004; Butler and Muir, 2006), while other show decreases (Meng et al., 2005; Bastida et al., 2008; Bi et al., 2009). The effect depends on original soil pH and type of organic materials (Diacono and Montemurro, 2010). For example, addition of compost made from broiler litter increased soil pH because of the high concentration of basic cations of broiler litter, ammonification and the generation of NH_3 during composting (Hubbard et al., 2008). On the other hand, application of composted rice straw mixed with agro-industrial wastes reduced soil pH due to release of H^+ during nitrification (Bolan and Hedley, 2003; Rashad et al., 2011).

Organic amendments can influence soil physical properties by increasing soil organic matter content. Aggregate stability plays a key role in soil fertility as it can increase and stabilise pore space which is important for gas exchange, water retention, root growth and microbial activity (Hudson, 1994; Van-Camp et al., 2004). Organic matter stabilises soil structure by two mechanisms: binding particles within aggregates (inter-cohesion) and enhancing their hydrophobicity (Cesarano et al., 2016), thus making them less prone to breakdown (Abiven et al., 2009). In addition, organic matter can improve soil structure by stimulating microbial activity and thus the generation of bacterial slimes, fungal hyphae and their binding with roots (Tisdall and Oades, 1982). However, the effect of organic amendments on soil structure may depend on soil type. Yüksek et al. (2010) and Duong et al. (2012) reported that composts increase soil aggregate stability in light and medium textured soils, but not in sandy and heavy textured soils. Soil aggregate stability is strongly influenced by the decomposability of organic amendments Monnier (1965), (Abiven et al., 2009). Readily decomposable organic materials such as green manure strongly increased aggregate stability within a month, while amendments that are partially decomposed such as composted manure had small initial but relatively large effect within years (Abiven et al., 2009). Organic amendments also have suppressive effects on soil-born pathogens. This effect can be indirect by improved supply of nutrients to the plant or by changing soil biochemical properties (Drinkwater et al., 1995) thereby enhancing plant health and resistance to pathogens (Hoitink et al., 1993). Direct effects of organic amendments on pathogens include release of compounds toxic to pathogens (Shetty et al., 2000), changes of soil properties influencing pathogen survival (Keinath and Lioria, 1989) and stimulation of biological control organisms (Lumsden et al., 1983). However, some organic amendments can increase plant diseases (Lazarovits et al., 2001). For example, fresh animal manures have been reported to increase the incidence of common scab disease of potato, thus it is usually recommended to avoid the use of fresh manures in intensive potato production (Sawant et al., 1991).

1.3 Factors affecting decomposition and nutrient release from organic amendments

In general, there are two important sets of factors affecting decomposition and nutrient dynamics of organic materials. One is the composition of the organic materials such as organic C composition and nutrient concentration (Prescott, 2010; Moore et al., 2011). The second is the environment in which organic materials decompose, including climatic factors such as water availability and temperature (Prescott, 2010; Moore et al., 2011) and soil factors such as soil texture and soil disturbance.

1.3.1 Chemical composition of organic materials

Properties of organic materials such as C/N ratio, N content, lignin and polyphenol concentration influence their decomposition and nutrient release (Vigil and Kissel, 1991;

Wang et al., 2004). Organic materials typically contain three main chemical groups with different decomposition rate: 1) easily decomposable, water-soluble sugars and amino acids, 2) slowly decomposable compounds including hemicellulose and cellulose; 3) recalcitrant materials such as lignin (Van Veen et al., 1984). The concentration of water-soluble compounds influences mainly the initial stages of decomposition, whereas lignin is the most important determinant of decomposition when easily decomposable compounds are depleted (Berg and Staaf, 1980; Rahman et al., 2013; Hall et al., 2015). Other factors such as polyphenol concentration and polyphenol/N ratio are also related to decomposition rate (Palm and Sanchez, 1991; Kumar and Goh, 1999). According to Palm and Sanchez (1991), organic materials with high polyphenol concentration and polyphenol/N ratio have low N mineralisation rates due to the formation of stable polymers between polyphenols and amino groups which are resistant to decomposition.

An important property that influences both decomposition rate and nutrient release is the C/N ratio. High C/N materials are decomposed more slowly than low C/N materials. Microbes have a low C/N ratio compared to most organic materials (Bolan et al., 2004; USDA, 2011). Organic materials with low C/N ratio supply sufficient N for rapid build-up of microbial biomass whereas N is a limiting factor for microbes when high C/N materials are added. Further, low C/N materials (C/N < 25) can supply N in excess of microbial demand and lead to net mineralisation (increase in available N) (Janssen, 1996; Hadas et al., 2004). On the other hand, materials with C/N ratio > 25 result in at least temporary net immobilisation in the microbial biomass and a decrease in available N (Moritsuka et al., 2004). However, the C/N ratio may not fully explain decomposition and nutrient release of organic materials (Cartenì et al., 2018; Bonanomi et al., 2019). For example, C/N was shown to be a poor predictor of leaf litter decomposition in tropical (Hättenschwiler et al., 2011) and temperate forests (Bonanomi et al., 2013). (Bonanomi et al., 2019) found that the biochemical quality of organic C, which was determined ^{13}C cross-polarization magic angle spinning (CPMAS) nuclear magnetic resonance (NMR), was better than C/N ratio in predicting N dynamics after organic amendments. They showed that N mineralisation was fast with organic amendments of high C quality and high N content (e.g. meat powder, fish meal and alfalfa litter). On the other hand, N mineralization was slow with organic amendments that were nitrogen-rich but had low C quality (e.g. humus, alfalfa biochar). Organic amendments with low N content but high C quality (e.g. glucose) caused rapid, short-term immobilisation whereas organic amendments with low N content and low C quality (e.g. sawdust, cellulose, wood biochar and grass litter) induced a slow, long-lasting immobilization.

Manures may have low C/N ratio but decompose more slowly than other organic materials having similar C/N ratio such as plant residues. For example, Daudu et al. (2006) found that *Mucuna pruriens* residue (C/N 16) decomposed rapidly, losing more than 60% of its dry weight one month after addition to the soil, whereas cow manure (C/N 22) decomposed more slowly with a mass loss of 50% within six weeks. The slow mineralization of manure can be explained by its highly decomposed nature. Manures are decomposed in the digestive tract of animals as well as during storage (Hungate, 1966; Janssen, 1996). This decomposition results in loss of C and therefore low C/N and C/P ratio as well as depletion of easily decomposable compounds, and accumulation of recalcitrant compounds.

The C/P ratio of organic amendments determines whether P is mineralised or immobilised (Curtin et al., 2003). About 40 – 60% of P in residues is water-soluble and released quickly after incorporation into soil (Martin and Cunningham, 1973), but the remaining P has to be mineralised. In general, net immobilisation of P occurs when the C/P ratio of organic materials > 300 (Brady and Weil, 1996; Iyamuremye et al., 1996) whereas net P mineralisation is induced at C/P ratio <200 (Nachimuthu et al., 2009; Hasbullah et al., 2011; Alamgir et al., 2012). Organic amendments with low C/N and C/P and low lignin concentration decompose more rapidly and release more available P during decomposition than those with high C/P ratio (Silver and Miya, 2001). Net P mineralisation was negatively correlated with C/P ratio (Hundal et al., 1987) and lignin content (Lupwayi and Haque, 1999).

1.3.2 Soil water availability

Water availability influences decomposition and nutrient release of organic materials mainly through its effect on soil microbes (Liu et al., 2009; Moyano et al., 2013). Water availability is an important factor affecting soil microbial activity (Geisseler et al., 2011). Generally, low water availability reduces soil respiration (Stott et al., 1986; Quemada and Cabrera, 1997) and net N mineralisation (Stanford and Epstein, 1974; Paul et al., 2003). The low microbial activity in dry soil is due to two main mechanisms. Firstly, low water availability may decrease microbial activity by restricting substrate supply (Stark and Firestone, 1995; Geisseler et al., 2011). At low water content water is held more tightly to soil particles, therefore water becomes less available, water films on soil particle surfaces become thinner and increasingly disconnected. As a result, substrate molecules must follow a more tortuous and longer path to diffuse to cells which reduces substrate flux towards microorganism (Papendick and Camprell, 1981; Moldrup et al., 2001). Secondly, low water availability can restrict microbial activity by reducing extracellular water potential which draws water out of the cells, reducing hydration and activity of enzymes (Stark and Firestone, 1995). When soil availability is low, microorganisms can minimise water loss from the cells by increasing intracellular solutes to concentrations which are slightly higher than extracellular concentrations (Brown, 1990). High internal solute concentrations are created by either synthesis of compatible organic solutes or ion uptake from the extracellular solution (Csonka, 1989). This may result in enzyme inhibition as the degree of enzyme hydration is reduced (Skujins and McLaren, 1967; Lanyi et al., 1979; Csonka, 1989). Further, solute accumulation requires high amounts of energy which can reduce energy available for biomass synthesis (Geisseler et al., 2011).

Effects of high water content on mineralization of organic materials have also been studied extensively. High water content reduces oxygen supply in soils because diffusion of oxygen in water is orders of magnitude lower than in air (Richard, 1969; Drew, 1981). Initially after flooding, oxygen is consumed by aerobic microbes, but then the soil becomes anaerobic because oxygen diffusion from the atmosphere into the soil is restricted. High water content increases the activity of anaerobic microbes and inhibits aerobes (Kozlowski, 1984; Skopp et al., 1990). For example, Hassan (2013) found higher decomposition rate of added organic materials at 50% of soil water holding capacity than at 100% water holding capacity. The enhanced decomposition at 50% water holding capacity resulted in higher concentrations of NH_4-N, dissolved organic C and dissolved organic N. Submerged conditions reduce decomposition rate of organic amendments and increase organic C sequestration (Hassan, 2013). Long-term flooding in rice paddies has been found to increase organic matter content due to slow decomposition of organic inputs (Zhongpei et al., 2004).

1.3.3 Temperature

Between 0 and 50^o C, increasing temperature enhances decomposition (Davidson and Janssens, 2006; Craine et al., 2010). Optimal temperature for decomposition will lead to high microbial activity if water availability is sufficient (Berg and McClaugherty, 2008). When soil temperature is higher than 50^o C as in fires, soil properties may change in the short-term, long-term or permanently (Certini, 2005). High soil temperatures can lead to microbial death, loss of organic C and N via volatilization, increase in available N, P and pH as well as changes to soil physical properties (e.g decreased soil permeability and changes in soil colour) (Certini, 2005).

1.3.4 Soil texture

Fine textured soils have higher organic C and N contents than soils with coarse texture under similar vegetation and climatic conditions (Jenny, 1941). This is because clay can protect organic matter from decomposition (Krull et al., 2003; Lützow et al., 2006; Six and Paustian, 2014). High clay content inhibits decomposition by reducing accessibility of organic matter to microbes through several mechanisms. Firstly, clay soils have a large number of small pores that are not accessible to microorganisms and thus, organic matter confined in these pores is physically unavailable for microbes (Oades, 1988; Krull et al., 2003; Lützow et al., 2006). Secondly, due to their high charge and specific surface area, clay particles can bind organic matter to generate organo-mineral complexes (Krull et al., 2003; Lützow et al., 2006). And thirdly, soil organic matter is entrapped within soil aggregates formed by clay (Hassink et al., 1993).

1.3.5 Soil disturbance

Disturbance can aerate soil, break organic materials and aggregates and mix the top soil layer which is rich in organic materials with deeper layers (Larney and Bullock, 1994; Khan, 1996). Mixing and aggregate breakdown can increase the accessibility of organic materials for soil organisms, resulting in faster decomposition (De Varennes et al., 2007). An increase in CO_2 emission after tillage was observed within hours or days after tillage (Reicosky, 1997; Reicosky et al., 1997; Ellert and Janzen, 1999; Calderón et al., 2000).

Changes in nutrient availability have been observed after soil disturbance. Tillage can lead to increased N availability (De Varennes et al., 2007). Calderón et al. (2000) simulated tillage by mixing and then sieving grassland and vegetable soils. They reported increased net N mineralisation and nitrification occured after disturbance in both soils, but the stimulation depended on landuse. The relative increase of NO_3-N and dissolved organic N was greater in grassland soil than vegetable soil. They explained that disturbance-induced exposure of previously protected N may increase dissolved organic N and then net N mineralisation and nitrification. Grassland soil which had a higher amount of organic matter protected in macro-aggregates released more available N than cultivated vegetable soil where less organic matter was protected in macro-aggregates (Tisdall and Oades, 1982; Elliott, 1986; Cambardella and Elliott, 1994). Further, in the field, tillage induces incorporation of fresh organic materials into soil, resulting in higher substrate availability for soil microbes (Kristensen et al., 2000) and thus enhanced N mineralisation (Dharmakeerthi et al., 2004). N availability can be increased for several days after tillage (Grace et al., 1993; Reicosky et al., 1997; Calderón et al., 2000; Dharmakeerthi et al., 2004) particularly in soil which had not been tilled for a long time (Kristensen et al., 2000). However, the increase in available N after tillage with residue incorporation may be transient. For example, De Varennes et al. (2007) disturbed soil after harvesting the first crop (oat or lupin), added oat or lupin residues (root only or roots plus aboveground residues) and then determined changes in total and available N and other factors such as dehydrogenase activity and fungi after harvesting the second crop (oat). They showed that at the end of the crop rotations in year 2, total N and NO_3–N concentration in the soil were not significantly affected by tillage.

Regarding P, a long history of no-till results in a gradual accumulation of P from fertilisers and organic materials in the upper-most soil layer and P stratification within the soil profile because there is little mixing between soil layers (Rasmussen, 1999; Sharpley, 2003; Mathers and Nash, 2009; Cade-Menun et al., 2010). This increases the risk of loss of dissolved reactive P with runoff water (Sharpley and Smith, 1994; Pote et al., 1999). Mixing high-P surface soil with low-P subsoil can reduce the loss of dissolved reactive P (Sharpley, 2003). Tillage has been shown to reduce water soluble P content in surface flow on grassland soil that had received high amounts of manure, but the influence was significant only for the first year after tillage (Schärer et al., 2007). Moreover, soil disturbance such as mixing and sieving can increase available P as a result of greater microbial mineralisation of organic P previously occluded within aggregates (Maltais-Landry and Frossard, 2015). Available P may also be indirectly increased after tillage by organic acids generated from residue decomposition (Sharpley and Smith, 1989). Organic acid anions can replace adsorbed P from binding sites and release P bound to Fe/Al by chelating Fe or Al (Ayaga et al., 2006).

1.4 Effects of organic amendments on soil nutrient availability, microbial activity and plant growth

1.4.1 Effects of organic amendments incorporated into soils

Organic amendments can be incorporated into soil once, e.g., after harvest of a crop, or added repeatedly during a crop season in agricultural ecosystems and in the form of litter fall and root turnover in natural ecosystems. Single and repeated addition of organic materials have different effects on soil nutrient availability, microbial activity and plant growth.

1.4.1.1 Single organic amendments

In this literature review, single organic amendment refers to one-off addition of one organic material into soil. With single organic amendments, respiration rates are high within the first few days after amendment and then decrease over time (Duong et al., 2009; Zheng and Marschner, 2017). When organic materials are added once, respiration rates are initially high due to decomposition of easily available compounds. Thereafter, respiration rates decrease as these compounds are depleted. Respiration rates are low in the later stages of decomposition when only more recalcitrant compounds are left (Wang et al.,2004). N availability decreased after the addition of high C/N residue (C/N 122), gradually over the first 16 days after addition, whereas microbial biomass N increased until day 80, indicating net immobilisation (Duong et al., 2009). Compared to addition of high C/N residue (C/N 120), the addition of low C/N residue (C/N 22) resulted in higher microbial biomass C and

P and available N and P concentrations until 30 days after the addition (Nguyen et al., 2016). Nguyen et al. (2016) explained that the low C/N residue supplied sufficient N and P for microbial activity and growth, but also resulted in higher nutrient availability than the high C/N residue where low N and P availability limited microbial activity and growth.

1.4.1.2 Repeated amendments

A number of studies examined the effects of single addition and repeated addition on nutrient availability and microbial activity. Effects of repeated addition of organic amendments on nutrient availability varied depending on C/N ratio of materials added. In the study by Duong et al. (2009) wheat straw (C/N 122) was incorporated to the soil once or every 16 (four times), 8 (eight times) and 4 days (16 times) over a period of 60 days. Frequent addition did not increase available N compared to the single addition and unamended soil. However, in the study of Zheng and Marschner (2017), low C/N residue (C/N 19) was added once (day 16), twice (days 16 and 24) or four times (days 16, 20, 24 and 28) following high C/N residue (C/N 72) addition on day 0. The total amount of low C/N residue was the same in all treatments. Microbial biomass N on day 23 was higher with repeated addition than the single addition. But compared to single addition of low C/N residue, N and P availability on day 23 were lower with low C/N residue added two or four times, which was likely due to microbial N uptake and the lower amount of low C/N residue added in the latter. But on day 32 N and P availability with repeated additions were similar as with single addition which was attributed turnover of microbial biomass in all treatments by then. It was concluded that more frequent addition of low C/N residue after a high C/N residue enhances microbial nutrient uptake but reduces nutrient availability temporarily. Repeated addition of organic amendments increased soil respiration and microbial biomass C compared to a single addition (Duong et al., 2009; Cavalli et al., 2014; Elmajdoub and Marschner, 2015). It was explained that compared to a single addition, repeated addition of organic materials supplied more easily decomposable compounds to microbes and thus minimising the periods of low C availability (Elmajdoub and Marschner, 2015; Zheng and Marschner, 2017). Duong et al. (2009) showed that compared to a single addition, repeated addition increased soil respiration and that this stimulation increased with addition frequency. In a study of Bonanomi et al. (2017), compared to single amendment, repeated additions of organic materials increased fungal growth inhibition and reduced the time required for fungistasis restoration. The results were explained by higher microbial activity and functional diversity in soil with repeated addition of organic materials.

Nutrient availability after organic amendments also depends on the order in which materials are added. Marschner et al. (2015) found that when low C/N was followed by high C/N residue, nutrient availability after the second residue addition was higher than with high C/N residue alone or with high C/N following high C/N residue. This can be explained by nutrients released from decomposition of the previously added low C/N residue being still present in the soil when high C/N residue added. With high C/N followed by low C/N residue, the previously added high C/N residue reduced net nutrient release because microbes decomposing the remaining high C/N residues took up the released N. Marschner et al. (2015) referred to this as legacy effect.

1.4.1.3 Mixes of organic amendments

Organic amendments are not only added to soil singly or repeatedly, but also together in mixes. Decomposition and nutrient release of such mixtures may be similar or different from those expected based on the individual residues (Gartner and Cardon, 2004). The expected value for the measured parameters in organic material mixes is calculated based on average concentration of the separate organic amendments (e.g. A and B) according to the following equation (Gartner and Cardon, 2004):

Expected value = (Proportion of A in mix * concentration in sole A) + (Proportion of B in mix*concentration in sole B).

Decomposition of mixtures can be greater than expected (synergism) (Wardle et al., 2006; Barantal et al., 2011; De Marco et al., 2011), lower (antagonism) (Barantal et al., 2011; Coq et al., 2011; Maisto et al., 2011) or similar (no interaction) (Gartner and Cardon, 2004; Wardle et al., 2006). Gartner and Cardon (2004) reported that in 67% of all mixtures in 30 published papers, measured decomposition differed expected values. Of these 65% were synergistic and 35% were antagonistic. Similar measured and expected values could be due to spatial separation of the decomposer communities of organic materials in the mix (Morris and Dress, 2007). Greater than expected decomposition has been attributed to i) transfer of nutrients from rapidly decomposing organic materials to recalcitrant materials (Seastedt, 1984; Blair et al., 1990), ii) changed decomposer community composition (Blair et al., 1990), iii) niche complementarity or specialisation within the decomposer community (Hector et al., 1999; Chapman and Koch, 2007) and iv) priming effects in which decomposition of low-nutrient materials is stimulated by high-nutrient materials (Chapman et al., 1988; Wardle et al., 1997; Chapman and Koch, 2007). Lower than expected decomposition in a mix can be due to diffusion of inhibitory compounds such as phenolics and tannins from slow to fast decomposing materials (Dix, 1979; Hättenschwiler and Vitousek, 2000).

Effects of mixes of organic materials differing in C/N ratio on nutrient availability have been studied extensively. In these mixes, measured decomposition and nutrient release often differ from expected values. For example, Bonanomi et al. (2014) found that measured decomposition after 360 days of a 1:1 mix of *Hedera helix* leaf litter (C/N 21) with cellulose strips C/N (440) was higher than expected due to N transfer from low C/N litter to high C/N cellulose. In another study, low C/N (C/N 20) and various high C/N litter (C/N 50-80) were incubated singly or as 1:1 mixes in litter bags buried in soil for two or nine months (Cuchietti et al., 2014). Cuchietti et al. (2014) reported that measured decomposition and nutrient release in most mixes were higher than expected because high C/N litters appeared to stimulate decomposition of low C/N litters. Mao and Zeng (2012) also found that measured mass loss and N release were higher than expected in a 1:1 mix of residues differing in C/N ratio (high in poplar leaves and low in soy bean leaves) after 84 days of incubation. However, little is known about the effect of mixing of organic materials with similar C/N ratio, but differing in decomposability on decomposition and nutrient release.

Addition of mixes of organic materials also affects soil microbial activity. In a short term study (18 days) by Shi and Marschner (2014), cumulative respiration in mixture of *Stipa* shoot and root was higher than expected which was attributed to the relative similarity of these two residues in total N, C/N ratio and water soluble C content. They explained that the similarity of residues may increase the adaptation of the microbial community to both residue types. However, mixing *Stipa* shoots and roots did not increase microbial biomass C compared to individual residue. In mixes of two residues differing greatly in properties (total N, C/N and water soluble C), e.g. barley shoots and roots, cumulative respiration was lower than expected. The lower than expected respiration may occur in mixtures of lignin-rich and high-N litter where the mineralized N suppresses the formation of lignolytic enzymes or reacts with lignin residues to form complexes which are highly resistant to microbial degradation (Dijkstra et al., 2009).

Influences of mixes of organic material on plant growth and nutrient uptake differ from those of single amendments. Bunyasi (1997) examined the effects of mixing organic materials differing in C/N ratio on maize biomass and N uptake. Soil was amended with *Croton macrostachyus* residue (C/N 11) and rice (*Oryza sativa* L.) husks (C/N 52) singly or as 1:1 mix. Maize dry weight 7 weeks after planting was higher with the mix than with the single amendments, and was higher than expected in the mix. In another study where groundnut residue (C/N 18) and rice straw (C/N 83) were incorporated into the soil singly or as 1:0.5 and 1:1 mixes 1.5 months before rice seedlings were planted. Rice grain yield and N content of rice were highest with the mixes (Kaewpradit et al., 2009). They explained that mixing delayed N loss, prolonged N availability and led to better synchrony of N release with crop N demand and thereby improved rice yield.

1.4.2 Effects of organic amendments as mulches

1.4.2.1 Mulching compared to incorporation

With incorporation, organic materials are mixed into soils whereas in mulching, the soil surface is covered by a layer of organic materials. Application methods can influence decomposition rate and nutrient release of organic amendments (Schomberg et al., 1994; Stemmer et al., 1999). In general, decomposition rate of organic material mulches is lower than that of incorporated materials because mulched materials have smaller contact area to the soil and are exposed to alternating wet and dry periods that may limit microbial activity (Coppens et al., 2006). Incorporated organic materials are decomposed more quickly because they are surrounded by moist soils and in direct contact to soil microbes (Coppens et al., 2006; Nicolardot et al., 2007). Nutrients from mulches can get into the soil by leaching (Rosolem et al., 2005; Tu et al., 2006), soil animals e.g termites (Ibrahim et al., 2018) and earthworms (Ortiz-Ceballos et al., 2007) and fungal hyphae (Frey et al., 2003).

Incorporation and mulching of organic amendments have different effects on soil properties. Compared to incorporation and unamended soils, mulching of organic materials can improve the soil water balance by reducing evaporation, particularly in the top layer (Dahiya et al., 2007). This can contribute to the maintenance of microbial activity and decomposition rate in soils that are exposed to high evaporation rates. Mulching of organic materials can reduce the incidence of diseases and pests and suppress weeds compared to unamended control (Pinamonti, 1998), whereas incorporation may not (Campell and Sharma, 2003).

Incorporation of organic materials usually has greater effects on soil C and N contents than mulching which can be explained by the more intimate contact of organic materials and soil with the former (Cogger et al., 2008). However, these effects are not always consistent among soil types. In a field study with sandy and clay soils, Nishigaki et al. (2017) found that in clay soil, soil respiration rate was higher with maize straw incorporation than mulching, whereas in sandy soil, soil respiration did not differ between the two application methods. Nevertheless, in sandy soil, N availability was about 20% higher and crop N uptake was 25-60% higher with mulched maize straw than incorporation. The higher N availability in the sandy soil with mulching was attributed to higher soil water content compared to incorporation. In the study by Coppens et al. (2006), mulching increased the storage of fresh organic C in soil after 9 weeks compared to incorporation, but also increased the risk of nitrate leaching.

1.4.2.2 Effects organic mulch properties

Nutrient release from mulches into soil is related to the C/N ratio of the organic materials. In field experiments, N release was greater from organic materials with higher N content compared to those with low N content (Halde and Entz, 2016; Ibrahim et al., 2018). P release from mulches was also positively related to P concentration of the organic materials (Ibrahim et al., 2018). N release from a number of organic mulches was found to be greatest initially due to leaching of water-soluble compounds, later differences among organic mulches became smaller (Rosolem et al., 2005).

Application of organic amendments as mulches can influence microbial nutrient uptake. Tu et al. (2006) reported that mulching with low C/N organic materials increased microbial biomass C and N and soil respiration compared to the control with synthetic fertilisers over a two-year field experiment. Marinari et al. (2015) found that soil microbial C/N ratio after mulching of legume cover crops was higher than the non-mulched control which they attributed to a greater abundance of fungi in the mulched soils. Mulching soil with wheat straw increased microbial biomass C compared to the control; microbial biomass C was higher with a mulching rate of 9 Mg ha^{-1} than with 4.5 Mg ha^{-1} (Wang et al., 2018).

The influence of organic mulches on crop growth also depends on their C/N ratio. Higher crop yield with low C/N mulches (C/N < 20) compared higher C/N mulches (C/N > 20) was shown in a number of studies, e.g. Radicetti et al. (2016) and Ibewiro et al. (2000). However, Ibrahim et al. (2015) used mulches with C/N ratios between 20 and 64 and found that all mulches increased millet growth by 30-50% compared to the control. They explained this by the higher water retention capacity and therefore more efficient use of available rainfall in mulched soil compared to the control.

1.5 Research gaps and aims

The literature review showed that effects of organic amendments on soil nutrient availability and microbial activity and plant growth depend on their chemical composition, decomposition stage and application methods as well as on external factors such as soil water content, temperature, texture and disturbance. Nevertheless, several research questions remain which will be addressed in this project:

1. Are the effects of residue mixes on soil respiration, microbial biomass and nutrient availability over time influenced by amendment frequency and do the effects of mixes differ from that of the same residues added sequentially?

2. How does the addition frequency of residue mixtures of high C/N and low C/N residues in mixes influence soil respiration, microbial biomass and nutrient availability?

3. How does mixing of organic materials differing in C/N ratio and decomposition stage affect nutrient availability, microbial activity and plant growth?

4. How do nutrient availability and microbial activity in soil mulched with organic materials differing in C/N ratio and decomposition stage without or with high C/N residue incorporated into the soil change in the first weeks after mulching?

5. Are plant growth, nutrient availability and microbial activity influenced by mulch C/N ratio and decomposition stage and do the effects differ when high C/N residue is present in soil?

Therefore, the overall aim of the studies presented in this thesis was to determine the effect of incorporation and mulching of organic materials differing in C/N ratio and decomposition stage/decomposability on nutrient availability, microbial activity and plant growth (Fig.1). Specifically, the aims of the experiments were to:

1. Determine the influence of amendment rate, frequency and order on soil respiration, microbial biomass and nutrient availability after organic amendment (Chapter 2).

2. Assess the effect of amendment frequency on the interactive effect of high C/N and low C/N residues in mixes on soil respiration, microbial biomass and nutrient availability (Chapter 3).

3. Determine the influence of mixing of organic materials differing in C/N ratio and decomposition stage on nutrient availability, microbial activity and plant growth (Chapters 4 and 5).

4. Determine the effects of mulching with high or low C/N organic materials, in which low C/N materials differed in decomposability, and the incorporation of high C/N residues in the

soil on soil respiration, microbial biomass N and P and available N and P within the first few weeks after amendment (Chapter 6).

5. Assess the effect of mulch C/N ratio and its decomposition stage and the incorporation of high C/N residues in the soil on plant growth and nutrient availability (Chapter 7).

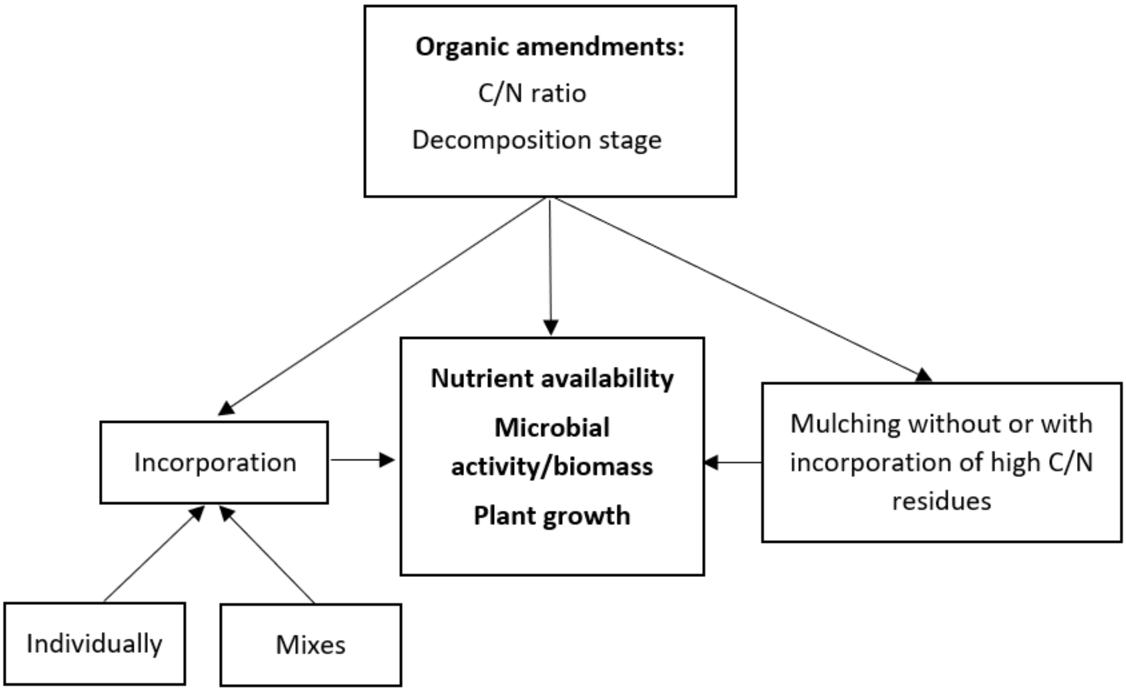

Figure 1. Schematic diagram of factors assessed in this thesis influencing nutrient availability, microbial activity and plant growth in soil amended with organic materials.

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CHAPTER 2

AMENDMENT WITH HIGH AND LOW C/N RESIDUES - INFLUENCE OF RATE, ORDER AND FREQUENCY

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Overall percentage (%)	70%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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By signing the Statement of Authorship, each author certifies that:

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ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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RESEARCH ARTICLE

Amendment with high and low C/N residues- Influence of rate, order and frequency

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Abstract

It is unclear if the effect of residue mixes on soil respiration, microbial biomass and nutrient availability over time is influenced by amendment frequency and how the effect differs from that of the same residues added sequentially. There were six treatments differing in number of amendments and order in which residues were added, total amendment rate in all treatments was 20 g kg^{-1} with 10 g kg^{-1} of each high (H) and low (L) C/N residue. In treatment names, order of letters indicates order of residues, e.g. HL is H followed by L. In treatments with two amendments, residues were added on days 0 and 20 at 10 g kg^{-1}: 10-LH, 10-HL or a 1:1 mixture of L and H added twice [10-(HL)x2]. In treatments with four amendments, residues were added on days 0, 10, 20 and 30 at 5 g kg^{-1}: 5-HLHL, 5-LHLH, and 5(HL)x4. In 5-HLHL, microbial biomass N (MBN) increased only after the first L addition although available N increased after both additions. Differences between measured and expected value depended on residue addition frequency and parameter. In 10-(HL)x2, MBN, microbial biomass P and C (MBP and MBC) were greater than expected and this was accompanied by lower than expected available N and P. In 5-(HL)x4 on the other hand, the difference between measured and expected MBN and available N changed over time, possibly because the proximity of microbes decomposing different residues changes. The study showed that with repeated addition of H and L, N availability and MBN are influenced by residue rate and order.

Keywords: Amendment rate, N immobilisation, N mineralisation, residue mixing

1. Introduction

It is well-known that composition of organic amendments determines decomposition rate and nutrient availability (Tian *et al*., 1992). For example, addition of low C/N organic materials (C/N < 20) results in net N mineralisation (Hadas *et al.*, 2004) whereas high C/N amendments induce net N immobilization (Moritsuka *et al*., 2004). The effect of simultaneous addition of plant residues of different composition has also been studied extensively (Gartner and Cardon, 2004; Cobo *et al*., 2008). In such residue mixes, expected nutrient availability can be calculated based on nutrient availability with each residue separately and the ratio of residues in the mixes.

Plant residues differing in composition can also be added to soil sequentially, e.g. in intercropping. In previous studies, we found that nutrient availability after the second residue addition is influenced by the C/N ratio of the first and the second residue amendment, termed legacy effect (Marschner *et al*., 2015). For example, N availability was higher after high C/N residue amendment when it followed low C/N residue than if high C/N residue was added to unamended soil. The extent of the legacy effect decreased with time between residue additions (Nguyen *et al.*, 2016; Nguyen and Marschner, 2016). In these experiments, residues were added at 10 g kg^{-1}. In Zheng and Marschner (2017a) the first residue was added at 2.5, 5 or 10 g kg^{-1} and the second residue at 10 g kg^{-1}. They found that the legacy effect decreased with addition rate of the first residue. These results suggest that the legacy effect depends on the amount of the first residue left in the soil when the second residue is added.

There are few studies investigating the effect of repeated soil amendments on soil respiration and microbial biomass. In the study by Cavalli *et al.* (2014), repeated slurry application compared to a single addition increased soil respiration and the proportion of CO_2 derived from slurry, but reduced the proportion of CO_2 from SOC. De Nobili *et al*. (2001) found that repeated addition of trace amounts of glucose increased CO_2 release compared to a single addition beyond the amount of C added with glucose. They suggested that trace amounts of substrate stimulate turnover of microbial biomass because biomass C was not increased by glucose addition. In the study by Duong *et al*. (2009), the same amount of high C/N residue was applied once, four, eight or 16 times (added every 16, 8 or 4 days, respectively) over a period of 60 days. They found that compared to a single addition, repeated addition increased cumulative respiration per g C added and that this stimulation increased with addition frequency. In their study, addition frequency did not influence microbial biomass C or available N. Zheng and Marschner (2017b) added high or low C/N residue once (d0), twice (d0, 8) or four times (d0, 4, 8, 12). Cumulative respiration, microbial biomass and nutrient availability compared to single addition were lower on d7 with residue added twice or four times, but higher on d15.

Residues differing in C/N ratio, can not only be added sequentially, but also together. Many studies investigated the effect of single addition of residue mixes on decomposition and nutrient availability (e.g. Gartner and Cardon, 2004; Cobo *et al*., 2008). But it is unclear if the effect of residue mixes on soil respiration, microbial biomass and nutrient availability over time is influenced by amendment frequency and how the effect differs from that of the same residues added sequentially. In the present study, soil was amended with high and low C/N residues or their 1:1 mixture to a total amendment rate of 20 g kg^{-1}. The aim was to determine the influence of amendment rate (5 or 10 g kg^{-1}), frequency: twice (days 0 and 20) or four times (days 0, 10, 20 and 30) and order on soil respiration, microbial biomass and nutrient availability after amendment. The first hypothesis was that the effect of amendment rate on nutrient availability and microbial biomass will be greater in the first 20 days than from day 20 to day 40. This hypothesis assumed that the difference in amount of residue in the soil between two and four additions will decrease over time, buffering the effect of the freshly added residue. The second hypothesis was that when mixes of H and L are added, differences between expected and measured value will remain the same irrespective of amendment rate and frequency.

2. Material and Methods

2.1. Soil and plant residues

The sandy clay loam used in this study was collected from 0 to 10 cm at Waite Campus, The University of Adelaide (Longitude 138°38'3.2" E, Latitude 34°58'0.2"S). The area is in a semi-arid region and has a Mediterranean climate with cool, wet winters, and hot and dry summers. The soil is a Red-brown Earth in Australian soil classification and a Rhodoxeralf according to US Soil Taxonomy. The soil has been managed as permanent pasture for over 80 years and has the following properties (for methods see section 2.3 below): sand 54%, silt 20% and clay 25%, pH (1:5 soil:water) 6.3, electrical conductivity (EC 1:5 soil:water) 143 µS cm^{-1}, total N 1.5 g kg^{-1} and total P 371 mg kg^{-1}, total organic carbon (TOC) 17 g kg^{-1}, available N 15 mg kg^{-1}, available P 10 mg kg^{-1}, maximum water holding capacity (WHC) 378 g kg^{-1} and bulk density 1.3 g cm^{-3}. The soil was collected from several randomly selected sites on the plot. In each sampling site, after removal of plants and surface litter, five samples of topsoil were collected. The soil was then air dried at 40 °C in a fan-forced oven. During summer, top soil in this area are often reach temperatures of 40-50 °C. After air-drying, visible plant debris was removed and the soil sieved to < 2 mm. Soil from all sampling sites was pooled and thoroughly mixed before subsamples were taken for the experiment.

Two types of plant residues were used: young faba bean (*Vicia faba* L., referred to as L) as low C/N residue, and mature wheat straw (*Triticum aestivum* L., referred to as H) as high C/N residue (Table 1). Legumes and cereals are often grown together in intercropping systems. The residues were dried at 40 °C in a fan-forced oven, finely ground and sieved to < 2 mm particle size. Total N and total P were 4-8 times higher in low C/N ratio residue (young faba bean) than in high C/N ratio residue (wheat straw). Therefore, low C/N residue had significantly lower C/N ratio and C/P ratio than high C/N residue. Total organic C was about 10% higher, but water extractable organic C was about 60% lower in high C/N residue than in low C/N residue.

Table 1. Total organic C, N, P, C/N ratio and C/P ratio, available N and P, water-extractable C, and pH of low C/N (young faba bean shoot) and high C/N (mature wheat straw) residues (n = 4). Different letters indicate significant differences between residues (P < 0.05).

	High C/N	Low C/N
Total organic C (g kg^{-1})	376.3^b	346.8^a
Total N (g kg^{-1})	4.6^a	38.5^b
Total P (g kg^{-1})	2.1^a	9.2^b
Organic C (g kg^{-1})	23.9^a	37.8^b
C/N ratio	82^b	9^a
C/P ratio	176^b	38^a
pH (1:10)	5.6^a	6.0^b

2.2. Experimental design

Before the start of the experiment, the air-dried soil was incubated for 13 days at 50% of maximum WHC at 20-25 °C in the dark to activate the soil microbes and to stabilise soil respiration after rewetting of air-dry soil. This water content was chosen because in previous studies with this soil, microbial activity is maximal at 50% WHC (Marschner *et al*., 2015).

There were six treatments (Table 2) differing in number of amendments (two or four) and order in which the residues (L and H) were added. The order of H and L in treatment names indicates residue addition order, e.g., HL is H followed by L. In treatments with two amendments, residues were added on day 0 and day 20 at 10 g kg^{-1}, either L followed by H (10-LH), H followed by L (10-HL) or a 1:1 mixture of L and H added twice [10-(HL)x2]. In treatments with four amendments, residues were added on day 0, 10, 20 and 30 at 5 g kg^{-1} in the following order: first H, then L, then H, then L (5-HLHL); first L, then H, then L, then H (5-LHLH); or four times a 1:1 mixture of L and H [5(HL)x4]. The treatments were designed so that (i) all treatments received both H and L, but at different rate and in different order, (ii) by day 20 and 40 the same amount of residue had been added (10 and 20 g kg^{-1}, respectively), and (iii) by day 40 all treatments had received the same amount of H and L residue. The terms 10-treatments or 5-treatments refer to all treatments with residues added at 10 or 5 g kg^{-1}, respectively. An unamended control was not included because the aim of the experiment was to compare different types of amendment, not the effect of amendment. The residue amendment rates were high compared to average expected amounts in the field. However, such high residue amounts are possible in the field, e.g. in windrows left by the harvester. The short period between residue additions was chosen based on our previous studies where we found that the legacy effect of the previous residue addition was greatest with a 10-day interval and small with a 30-day interval (Nguyen *et al*., 2016; Nguyen and Marschner, 2016). While such short intervals are unlikely to occur in rotations, they may occur during crop growth, through, e.g. senescent leaves or root turnover. In intercropping systems, this could lead to addition of residues differing in C/N ratio.

Table 2. Experimental design with treatment names and corresponding details about residue type (high or low C/N residue (H or L) or their 1:1 mixture (HL) and addition rate (10 or 5 g kg^{-1}) on days 0, 10, 20 and 30.

Treatment name	Residue types and rate (g kg^{-1} soil) added on day			
	0	10	20	30
10-HL	H-10		L-10	
10-LH	L-10		H-10	
10-(HL)x2	HL-10		HL-10	
5-HLHL	H-5	L-5	H-5	L-5
5-LHLH	L-5	H-5	L-5	H-5
5-(HL)x4	HL-5	HL-5	HL-5	HL-5

At each residue addition, residues were thoroughly mixed in 30 g soil (dry weight equivalent) in a small plastic bag. Then the amended soil was filled into PVC cores with 3.7 cm diameter, 5 cm height and a nylon net base (7.5 µm, Australian Filter Specialist) and packed to a bulk density of 1.3 g cm^{-3} by adjusting the height of the soil in the cores. In treatments with two amendments, where soils were not amended on days 10 and 30, soils were mixed similarly as amended soils. The cores were placed individually into 1 L jars with gas-tight lids equipped with septa to allow quantification of headspace CO_2 concentration as described below. The jars were incubated in the dark at 22-27 °C. Soil moisture was maintained at 50% of WHC by checking the water content every few days by weighing the cores and adding reverse osmosis (RO) water if necessary. Cores were destructively sampled on days 10, 20, 30 and 40 for analysis of available N and P, microbial biomass C, N and P.

In a given 10-day period, only the cores to be sampled at the end of the period were placed in the jars. The remaining cores were incubated under the same conditions in large plastic trays covered with aluminium foil. After removal of the cores from the jars for analysis, the cores to be harvested at the next sampling time were placed in the glass jars for respiration measurement.

2.3. Analyses and calculations

Soil analyses were carried out as described in Marschner *et al*. (2015). Briefly, soil texture was determined by the hydrometer method (Gee and Or, 2002). Soil pH was determined in a 1:5 soil:water extract after 1 h end-over-end shaking at 25 °C (Rayment and Higginson, 1992). Soil water holding capacity was determined using a sintered glass funnel connected to a 1 m water column (matric potential = -10 kPa) (Wilke, 2005). Total organic carbon content of soil and residues was determined by wet oxidation and titration (Walkley and Black, 1934). To determine total N and P in soil and residues, the material was digested with H_2SO_4 and a mixture of HNO_3 and $HClO_4$, respectively. Total N was measured by a modified Kjeldahl method (Bremner and Mulvaney, 1982). Total P in the digest was measured by the phosphovanado-molybdate method according to Hanson (1950). Available N (ammonium and nitrate) concentration was measured after 1 h end-over-end shaking with 2 M KCl at 1:10 soil extractant ratio. Ammonium-N was determined after Forster (1995). Nitrate-N was determined using a modification of Miranda *et al*. (2001). Available P was extracted by the anion exchange resin method (Kouno *et al*., 1995) and the P concentration was determined colorimetrically (Murphy and Riley, 1962).

Microbial biomass C and N were determined by chloroform fumigation-extraction with 0.5 M K_2SO_4 at 1:4 soil to extractant ratio (Moore *et al*., 2000). Organic C concentration in the extract was measured by titration with 0.033 M acidified $(NH_4)_2Fe(SO_4)2.6H_2O$ after dichromate oxidation (Anderson and Ingram, 1993). Chloroform-labile C concentration is the difference between fumigated and non-fumigated soil, which was multiplied by 2.64 to calculate MBC (Vance *et al*., 1987). Microbial biomass N was calculated as the difference in NH_4^+ concentration between fumigated and non-fumigated samples divided by 0.57 which is the proportionality factor to convert ammonium to MBN (Moore *et al*., 2000). Microbial biomass P was determined with the anion exchange method (Kouno *et al*.,1995) using hexanol as fumigant. Microbial biomass P is the difference in P concentration between fumigated and un-fumigated soil (Kouno *et al*., 1995). No correction factor was used for P because recovery of a P spike in this soil was 98% (Butterly *et al*., 2010).

Soil respiration was measured daily by quantifying the CO_2 concentration in the headspace of the jars using a Servomex 1450 infra-red analyser (Servomex Group, Crowborough, UK) as described in Setia *et al*. (2011). After each measurement (T1), jars were vented using a fan to refresh the headspace and then resealed followed by another CO_2 measurement (T0). CO_2 produced during this given interval is the difference in CO_2 concentration between T1 and T0 (Setia *et al*., 2011). Linear regression based on injection of known amounts of CO_2 into empty jars of the same size was used to define the relationship between CO_2 concentration and detector reading.

In treatments where residue mixes were applied, expected values for a given parameter were be calculated based on nutrient availability with each organic material separately and the proportion of each organic material in the mixes (Gartner and Cardon, 2004). In this study the proportion of each residue was 0.5.

2.4. Statistical analysis

There were four replicates per treatment and sampling time, arranged in a randomized block design with destructive sampling times as blocks. Data were analysed by one-way repeated measures ANOVA with time as repeated measure. The treatment x time interaction was significant. Then, one-way ANOVA was then carried out for each sampling date separately using Genstat 15th edition (VSN Int. Ltd, UK). Tukey's multiple comparison tests at 95% confidence interval was used to determine significant differences among treatments. One-way ANOVA was used to compare the properties of two plant residues.

3. Results

3.1. Cumulative respiration

Cumulative respiration in the first 10 days was higher in 10-treatments (10 g kg^{-1} added on day 0) compared to 5-treatments (5 g kg^{-1} added on day 0) (Figure 1). Among 10-treatments, cumulative respiration was about 10% lower in the treatment where only H was added on day 0 (10-HL) compared to those with L addition (10-LH and 10-(HL)x2). Cumulative respiration from day 11 to 20 was 50-75% lower in 10-treatments which were not amended on day 10 compared to 5-treatments that were amended with 5 g kg^{-1} residue on day 10 (5-HLHL, 5-LHLH, 5-(HL)x4). Among 10-treatments, cumulative respiration from day 11 to 20 was about two-fold higher in 10-HL than in those where L had been added. Cumulative respiration from day 21 to 30 was about two-fold higher in 10-treatments (amended with 10 g kg^{-1} on day 20) than 5-treatments which received only 5 g kg^{-1}. From day 31 to day 40, cumulative respiration was three-fold lower in 10-treatments (no residues were added on day 30) than 5-treatments (amended on day 30). Total cumulative respiration at the end of the experiment differed little among treatments. It was less than 5% higher in 10-HL than 10-LH and 5-LHLH.

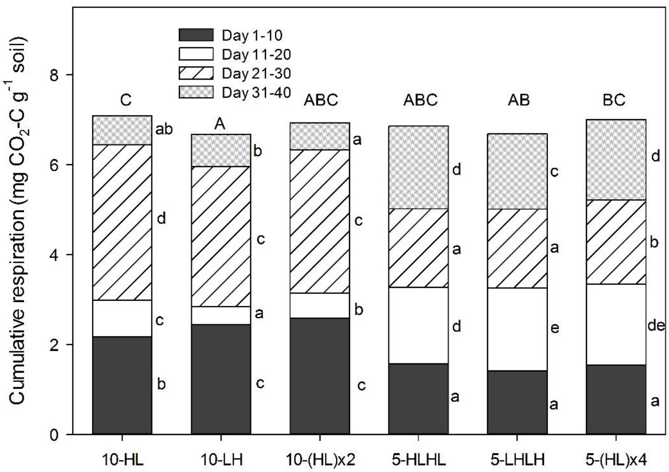

Figure 1. Cumulative respiration in 10-day intervals and total over 40 days in soil amended with high (H) and low C/N (L) residues or their 1:1 mixture at 5 or 10 g kg^{-1} (n=4). Different lower case letters indicate significant differences ($P \leq 0.05$) among treatments for a given 10-day interval. Different upper case letters indicate significant differences among treatments in total cumulative respiration. For treatment names see Table 2.

3.2. Microbial biomass

Microbial biomass C and N increased from day 10 to day 30 (Figure 2). On day 10, MBC was up to 25% higher in 10-treatments than 5-treatments (Figure 2A). MBC on day 20 was lowest in 10-HL. MBC on day 20 did not differ among treatments that had been amended with H and L irrespective of the timing of the amendment, once on day 0 [10-(HL)x2] or twice (5-treatments). MBC increased from day 10 to day 20 about two-fold in 5-treatments, but only by about 25% in 10-treatments. On day 30, MBC was highest in 10-LH which had been amended with H on day 20. MBC on day 40 was lowest in 10-HL where it was about 20% lower than in the other treatments.

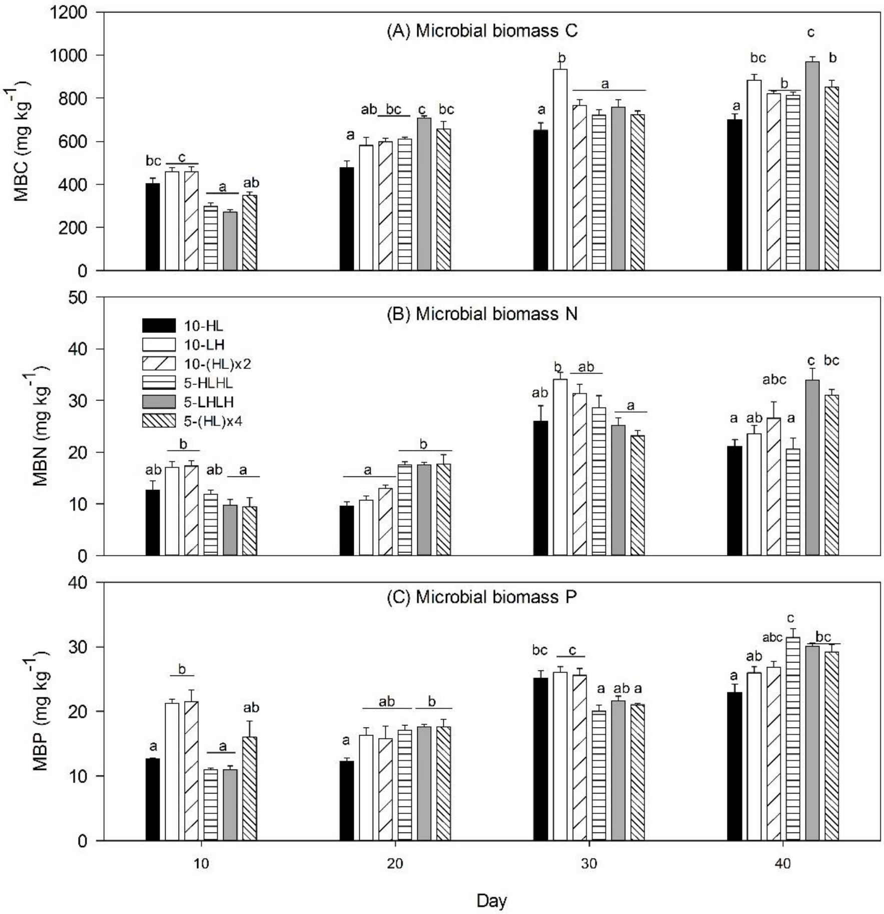

Figure 2. Microbial biomass C (A), N (B) and P (C) on days 10, 20, 30 and 40 in soil amended with high (H) and low C/N (L) residues or their 1:1 mixture (HL) at 5 or 10 g kg^{-1} (n=4, vertical lines indicate standard error). Different letters indicate significant differences ($P \leq 0.05$) among treatments at a given sampling day. For treatment names see Table 2.

In treatments with L or HL addition on day 0, MBN on day 10 was lower in 5-treatments [5-LHLH, 5-(HL)x4] than 10-treatments [10-LH, 10-(HL)x2] (Figure 2B). On day 20, MBN was 30-50% lower in 10-treatments than 5-treatments. In 5-treatments, MBN increased from day 10 to day 20 whereas it decreased in 10-treatments. MBN increased from day 20 to day 30 in all treatments, particularly 10-treatments where MBN increased about three-fold. On day 30, MBN was highest in 10-LH which had been amended with 10 g kg^{-1} of H on day 20 and low in treatments with 5 g kg^{-1} L or HL on day 20 [5-LHLH, 5-(HL)x4]. In the latter treatments, MBN increased from day 30 to day 40 whereas it decreased or remained unchanged in the other treatments. On day 40 among 5-treatments, MBN was about 30% higher when H or HL was added on day 30 [5-LHLH, 5-(LH)x4] than in the treatment amended with L on that day (5-HLHL).

On day 10, MBP differed only among 10-treatments where it was lowest in 10-HL (Figure 2C). MBP on day 20 was lower in 10-HL than in 5-treatments that were amended with H or HL on day 10 [5-LHLH and 5-(HL) x4]. MBP increased by about 30% from day 20 to day 30 in 10-treatments, but changed little in 5-treatments. On day 30, MBP was 15% higher in 10-treatments than 5-treatments. MBP increased from day 30 to day 40 by about 30% in 5-treatments that were, but remained unchanged in 10-treatments. On day 40, MBP was about 30% higher in 5-treatments than 10-treatments.

3.3. Available N and P

On day 10 and day 20, available N was highest in 10-LH (Figure 3A). On day 10, available N was about 30% higher in 10-LH compared to 5-LHLH which was amended with only 5 g kg^{-1} L on day 0. Available N differed little between 10-HLx2 and 5-(HL)x4 although two times more residue had been added in 10-(HL)x2. At a given residue addition rate, available N was lowest when only H had been added on day 0. It was lower in treatments amended with HL than with L alone, with greater differences in 10-treatments. Available N on day 20 was lowest in 10-HL and did not differ among treatments that had received both H and L by day 20 [10-(HL)x2, 5-treatments]. On day 30, available N was lowest in 5-HLHL and highest in 5-LHLH. Available N on day 40 was about 30% higher in 10-HL than in 10-LH and 10-(HL)x2 and about two-fold higher than in 5-treatments.

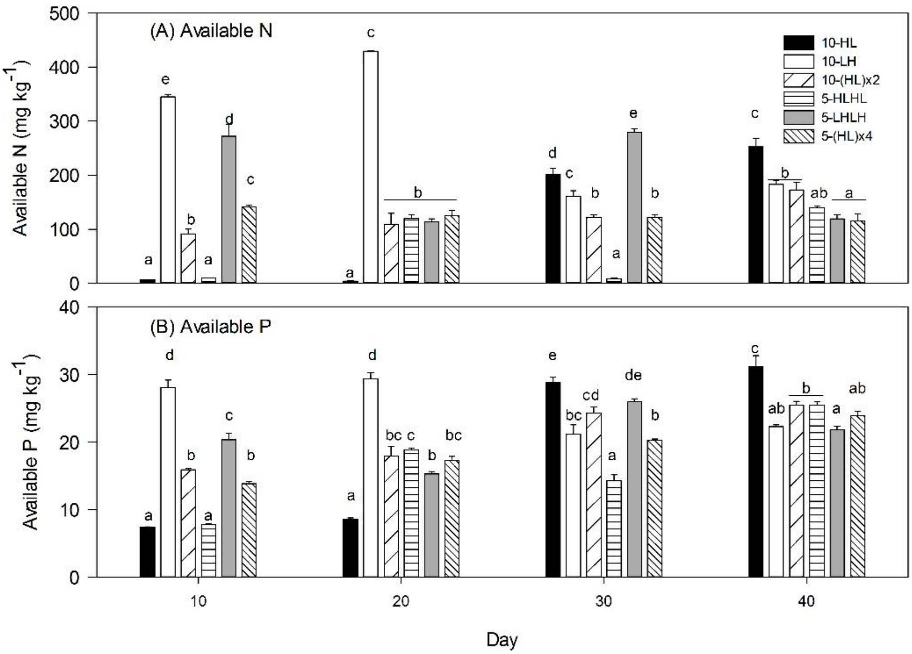

Figure 3. Available N (A) and P (B) on days 10, 20, 30 and 40 in soil amended with high (H) and low C/N (L) residues or their 1:1 mixture (HL) at 5 or 10 g kg^{-1} (n=4, vertical lines indicate standard error). Different letters indicate significant differences ($P \leq 0.05$) among treatments at a given sampling day. For treatment names see Table 2.

4. Discussion

The experiment showed that residue order had little effect on total cumulative respiration, but influenced MBC and MBN on day 40 and available N and P throughout the experiment. Residue addition frequency influenced distribution of cumulative respiration among 10-day intervals, and microbial biomass and nutrient availability throughout the experiment.

Some results are consistent with previous studies. For example, the higher N availability and MBN after L compared to H addition can be explained by the higher N concentration and greater decomposability of young faba bean residue (L) compared to mature wheat straw (H) (Tian *et al*., 1992). In agreement with other studies, N was immobilised after H addition and respiration rates declined over time after residue addition (Marschner *et al*., 2015; Nguyen *et al*., 2016; Zheng and Marschner, 2017a). The following discussion will focus on results indicating the importance of residue addition frequency and order on the measured parameters.

The first hypothesis (the effect of amendment rate on nutrient availability and microbial biomass will be greater in the first 20 days than from day 20 to day 40) can only be confirmed for available N and P after L addition which were much higher on days 10 and 20 in 10-LH than in 5-HLHL or 5-LHLH, than on day 40. However, differences between 10 and 5-treatments in cumulative respiration over 10 days remained the same. MBN was higher in 10-treatments only 10 days after amendments whereas it was greater in 5-treatments than 10-treatments on days 20 and 40 which can be explained by the time since residue addition. On days 20 and 40, the soil had been amended 10 days prior in 5-treatments, but 20 days prior in 10-treatments. In the latter, easily decomposable compounds had been largely decomposed resulting in biomass turnover.

4.1. Low following high C/N residue

Ten days after H addition on day 0, MBN was only slightly lower than after L addition, but available N was very low which indicates that almost all N available in the soil was immobilised and decomposition of H may be N limited. Changes in available N and MBN after L was added to soil previously amended with H depended on residue addition rate and timing.

In 5-HLHL where L was added the first time on day 10, MBN increased by about 25% from day 10 (prior L addition) to day 20, whereas available N increased about 10-fold. Similarly in 10-HL on day 30 (10 days after L addition), MBN was more than two-fold higher than on day 20, whereas available N about fifty-fold higher. This indicates that only a small proportion of N mineralised during L decomposition was immobilised. Nevertheless, available N in 10-HL on day 30 was lower than in 10-LH on day 10 whereas MBN was higher. This is in agreement with our previous studies on the legacy effect (Marschner *et al*., 2015; Nguyen *et al*., 2016). The lower available N in 10-HL can be explained by the remaining H in the soil after L addition. Microbes decomposing H will immobilise N released during decomposition of L. In 5-HLHL after the second L addition on day 30, available N increased to the same extent as after the first L addition, but MBN on day 40 was slightly lower than on day 30. The lack of increase in MBN from day 30 to 40 in 5-HLHL indicates that microbes exposed to repeated N limitation (day 10 and day 30) have limited capacity to take up available N.

Changes in available N and MBN when H was added after L were similar in 10-LH and 5-LHLH: 10 days after H addition, MBN increased whereas available N decreased. The relative increase in MBN and decrease in available N was greater in 10-LH than 5-LHLH which is likely due to the greater amount of residue added in the former. This suggests that irrespective of residue addition rate, microbes were not N limited after H addition because the previously added L supplied sufficient N even when large amounts of H were added.

4.2. Comparison of 5-HLHL and 5-LHLH

In 5-HLHL, native soil N availability was apparently sufficient to allow decomposition of the first H amendment, but it resulted in depletion of available N. MBN then further increased after the first L addition on day 10. Sufficient N was left on day 20 to allow an increase in MBN after the second H addition on day 20, again resulting in depletion of available N. Changes in MBN from day 30 to day 40 were not as expected. L was added on day 30 and available N increased, but MBN did not further increase. The increase in MBP from day 30 to day 40 suggests a change in the stochiometry of the microbial biomass compared to the first L addition after which both MBN and MBP increased.

Although L was added on day 0 in 5-LHLH, MBN on day 10 was similar as in 5-HLHL where H had been added. However, available N was much higher in 5-LHLH which suggests microbial N uptake was limited. Changes in MBN and available N from day 10 onwards were as expected from previous studies (Tian *et al.*, 1992). The first and second H addition resulted in an increase in MBN and depletion of available N.

4.3. H and L added at the same time

The effect of mixes of plant residues with different composition has been studied extensively (Gartner and Cardon, 2004; Cobo *et al.*, 2008). In residue mixes, expected values (e.g. mass loss, nutrient content, nutrient availability) can be calculated based on values with each residue separately and the ratio of residues in the mixes. Measured values have been found to be similar, higher or lower than expected values (Gartner and Cardon, 2004; Chapman and Koch, 2007; Ball *et al*., 2008; Mao and Zeng, 2012). It is likely that particles of different residues are decomposed by distinct microbial communities that may interact through nutrient exchange (Schneckenberger and Kuzyakov, 2007) or diffusion of metabolites (McTiernan *et al.*, 1997; Gartner and Cardon, 2004). Greater than expected decomposition has been explained by (i) nutrient transfer from high-nutrient to low-nutrient residues (ii) differential decomposer community composition (Blair *et al*., 1990), (iii) niche complementarity (Chapman and Koch, 2007), and (iv) priming (Blair *et al*., 1990; Chapman and Koch, 2007).

The second hypothesis (when mixes of H and L are added, differences between expected and measured value will remain the same irrespective of amendment rate and frequency) has to be declined. Measured and expected values matched for cumulative respiration, available P and MBP irrespective of residue addition frequency (Table 3). However, differences between measured and expected available N and MBN (Figure 4) depended on residue addition frequency and parameter. In 10-(HL)x2 after both the first and second addition of the HL mix, available.

Table 3. Measured and expected values of cumulative respiration, microbial biomass C, P on days 10, 20, 30 and 40 for treatments where 1:1 mixes of H and L were added (10-(HL)x2, 5-(HL)x4). Measured and expected values were not significantly different.

Day		10	20	30	40
10-(HL)x2					
Cumulative respiration	**Measured**	**2.6**	**0.6**	**3.2**	**0.6**
(mg CO_2-C g^{-1})	Expected	2.3	0.6	3.3	0.7
MBC (mg kg^{-1})	**Measured**	**459**	**597**	**766**	**822**
	Expected	433	542	802	791
MBP (mg kg^{-1})	**Measured**	**21.5**	**15.8**	**25.6**	**26.8**
	Expected	16.9	14.3	27.8	24.5
Available P (mg kg^{-1})	**Measured**	**15.9**	**18.0**	**24.3**	**25.5**
	Expected	17.7	19.0	25.0	26.7
5-(HL)x4					
Cumulative respiration	**Measured**	**1.5**	**1.8**	**1.9**	**1.8**
(mg CO_2-C g^{-1})	Expected	1.5	1.8	1.7	1.8
MBC (mg kg^{-1})	**Measured**	**348**	**658**	**723**	**850**
	Expected	284	659	740	891
MBP (mg kg^{-1})	**Measured**	**16.0**	**17.6**	**25.0**	**29.2**
	Expected	11.0	17.3	20.8	30.8
Available P	**Measured**	**13.9**	**17.3**	**20.3**	**24.0**
	Expected	14.1	17.1	20.1	23.7

N was lower than expected. Measured MBN was significantly higher than expected only on day 20, but slightly higher on days 10 and 40. This suggests that mixing of H and L at 10 g kg^{-1} enhanced nutrient transfer from microbes decomposing L to those decomposing H compared to single residues or when residues were added sequentially. Thus at this residue addition rate, when H and L undergo the same decomposition stages together, nutrient transfer from L to H was greater than if residues are added one after the other. In the latter case, the freshly added residue will undergo early decomposition stages while the residue remaining from the previous amendment is already in later stages of decomposition.

In 5-(HL)x4, comparisons between measured and expected values were different from those in 10-(HL)x2 and changed over time (Table 3). For available N, measured values matched expected values on days 10 and 20, but measured values were smaller than expected on day 30 and slightly lower on day 40. Measured MBN matched expected values. This indicates that when smaller amounts of residue mixes are added repeatedly, interactions between microbial communities decomposing H and L change; possibly because their proximity to each other changes. In the first 20 days, when small amounts of residues are added, decomposer communities around each residue particle may be too far apart for interactions. As more residues are added over time, microbial communities decomposing freshly added residue are more likely to be close to those decomposing previously added residues resulting in N depletion as microbes decomposing H take up N released by microbes decomposing L.

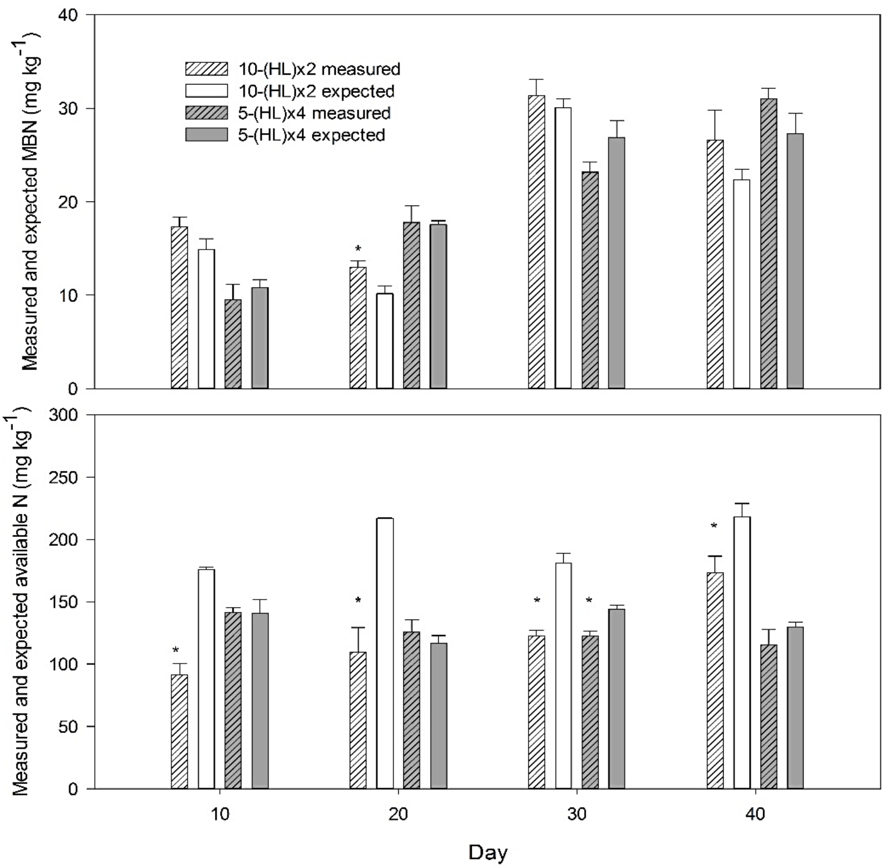

Figure 4. Measured and expected microbial biomass N (MBN) and available N on days 10, 20, 30 and 40 for treatments where 1:1 mixes of H and L were added (10-(HL)x2, 5-(HL)x4). Asterisk indicates significant difference between measured and expected value.

5. Conclusion

The study showed that with repeated addition of H and L, N availability and MBN are influenced by residue rate and order. Further the results suggest that differences between measured and expected values in residue mixes depend on residue addition frequency, and may change over time when small amounts of residues are added.

In the present study, the source of C, N and P in respired CO_2, microbial biomass and available nutrients could not be determined. In future studies with repeated residue additions, one of the amendments could be ^{13}C, ^{15}N or ^{32}P-labelled. Future studies could also assess microbial community structure at different times during decomposition, which may explain changes in nutrient availability and microbial biomass.

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CHAPTER 3

ADDITION OF RESIDUES WITH DIFFERENT C/N RATIO IN SOIL OVER TIME INDIVIDUALLY OR AS MIXES - EFFECT ON NUTRIENT AVAILABILITY AND MICROBIAL BIOMASS DEPENDS ON AMENDMENT RATE AND FREQUENCY

Statement of Authorship

Title of Paper	Addition of residues with different C/N ratio in soil over time individually or as mixes - effect on nutrient availability and microbial biomass depends on amendment rate and frequency
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Name of Principal Author (Candidate)	Thi Hoang Ha Truong		
Contribution to the Paper	Performed experiment and analyses of all samples, data analysis and interpretation and manuscript writing. I hereby certify that the statement of contribution is accurate.		
Overall percentage (%)	70%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	22/2/2020

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

iv. the candidate's stated contribution to the publication is accurate (as detailed above);

v. permission is granted for the candidate in include the publication in the thesis; and

vi. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Petra Marschner		
Contribution to the Paper	Supervised development of the work, data interpretation and manuscript evaluation and correction. I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.		
Signature		Date	22/02/2020

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RESEARCH ARTICLE

Addition of residues with different C/N ratio in soil over time individually or as mixes - effect on nutrient availability and microbial biomass depends on amendment rate and frequency

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Abstract

Residues with different properties may be added to soil simultaneously (as mixes) or after each other. In most previous studies on the effect of mixes on decomposition and nutrient release, residues were added once at the start of the experiment. Less is known about the effect of mixtures added more than once on soil respiration, microbial biomass and nutrient availability and how the addition frequency influences interactions between high C/N (H) and low C/N (L) residues in mixes. In the 48-day incubation experiment with total amendment rate in all treatments was 20 g kg^{-1}, treatments differed in addition frequency (twice, four or eight times), rate (10, 5 or 2.5 g kg^{-1}) as well as order of H and L. Soil was sampled every 12 days. Treatments had similar total cumulative respiration, but differed in distribution over the 12 day periods. Available N and MBN changed strongly over time in treatments with 10 or 5 g kg^{-1}, depending on the C/N ratio of the residue added before sampling. Frequent addition of small amounts of residues (2.5 g kg^{-1}) limited microbial N uptake initially compared to higher amendment rates, but resulted in similar available N irrespective of the C/N ratio of the residue added. It can be concluded that with less frequent residue addition, microbes decompose mainly the recently added residue. With more frequent addition, microbes decompose recently added residue together with residue added before the most recent amendment.

Keywords: Amendment, addition frequency, C/N ratio; MBN, microbial biomass; mixes, N availability.

1. Introduction

It is well-known that composition of organic amendments determines decomposition rate and nutrient availability (Tian *et al*., 1992; Bending and Turner, 1999). One of the most important properties is the N concentration of the organic materials, commonly expressed as C/N ratio. Addition of organic materials with high N concentration (C/N < 20) results in net N mineralization (Hadas *et al*., 2004) whereas amendments with low N concentration (high C/N) induce net N immobilization (Moritsuka *et al*., 2004). However, other residue properties such as organic C composition also influence decomposition rate and nutrient release (Cartenì *et al*., 2018). Nevertheless, the C/N ratio is considered as a good indicator of N availability and immobilization.

Plant residues differing in C/N ratio can be added to soil one after another, e.g. in intercropping. In previous studies, we found that nutrient availability after the second residue addition is influenced by the C/N ratio of the first and the second residue amendment, termed legacy effect (Marschner *et al*., 2015). For example, N availability was higher after high C/N residue amendment when it followed low C/N residue than if high C/N residue was added to unamended soil. The extent of the legacy effect depended on the amount of the first residue left in the soil when the second residue is added (Zheng and Marschner, 2017). In another study, repeated slurry application compared to a single addition increased soil respiration and the proportion of CO_2 derived from slurry, but reduced the proportion of CO_2 from SOC (Cavalli *et al*., 2014). Bonanomi *et al*. (2017) reported that repeated applications of organic materials increased inhibition of germination and growth of soil-borne fungi.

Residues differing in C/N ratio can not only be added sequentially, but also together. The effect of simultaneous addition of plant residues of different composition has been studied extensively (Gartner and Cardon, 2004; Xiang and Bauhus, 2007; Cobo *et al*., 2008). In such residue mixes, expected decomposition or nutrient availability can be calculated based on decomposition/nutrient availability with each residue separately and the ratio of residues in the mixes. For example, in a 360-day study where a 1:1 mix of Hedera helix leaf litter with low C/N ratio (21) with cellulose strips that had high C/N (440), measured decomposition in the 1:1 mix was higher than expected due to N transfer from low C/N litter to high C/N cellulose (Bonanomi *et al*., 2014). In another study, low C/N litter (20) and high C/N litter (50-80) were incubated singly or as 1:1 mixes in litter bags which were placed in soil for two or nine months (Cuchietti *et al*., 2014). Measured decomposition in mixes was usually higher than expected because high C/N litters appeared to increase decomposition rate of low C/N litters. In a shorter term study, with litters differing in C/N ratio (high in poplar leaves and low in soybean leaves) mixed at a 1:1 ratio, mass loss and N release after 84 days were higher than expected in the mix (Mao and Zeng, 2012).

In previous studies on mixing litters with different C/N ratio, the litters were added only once at the start of the experiment. However, litter mixes may also be added to soil repeatedly, e.g. through the action of ants or earthworms. It is unclear if the effect of residue mixture on soil respiration, microbial biomass and nutrient availability is influenced by addition frequency and how the addition frequency influences interactions between high C/N and low C/N residues in mixes. In the present study, soil was amended with high and low C/N residues or their 1:1 mixture to a total amendment rate of 20 g kg^{-1} over a period of 42 days. Residues were added twice (day 0, 24) at 10 g kg^{-1}, four times (day 0, 12, 24, 36) at 5 g kg^{-1} or eight times (day 0, 6, 12, 18, 24, 30, 36, 42) at 2.5 g kg^{-1}.

We tested the following hypotheses which are about available N and MBN because they responded most to C/N ratio of amendment in our previous studies.

(i) At a given sampling time, available N and MBN will be less influenced by the C/N ratio of the residue added last before sampling with frequent addition of low C/N (L) and high C/N (H) residue at low rate (four or eight times) than less frequent amendment (two times) at high rate. This hypothesis assumes that with frequent addition of L and H at low rate, changes in available N and MBN after a given amendment will be small due to the small amount of residue added. Further, due to the short time between residue additions, the soil contains previously added residues that are only partially decomposed. With less frequent amendment, previously added residues are largely decomposed by the time the next residue is added and microbes are likely to nutrient-starved. This will lead to rapid decomposition of the freshly added amendment.

(ii) In 1:1 mixes of H and L, available N and MBN will initially increase with amendment rate, but differences between rates will become smaller over time as lower rates are added more frequently leads to a build-up of the amount of residue in the soil during incubation.

(iii) In the mixes, differences between measured and expected values, if any, will be greater when residues are added at high rates twice than if they are added at lower rates more frequently. When residues are added at high rate, but less frequent, L and H will decompose separately whereas if they are added as mix, they will be decomposed together. With frequent amendment at low rate, the small amendments will induce small changes in measured properties and due to the short interval between additions, both H and L will be present in the soil even when added sequentially. This will occur earlier the more frequent the residues are added.

2. Materials and Methods

2.1. Soil and plant residues

The sandy clay loam used in this study was collected from 0 to 10 cm at Waite Campus, The University of Adelaide (Longitude 138˚38'3.2'' E, Latitude 34˚58'0.2''S). The area is in a semi-arid region and has a Mediterranean climate with cool, wet winters, and hot and dry summers. The soil is a Red-brown Earth in Australian soil classification and a Rhodoxeralf according to US Soil Taxonomy. The soil has been managed as permanent pasture for over 80 years. The soil was collected from several randomly selected sites on the plot. After removal of plants and surface litter, five samples of topsoil were collected at each sampling site. The soil was then air dried at 40 °C in a fan-forced oven. During summer, top soil in this area are often reach temperatures of 40-50 °C. After air-drying, visible plant debris was removed and the soil sieved to < 2 mm. Soil from all sampling sites was pooled and thoroughly mixed before subsamples were taken for the experiment. It has the following properties (for methods see section 2.3 below): sand 54%, silt 20% and clay 25%, pH (1:5 soil:water) 6.3, electrical conductivity (EC 1:5 soil:water) 143 $\mu S\ cm^{-1}$, total N 1.5 $g\ kg^{-1}$ and total P 371 $mg\ kg^{-1}$, total organic carbon (TOC) 17 $g\ kg^{-1}$, available N 15 $mg\ kg^{-1}$, available P 10 $mg\ kg^{-1}$, maximum water holding capacity (WHC) 378 $g\ kg^{-1}$ and bulk density 1.3 $g\ cm^{-3}$.

Two types of plant residues were used: young faba bean (*Vicia faba* L., referred to as L) as low C/N residue, and mature wheat straw (*Triticum aestivum* L., referred to as H) as high C/N residue (Table 1). Legumes and cereals are often grown together in intercropping systems. The residues were dried at 40 °C in a fan-forced oven, finely ground and sieved to particle size 0.25-2 mm. Total N and total P were 4-8 times higher in low C/N ratio residue (young faba bean) than in high C/N ratio residue (wheat straw). Therefore, low C/N residue had significantly lower C/N ratio and C/P ratio than high C/N residue. Total organic C was about 10% higher, but water extractable organic C was about 60% lower in high C/N residue than in low C/N residue.

Table 1. Total organic C, N, P, C/N ratio and C/P ratio, available N and P, water-extractable C, and pH of low C/N (young faba bean shoot) and high C/N (mature wheat straw) residues (n = 4). Different letters indicate significant differences between residues (P < 0.05).

	High C/N	Low C/N
Total organic C (g kg^{-1})	376.3^b	346.8^a
Total N (g kg^{-1})	4.6^a	38.5^b
Total P (g kg^{-1})	2.1^a	9.2^b
WEOC (g kg^{-1})	23.9^a	37.8^b
C/N ratio	82^b	9^a
C/P ratio	176^b	38^a
pH (1:10)	5.6^a	6.0^b

2.2. Experimental design

Before the start of the experiment, the air-dried soil was incubated for 10 days at 50% of maximum WHC at 20-25 °C in the dark to activate the soil microbes and to stabilise soil respiration after rewetting of air-dry soil. This water content was chosen because in previous studies with this soil, microbial activity was maximal at 50% WHC (Marschner *et al*., 2015).

There were nine treatments (Table 2) differing in number of amendments (two, four or eight) and order in which the residues (L and H) were added. In treatments with two amendments, residues were added on day 0 and day 24 at 10 g kg^{-1}, either L followed by H (10-L-H), H followed by L (10-H-L) or a 1:1 mixture of L and H added twice [10-(HL)x2]. In treatments with four amendments, residues were added on day 0, 12, 24 and 36 at 5 g kg^{-1} in the following order: first H, then L, then H, then L [5-(H-L)x2]; first L, then H, then L, then H [5-(L-H)x2]; or four times a 1:1 mixture of L and H [5-(HL)x4]. In treatments with eight amendments, residues were added on day 0, 6, 12, 18, 24, 30, 36 and 42 at 2.5 g kg^{-1}, either four times of H then L [2.5-(H-L)x4], four times of L then H [2.5-(L-H)x4] or eight times a 1:1 mixture of H and L [2.5-(HL)x8]. The treatments were designed so that (i) all treatments received both H and L, but at different rate and in different order, (ii) by day 24 and 48 the same amount of residue had been added (10 and 20 g kg^{-1}, respectively), and (iii) by day 48 all treatments had received the same amount of H and L residue. The terms 10-treatments, 5-treatments or 2.5-treatments refer to all treatments with residues added at 10, 5 or 2.5 g kg^{-1}, respectively. An unamended control was not included because the aim of the experiment was to compare different types of amendment, not the effect of amendment. The residue amendment rates were high compared to average expected amounts in the field. However, such high residue amounts are possible in the field, e.g. in windrows left by the harvester or in home gardens. The high residue mixing frequency (every eight days) may not be realistic in agriculture, but could occur in home gardens or through action of earthworms or ants.

Table 2. Experimental design with treatment names and corresponding details about residue type (high or low C/N residue (H or L) or their 1:1 mixture (HL), addition rate (10, 5 or 2.5 g kg^{-1} soil) and amendment dates

Treatment name	Residue types and rate (g kg^{-1} soil) and added on day							
	0	6	12	18	24	30	36	42
10-H-L	H-10				L-10			
10-L-H	L-10				H-10			
10-(HL)x2	HL-10				HL-10			
5-(H-L)x2	H-5		L-5		H-5		L-5	
5-(L-H)x2	L-5		H-5		L-5		H-5	
5-(HL)x4	HL-5		HL-5		HL-5		HL-5	
2.5-(H-L)x4	H-2.5	L-2.5	H-2.5	L-2.5	H-2.5	L-2.5	H-2.5	L-2.5
2.5-(L-H)x4	L-2.5	H-2.5	L-2.5	H-2.5	L-2.5	H-2.5	L-2.5	H-2.5
2.5-(HL)x8	HL-2.5	HL-2.5	HL-2.5	HL-2.5	HL-2.5	HL-2.5	HL-2.5	HL-2.5

At each residue addition, residues were thoroughly mixed in 30 g soil (dry weight equivalent) in a small plastic bag. Then the amended soil was filled into PVC cores with 3.7 cm diameter, 5 cm height and a nylon net base (7.5 µm, Australian Filter Specialist) and packed to a bulk density of 1.3 g cm^{-3} by adjusting the height of the soil in the cores. In 10-treatments on days 6, 12, 18, 30, 36 and 42 and in 5-treatments on days 6, 18, 30 and 42, soils were not amended but mixed similarly as the 2.5-treatments. The cores were placed individually into 1 L jars with gas-tight lids equipped with septa to allow quantification of headspace CO_2 concentration as described below. The jars were incubated in the dark at 20-24 °C. Soil moisture was maintained at 50% of WHC by checking the water content every few days by weighing the cores and adding reverse osmosis (RO) water if necessary. Cores were destructively sampled on days 12, 24, 36 and 48 for analysis of available N and P, and microbial biomass N and P.

In a given 12-day period, only the cores to be sampled at the end of the period were placed in the jars. The remaining cores were incubated under the same conditions in large plastic trays covered with aluminium foil. After removal of the cores from the jars for analysis, the cores to be harvested at the next sampling time were placed in the glass jars for respiration measurement.

Soil analyses were carried out as described in Marschner *et al*. (2015). Briefly, soil texture was determined by the hydrometer method (Gee and Or, 2002). Soil pH was determined in a 1:5 soil:water extract after 1 h end-over-end shaking at 25 °C (Rayment and Higginson, 1992). Soil water holding capacity was determined using a sintered glass funnel connected to a 1 m water column (matric potential =

-10 kPa) (Wilke, 2005). Total organic carbon content of soil and residues was determined by wet oxidation and titration (Walkley and Black, 1934). To determine total N and P in soil and residues, the material was digested with H_2SO_4 and a mixture of HNO_3 and $HClO_4$, respectively. Total N was measured by a modified Kjeldahl method (Bremner and Mulvaney, 1982). Total P in the digest was measured by the phosphovanado-molybdate method (Hanson 1950). Available N (ammonium and nitrate) concentration was measured after 1 h end-over-end shaking with 2 M KCl at 1:10 soil extractant ratio. Ammonium-N was determined after Forster (1995). Nitrate-N was determined using a modification of Miranda *et al*. (2001). Available P was extracted by the anion exchange resin method (Kouno *et al*., 1995) and the P concentration was determined colorimetrically (Murphy and Riley, 1962).

Microbial biomass N was determined by chloroform fumigation-extraction with 0.5 M K_2SO_4 at 1:4 soil to extractant ratio (Moore *et al*., 2000). Microbial biomass N was calculated as the difference in NH_4^+ concentration between fumigated and non-fumigated samples divided by 0.57 which is the proportionality factor to convert ammonium to MBN (Moore *et al*., 2000). Microbial biomass P was determined with the anion exchange method (Kouno *et al*., 1995) using hexanol as fumigant. Microbial biomass P is the difference in P concentration between fumigated and un-fumigated soil (Kouno *et al*., 1995). No correction factor was used for P because recovery of a P spike in this soil was 98% (Butterly *et al*., 2010).

Soil respiration was measured daily by quantifying the CO_2 concentration in the headspace of the jars using a Servomex 1450 infra-red analyser (Servomex Group, Crowborough, UK) (Setia *et al*., 2011). After each measurement (T1), jars were vented using a fan to refresh the headspace and then resealed followed by another CO_2 measurement (T0). CO_2 produced during this given interval is the difference in CO_2 concentration between T1 and T0 (Setia *et al*., 2011). Linear regression based on injection of known amounts of CO_2 into empty jars of the same size was used to define the relationship between CO_2 concentration and detector reading.

In treatments where residue mixes were applied, expected values for a given parameter were calculated based on nutrient availability with each organic material separately and the proportion of each organic material in the mixes (Gartner and Cardon, 2004). In this study the proportion of each residue in the mixes was 0.5.

2.3. Statistical analysis

There were four replicates per treatment and sampling time, arranged in a randomized block design with destructive sampling times as blocks. After confirming normal distribution, data were analysed by two-way ANOVA (residue treatment x rate) for each sampling date separately using Genstat 15th edition (VSN Int. Ltd., UK). Tukey's multiple comparison tests at 95% confidence interval was used to determine significant differences among treatments. One-way ANOVA was used to compare the properties of the two plant residues.

3. Results

3.1. Cumulative respiration

Throughout the experiment, cumulative respiration within 12-day intervals differed little among 5- and 2.5-treatments that, by the end of the intervals, had both been amended with 5 g kg^{-1} residue (Figure 1). Except for the first 24 days in the 10-treatments, the type of residue added had little effect on cumulative respiration. Cumulative respiration in the first 12 days was higher in 10-treatments (10 g kg^{-1} added on day 0) compared to 5- and 2.5-treatments (5 g kg^{-1} added by day 12). Although all treatments had received 10 g residue kg^{-1} by day 24, cumulative respiration was about two- to three-fold higher in 5-treatments and 2.5-treatments than in the 10-treatments. Among 10-treatments, cumulative respiration was two-fold higher in 10-H-L than in 10-L-H. Cumulative respiration from day 25 to 36 was about two-fold higher in 10-treatments that were amended with 10 g kg^{-1} residue on day 24 compared to 5-treatments (5 g kg^{-1} added on day 24) and 2.5- treatments (2.5 g kg^{-1} added on days 24 and 30). From day 37 to 48, when all treatments had received 20 g kg^{-1}, cumulative respiration was about two- to three-fold higher in 5-treatments and 2.5-treatments compared to 10-treatments which had received no residue addition since day 24. Total cumulative respiration at the end of the experiment did not differ among treatments.

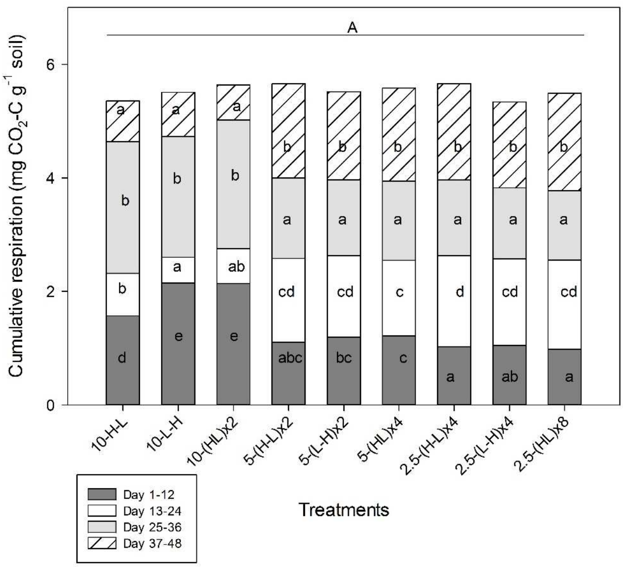

Figure 1. Cumulative respiration in 12-day intervals and total over 48 days in soil amended with high (H) and low C/N (L) residues or their 1:1 mixture (HL) eight times at 2.5 g kg^{-1}, four times at 5 or twice at 10 g kg^{-1} (n=4). Different lower case letters indicate significant differences ($P \leq 0.05$) among treatments for a given 12-day interval. Different upper case letters indicate significant differences among treatments in total cumulative respiration. For treatment names see Table 2.

3.2. Microbial biomass N and P

In 10- and 5-treatments, MBN on day 12 was similar in treatments with H and HL addition on day 0 [10-H-L, 10-(HL)x2, 5-(H-L)x2 and 5-(HL)x4] where it was between two and four-fold higher than in the 2.5-treatments (Figure 2A). MBN did not differ between 10-L-H and 5-(L-H)x2 although two times more L had been added in 10-L-H. MBN was similar among 2.5-treatments that had all received both H and L by day 12. MBN changed little from day 12 to 24 in 10- and 5-treatments except for 5-(L-H)x2 where it increased by about 80%. During that period, MBN increased up to three-fold in 2.5-treatments. In 10-treatments, MBN on day 24 was about 40% lower in 10-L-H which had only received L by then compared to the other two treatments that had been amended with H. On day 36, MBN in the 10-treatments was about 30% lower in 10-H-L which had received L on day 24 compared to the other two treatments. MBN differed little among 5- and 2.5 treatments. MBN on day 48 differed little among treatments at a given amendment rate, but was about 30% higher in 2.5-(H-L)x4 and 2.5-(L-H)x4 than in the 10-treatments.

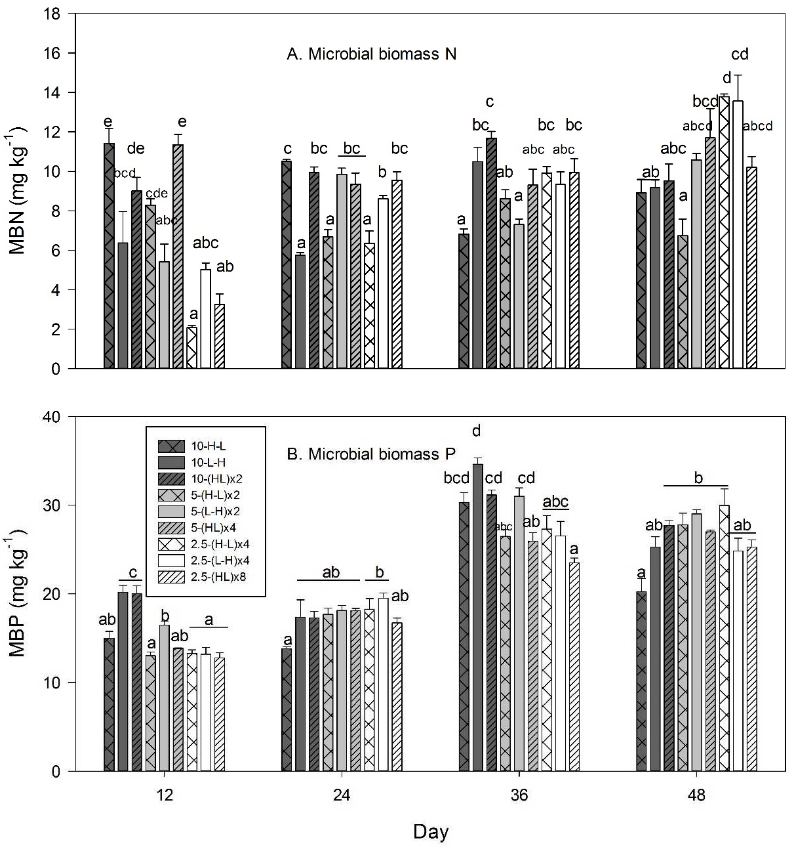

Figure 2. Microbial biomass N (A) and P (B) on days 12, 24, 36 and 48 in soil amended with high (H) and low C/N (L) residues or their 1:1 mixture (HL) eight times at 2.5 g kg^{-1}, four times at 5 or twice at 10 g kg^{-1} (n=4, vertical lines indicate standard error). Different letters indicate significant differences ($P \leq 0.05$) among treatments at a given sampling day. For treatment names see Table 2.

In all treatments, MBP was lower on day 12 than on days 36 and 48 (Figure 2B). On day 12, MBP differed little among 5- and 2.5-treatments. It was 10-20% higher in 10-L-H and 10 (HL)x2 than the other treatments. MBP on day 24 differed little among treatments except that it was 20% lower in 10-H-L than in 2.5-(L-H)x4. On day 36, MBP was about 20% higher in 10-L-H than the 2.5-treatments, but differed little among treatments at a given residue rate. On day 48, MBP was about 25% lower in 10-H-L than the 5-treatments.

3.3. Available N and P

Throughout the experiment, available N in 2.5-treatments differed little among treatments, but it was higher on day 48 than day 12 (Figure 3A). On days 12 and 24, available N was highest in 10-L-H. On day 12, available N in 10-L-H was about four-fold higher than in 10-(HL)x2 (5 g kg^{-1} L added on day 0) and about 30-fold higher than in 10-H-L where only H was added on day 0. Available N on day 12 in the 5-treatments was highest in the treatment where only L had been added on day 0 (5-(L-H)x2) where it was about 20-fold higher than when only H had been added on day 0 (5-(H-L)x2) and two-fold higher than when both H and L had been added on day 0 (5-(HL)x4). Available N changed little from day 12 to day 24 in the 10-treatments (no residue addition since day 0). But in the 5-treatments, available N in 5-(H-L)x2 on day 24 was about eight-fold higher than on day 12 (L added on day 12), whereas it was 50% lower in 5-(L-H)x2 (H added on day 12). On day 24, available N was about 30% higher in 5-(H-L)x2 (L added on day 12) than in the other two treatments. Available N was similar in the 10-treatments on day 36 because it increased about 10-fold from day 24 to 36 in 10-(H-L) and decreased about 60% in 10-(L-H), where L or H added on day 24, respectively. Similarly, available N increased more than two-fold from day 24 to 36 in 5-(L-H)x2 and decreased by about 70% in 5-(H-L)x2. Available N in all 10-treatments was about two-fold higher on day 48 than day 36. In the 5-treatments compared to day 36, available N increased five-fold in 5-(H-L)x2 and decreased by about 20% in 5-(L-H)x2 where L or H had been added on day 36, respectively. Available N on day 48 differed little among treatments at a given residue rate, but it was 20-50% higher in the 10- than the 2.5-treatments.

Changes over time of available P were similar as those in available N (increasing when L had been added before sampling, decreasing when H was added), but differences were less pronounced than in available N (Figure 3B).

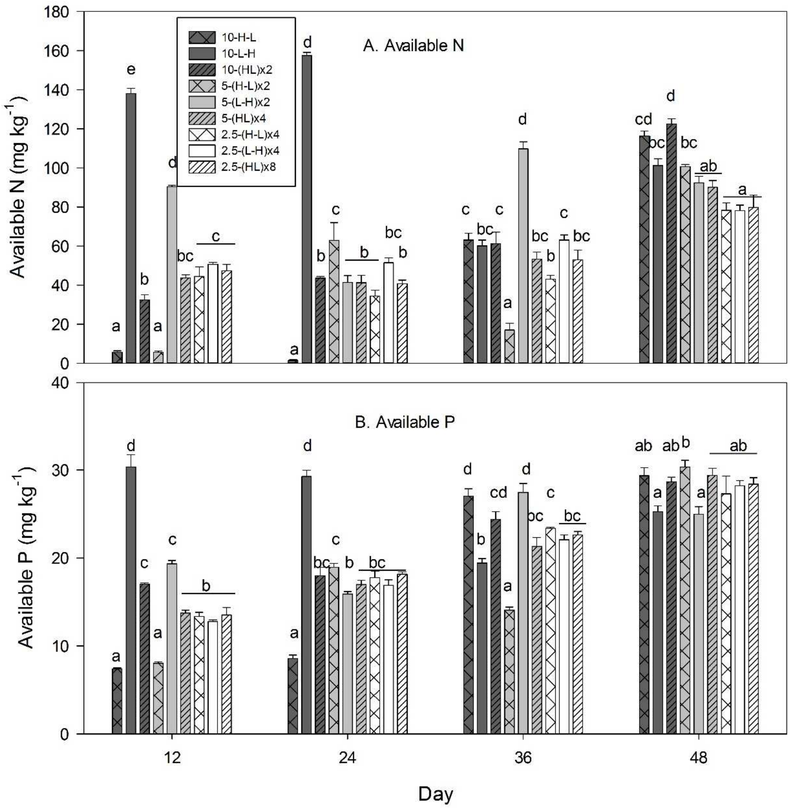

Figure 3. Available N (A) and P (B) on days 12, 24, 36 and 48 in soil amended with high (H) and low C/N (L) residues or their 1:1 mixture (HL) eight times at 2.5 g kg^{-1}, four times at 5 or twice at 10 g kg^{-1} (n=4, vertical lines indicate standard error). Different letters indicate significant differences ($P \leq 0.05$) among treatments at a given sampling day. For treatment names see Table 2.

3.4. Measured and expected values

The difference between measured and expected values differed among on parameters and sampling time (Table 3). In the following, only treatments and sampling times where expected and measured values differed significantly are described. In 10-(HL)x2, measured cumulative respiration was about 20% higher than expected on day 12, but 10% lower on day 48. Measured MBN was about 25% higher than expected on days 24 and 36. Measured MBP was about 25% higher than expected on day 48. For available N in 10-(HL)x2, measured values were about half of expected values on days 12 and 24, but 10% higher on day 48. In 5-(HL)x4, measured and expected values of cumulative respiration and MBN differed only on day 12 where measured values were 10% and about two-fold higher, respectively. On day 36, measured MBP and available N in 5(HL)x4 were about 20% lower than expected. In 2.5-(HL)x8, measured cumulative respiration was slightly lower than expected on day 12. For MBN, the measured value was about 25% higher than expected on day 24, but 25% lower on day 48. Measured MBP in 2.5-(HL)x8 on days 24 and 36 was about 20% lower than expected. Measured available N matched expected values. Measured and expected available P matched in all three HL treatments at all sampling dates.

Table 3. Measured and expected values of measured parameters on days 12, 24, 36 and 48 for treatments where H and L were mixed. At a given sampling date, asterisks indicate significant differences between measured and expected values.

Parameter	Day	Values	10-(HL)x2	5-(HL)x4	2.5-(HL)x8
	12	**Measured**	**2.14***	**1.22***	**0.98***
		Expected	1.86	1.15	1.03
	24	**Measured**	**0.61**	**1.33**	**1.57**
Cumulative respiration		Expected	0.60	1.46	1.57
(mg CO_2-C g^{-1})	36	**Measured**	**2.27**	**1.40**	**1.23**
		Expected	2.22	1.38	1.29
	48	**Measured**	**0.62***	**1.64**	**1.72**
		Expected	0.75	1.61	1.61
	12	**Measured**	**9.01**	**11.35***	**3.25**
		Expected	8.90	6.85	3.55
	24	**Measured**	**9.94***	**9.35**	**9.55***
MBN		Expected	8.13	8.27	7.48
(mg kg^{-1})	36	**Measured**	**11.68***	**9.30**	**9.94**
		Expected	8.65	7.95	9.62
	48	**Measured**	**9.52**	**11.70**	**10.20***
		Expected	9.04	8.65	13.67
	12	**Measured**	**20.01**	**13.84**	**12.76**
		Expected	17.57	14.76	13.23
	24	**Measured**	**17.27**	**18.11**	**16.71***
MBP		Expected	15.57	17.90	18.88
(mg kg^{-1})	36	**Measured**	**31.17**	**25.92***	**23.49***
		Expected	32.46	28.73	26.90
	48	**Measured**	**27.71***	**26.97**	**25.24**
		Expected	22.75	28.40	27.39
	12	**Measured**	**32.48***	**43.74**	**47.41**
		Expected	71.91	48.01	47.55
	24	**Measured**	**43.58***	**41.23**	**40.78**
Available N		Expected	79.52	52.15	42.97
(mg kg^{-1})	36	**Measured**	**61.14**	**53.44***	**53.08**
		Expected	61.62	63.42	53.08
	48	**Measured**	**122.45***	**90.17**	**79.74**
		Expected	108.77	96.51	78.19
	12	**Measured**	**17.00**	**13.74**	**13.53**
		Expected	18.87	13.67	13.07
	24	**Measured**	**17.98**	**16.96**	**18.15**
Available P		Expected	18.92	17.41	17.32
(mg kg^{-1})	36	**Measured**	**24.37**	**21.31**	**22.60**
		Expected	23.21	20.75	22.72
	48	**Measured**	**28.66**	**29.37**	**28.39**
		Expected	27.29	27.64	27.74

4. Discussion

This study showed that available N and MBN were influenced by residue C/N ratio, amendment rate and frequency whereas cumulative respiration in a given 12-day period was influenced only by amendment rate. Addition of H generally increased microbial N uptake which was, in some cases, accompanied by a decrease in available N. Amendment with L increased available N. But the magnitude of these changes depended on sampling time and differed among treatments.

In the 10-treatments, cumulative respiration was much higher in the first 12 days after amendment than from day 13 to 24 which is consistent with other studies (McTiernan *et al*., 1997; Marschner *et al*., 2015). This indicates that easily decomposable compounds were depleted in the first 12 days. In contrast, cumulative respiration differed little among 12-day periods in the 5 and 2.5 treatments. This is likely because residue addition every 12 or six days prevented or minimized depletion of easily decomposable compounds. The finding that cumulative respiration was not influenced by the C/N ratio suggests that N availability did not limit decomposition of high C/N residue in this experiment.

The difference between measured and expected cumulative respiration in the mixes depended on amendment rate. On day 12, where only either H or L had been added of H-L or L-H, measured was higher than expected which is. The higher than expected cumulative respiration on day 12 in the 10 or 5 treatments in agreement with previous studies, e.g., Mao and Zeng (2012), Cuchietti *et al*. (2014), likely due to the supply of N from L to microbes decomposing H. After day 12, expected and measured cumulative respiration generally matched, probably because H and L had been added in all treatments.

In the following discussion, we will focus on available N and MBN because available P and MBP differed little between treatments or sampling times.

The first hypothesis (at a given sampling time, available N and MBN will be less influenced by the C/N ratio of the residue added last before sampling with frequent addition of L and H at low rate four or eight times than less frequent amendment at high rate) can be confirmed. The second hypothesis (in 1:1 mixes of H and L, available N and MBN will initially increase with amendment rate, but differences between rates will become smaller over time) can be confirmed only for MBN on day 12 where it was higher in treatments where mixes were added eight times than where they were added twice. And the third hypothesis (in the mixes, differences between measured and expected values will be greater when residues are added at high rates twice than if they are added at lower rates more frequently) can be confirmed for the comparison of mix added eight times (2.5-(HL)x8) compared to added twice (10-(HL)x2), particularly for available N.

In the first 24 days of the 10 and 5 treatments, available N was very low after H addition which can be explained by N immobilization which is in agreement with many previous studies, e.g. Tian *et al.* (1992), Hadas *et al.* (2004). As expected from previous studies, available N was high after L addition suggesting high net N release from the low C/N residue Moritsuka *et al.* 2004. However, MBN was quite low indicating that microbial growth was limited by C availability, likely due to the very rapid decomposition of L in the first few days after amendment. MBN may have been higher a few days after residue addition (Hoyle and Murphy, 2011; Nguyen *et al*., 2016). But by day 12, a proportion of the microbial biomass had died. However, in the 10-treatments, after the second addition of residues on day 24, the C/N ratio of the added residue had no effect on available N and only temporarily influenced MBN. The lack of differences between treatments in day 36 is due to a strong increase in available N in 10-H-L compared to day 24 whereas available N decreased during this period in 10-L-H. It is likely that a proportion of the residue added on day 0 was still in the soil and was decomposed together with the residue added on day 24. The decrease in available N in 10-L-H can be explained by N immobilization, as microbes decomposed H added on day 24. N transfer from low to high C/N litter was shown in previous studies (e.g. Schwendener *et al*. (2005)). A strong decrease in available N when high C/N residue is added after low C/N residue is in agreement with our previous studies, e.g. Marschner *et al.* (2015), Zheng and Marschner (2017).

In the 5-treatments, the C/N ratio of the residue added 12 days before the sampling on day 36 had a similar effect on available N and MBN as when added on day 0 (net immobilization after H addition, net release after L addition). This suggests that at the lower amendment rate, little of the previously added residue remained in the soil by the time of the next amendment. In 5-treatments, differences between treatments in available N only disappeared on day 48, probably because more residues were present in the soil than earlier and H and L decomposed together as also indicated by the smaller differences among treatments in MBN compared to day 12 and day 24.

Until day 36 compared to expected values, measured available N in 10-(HL)x2 was lower whereas MBN was generally higher. This indicates that decomposition of H and N together stimulated net immobilization compared to treatments where H and L were added sequentially. In the 5-treatments, differences between expected and measured values in available N and MBN were transient, probably because in all treatments after day 12, both H and L had been added. In the 2.5 treatments, differences between available N and MBN after H and L addition were small because before each sampling date, both H and L had been added within the last 12 days. This also explains why measured and expected available N matched throughout the experiment and only transiently differed with respect to MBN. This is likely because soil in all 2.5-treatments contained H and L at different stages of decomposition which buffered the effect of freshly added residues. The presence of easily decomposable compounds in undecomposed or partially decomposed residues in the soil make it less likely that microbes will preferentially decompose freshly added residues. In the 5 and 10-treatments on the other hand, the freshly added residue was the main substrate source for microbes because the previously added residue left in the soil was largely decomposed. Therefore, with frequent residue addition (every 6 days) H and L are decomposed together, even when added sequentially. MBN on day 12 was lower in the 2.5-treatments than in the 5 and 10-treatments, probably because of the smaller amount of residue added on day 0. The build-up of MBN after day 12 to become similar as in the 5 and 10-treatments on days 24 and 36 can be explained by the frequent addition of residues that sustained microbial growth although only small amounts were added each time. The frequent addition can also explain the higher MBN than in the 10-treatments on day 48. Whereas available substrate was likely depleted in the latter, residues added six days before in the 2.5 treatments supplied sufficient available substrate for microbes.

5. Conclusion

This study showed that N availability and MBN with repeated addition of high and low C/N residues are influenced by amendment frequency and change over time. Amendment frequency also influenced interactions in mixes regarding N availability and MBN. Frequent addition of small amounts of residues limited microbial N uptake initially compared to higher amendment rates, but resulted in similar available N irrespective of the C/N ratio of the residue added. Less frequent addition of large amounts of residues on the other hand resulted in large fluctuations in available N and MBN depending on the C/N ratio of the residue added previously. In the present study, the source of C, N and P in respired CO_2, microbial biomass and available nutrients could not be determined. In future studies with repeated residue additions, one of the addition could be in the form of ^{13}C, ^{15}N or ^{32}P-labelled residues.

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CHAPTER 4

RESPIRATION, AVAILABLE N AND MICROBIAL BIOMASS N IN SOIL AMENDED WITH MIXES OF ORGANIC MATERIALS DIFFERING IN C/N RATIO AND DECOMPOSITION STAGE

Statement of Authorship

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Name of Principal Author (Candidate)	Thi Hoang Ha Truong		
Contribution to the Paper	Performed experiment and analyses of all samples, data analysis and interpretation and manuscript writing. I hereby certify that the statement of contribution is accurate.		
Overall percentage (%)	70%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	22/2/2020

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

vii. the candidate's stated contribution to the publication is accurate (as detailed above);

viii. permission is granted for the candidate in include the publication in the thesis; and

ix. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Petra Marschner		
Contribution to the Paper	Supervised development of the work, data interpretation and manuscript evaluation and correction. I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.		
Signature		Date	22/02/2020

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Respiration, available N and microbial biomass N in soil amended with mixes of organic materials differing in C/N ratio and decomposition stage

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ABSTRACT

The effect of C/N ratio on decomposition after amendment with individual litters and their mixes has been studied extensively. But less is known about the effect of highly decomposed organic materials such as manures on interactions in mixes. In this study, a sandy clay loam was amended with finely ground young faba bean shoots (FB, C/N 9), sheep manure (SM, C/N 6) and mature wheat straw (W, C/N 82) either individually (referred to as 100FB, 100SM, 100SM) or as mixes (e.g. 75FB-25SM, 50FB-50SM, 25FB-75SM, where the value represents the weight percentage of the organic materials). Soil was sampled on days 16, 32 and 48. Cumulative respiration after 48 days was similar with 100FB and 100 W, where it was about seven-fold higher than in 100SM. It decreased with percentage SM in the mixes and was about two-fold higher in 25SM-75 W than in 75SM-25 W. Available N was low with 100 W and microbial biomass N (MBN) was low with 100SM (five-fold lower than with FB). In mixes of W with FB or SM, available N was between two and 40-fold lower than expected with greater differences between measured and expected values FB-W than with SM-W. In FB-W and SM-W mixes, MBN was between 50% and two-fold higher than expected. In mixes of FB and SM, MBN on day 16 was 50% higher than expected in 75FB-25SM, but 30–50% lower than expected in mixes with ≥50% SM. We conclude that mixing of W with FB can provide plants with N, but also reduce N loss via leaching or denitrification.

1. Introduction

It is well-known that composition of organic amendments determines decomposition rate and nutrient availability (Bending and Turner, 1999; Tian et al., 1992). For example, N availability after amendment depends on C/N ratio with low C/N organic materials (C/N < 20) resulting in net N mineralisation (Hadas et al., 2004) whereas high C/N amendments inducing net N immobilisation (Moritsuka et al., 2004). Decomposition of mixes of organic materials has been studied extensively (Cobo et al., 2008; Gartner and Cardon, 2004; Xiang and Bauhus, 2007). In such mixes, expected nutrient availability can be calculated based on nutrient availability with each organic material individually and the ratio of each organic material in the mixes. In an additive response, measured and expected values such as mass loss and nutrients in the mix are similar, whereas in a non-additive response, measured decomposition in the mix is either greater or lower than expected (Gartner and Cardon, 2004). Similar measured and expected values could be due to spatial separation of the decomposer communities of organic materials in the mix (Shi et al., 2013). Greater than expected decomposition has been attributed to transfer of nutrients from rapidly decomposing organic materials to recalcitrant materials (Blair et al., 1990; Seastedt, 1984), changed decomposer community composition (Blair et al., 1990), niche complementarity or specialisation within the decomposer community (Chapman and Koch, 2007; Hector et al., 1999) and priming effects in which decomposition of low-nutrient materials is stimulated by high-nutrient materials (Chapman et al., 1988; Chapman and Koch, 2007; Wardle et al., 1997). Lower than expected decomposition in a mix can be due to diffusion of inhibitory compounds such as phenolics and tannins from slow decomposing materials to fast decomposing litter (Dix, 1979; Hättenschwiler and Vitousek, 2000).

A number of studies investigated mass loss or N content in mixes of leaf litters differing in decomposability. In most of these studies, the N concentration of the fast decomposing litter was higher than slow decomposing litter. For example, measured decomposition of 1:1 mixtures of fast (soybean) and slow decomposing (poplar) leaves was greater than expected (Mao and Zeng, 2012). In a study with leaf litter from a number of species, fast decomposing litters had a C/N ratio of about 20, slow decomposing litters a C/N ratio of 50–80 (Cuchietti et al., 2014). In that study, intact litter was added individually or mixed into litter

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bags which were placed in soil for two or nine months. At harvest, they were able to separate and individually weigh the different litter types in mixes. Cuchietti et al. (2014) found that in 1:1 mixes of fast and slowly decomposing litter, measured decomposition was usually greater than expected due to enhanced decomposition of the fast decomposing litter. Harguindeguy et al. (2008) buried litter bags with mixtures of two to five litter types and placed them in soil for 80 days. They reported that in mixtures of fast (high N) and slow (low N) decomposing litter, measured decomposition was greater than expected irrespective of the proportion of the fast decomposing litter in the mix. De Marco et al. (2011) mixed leaf litters with C/N about 100 which differed in decomposition rate due to differences in lignin content and lignin/N ratio (high in Quercus leaves, low in Cistus leaves). They reported lower than expected decomposition in the initial stages (days 0–90) of decomposition, but greater than expected decomposition later (day 90–400). De Marco et al. (2011) suggested that initially, inhibitory compounds from Quercus leaves reduced decomposition, but later, decomposition of lignin in Quercus leaves was enhanced as a result of greater microbial activity induced by the fast decomposing litter.

Previous studies on mixing organic materials have focused on CO_2 release, mass loss and nutrient contents of mixes using fresh or dried litter, usually with only one or two sampling times. There is limited understanding about the influence of mixing plant residues and manures, which may occur in agricultural ecosystems where manures are added after harvest of a crop or termination of a cover crop. Manures can have low C/N ratio, but are strongly decomposed during passage through the digestive system of animals and may be further decomposed during storage (Hungate, 1966). Therefore, they are at a late stage of decomposition and thus may decompose more slowly when added to soil and release less N than plant residues with similar C/N ratio. Further, little is known about changes in nutrient availability and microbial biomass during decomposition of mixes (Blair et al., 1990; Schwendener et al., 2005). The aim of this study was to determine the effect of low C/N organic amendments differing in decomposability and high C/N residue added to soil separately or as mixes on soil cumulative respiration, microbial biomass N and P and available N and P. Young faba bean shoot, sheep manure and mature wheat straw were added into soil singly or mixed. The selection of these organic materials was based on a preliminary experiment which showed that although young faba bean shoot and sheep manure had a similar low C/N ratio, sheep manure decomposed much more slowly than faba bean. Mature wheat straw was chosen as high C/N amendment.

The first hypothesis was that in mixes of high C/N wheat straw with low C/N faba bean or sheep manure, N availability and microbial biomass N will decrease with proportion of wheat straw. Based on the differences in decomposability of faba bean and sheep manure, the second hypothesis was that in mixes with mature wheat straw, the effect of faba bean on N availability and microbial biomass N is greater than with sheep manure. The third hypothesis was that in mixes of faba bean and sheep manure, N availability and microbial biomass N increase with proportion of faba bean in the mix. The fourth hypothesis was that interactions in mixes depend on ratio of organic materials and that they change over time.

2. Materials and methods

2.1. Soil and organic materials

The sandy clay loam used in this study was collected from 0 to 10 cm at Waite Campus, The University of Adelaide (Longitude 138°38′3.2″ E, Latitude 34°58′0.2″ S). The area is in a semi-arid region and has a Mediterranean climate with cool, wet winters, and hot and dry summers. The soil is a Red-brown Earth in Australian soil classification and a Rhodoxeralf according to US Soil Taxonomy. The soil has been managed as permanent pasture for over 80 years and has the following properties (for methods see below): sand 54%, silt 20% and clay 25%, pH (1:5 soil:water) 6.3, electrical conductivity (EC 1:5 soil:water) 143 $\mu S\ cm^{-1}$, total N 1.5 $g\ kg^{-1}$ and total P 371 $mg\ kg^{-1}$, total organic carbon (TOC) 17 $g\ kg^{-1}$, available N 15 $mg\ kg^{-1}$, available P 10 $mg\ kg^{-1}$, maximum water holding capacity (WHC) 378 $g\ kg^{-1}$ and bulk density 1.3 $g\ cm^{-3}$. The soil was collected from several randomly selected sites on the plot. In each sampling site, plants and surface litter were removed and five samples (0–10 cm) were collected. The soil was then air dried at 40 °C in a fan-forced oven. The drying is not unusual for soils in this area because during summer, top soil often reaches temperatures of 40–50 °C on hot sunny days. After air-drying, visible plant debris was removed and the soil sieved to < 2 mm. Soil from all sampling sites was pooled and thoroughly mixed before taking soil for the experiment.

Three types of organic materials were used: young faba bean (*Vicia faba* L., referred to as FB) as fast decomposing low C/N material, sheep manure (referred to as SM) as slow decomposing low C/N material and mature wheat straw (*Triticum aestivum* L., referred to as W) as high C/N material. The plant residues were chosen because they are common crops in southern Australia and often follow each other in crop rotations. Sheep manure was used because a preliminary experiment showed that sheep manure was slowly decomposing as compared to other low C/N organic materials such as young faba bean, composts and poultry manure. The organic materials were dried at 40 °C in a fan-forced oven, finely ground and sieved to 0.25–2 mm particle size. The organic materials were finely ground to maximise contact of organic materials in mixes and with the soil. In the field, applied organic materials are usually much coarser. Larger particles will decompose more slowly than fine particles because of physical protection and smaller surface area to volume ratio (Ambus and Jensen, 1997; Angers and Recous, 1997). Therefore mineralisation rates and nutrient transfer between organic materials in this study are likely to be higher than in the field.

2.2. Experimental design

Before the start of the experiment, the air-dried soil was incubated for 10 days at 50% of maximum WHC at 20–25 °C in the dark to activate the soil microbes and stabilise soil respiration after rewetting of air-dry soil. This water content was chosen because in previous studies with this soil, microbial activity was maximal at 50% (Marschner et al., 2015). Soil was amended with organic materials on day 0 at the rate of 20 $g\ kg^{-1}$ soil. This rate was high compared to average amounts used in the field, but it could occur in some situations, e.g. in windrows left by harvesting machines, uneven spreading of manure or in nurseries. There were twelve treatments in which organic materials were added to the soil singly or in mixes (Table 1). In treatments with single

Table 1
Experimental design with treatment names and corresponding details about organic amendments: young faba bean shoot (FB), sheep manure (SM) and mature wheat straw (W) and their mixing proportions.

Treatment name	Faba bean	Sheep manure	Wheat
	% in mix		
100FB	100		
100SM		100	
100 W			100
75FB-2.5SM	75	25	
50FB-50SM	50	50	
25FB-75SM	25	75	
75FB-25 W	75		25
50FB-50 W	50		50
25FB-75 W	25		75
75SM-25 W		75	25
50SM-50 W		50	50
25SM-75 W		25	75

amendment, 100% of FB, SM or W was added to the soil, giving treatments: 100FB, 100SM and 100 W. In treatments with mixes, two types of organic materials were mixed at different proportions prior to addition to soil, giving 75FB-25SM, 50FB-50SM and 25FB-75SM for FB-SM mixes, 75FB-25 W, 50FB-5 W and 25FB-75 W for FB-W mixes and 75SM-25 W, 50SM-50 W and 25SM-75 W for SM-W mixes. Unamended control was not included because the aim of this experiment was to compare amendments, not the effect of amendments compared to unamended soil.

Organic materials were thoroughly mixed in 30 g soil (dry weight equivalent) in a small plastic bag. Then the amended soil was filled into PVC cores with 3.7 cm diameter, 5 cm height and a nylon net base (7.5 μm, Australian Filter Specialist) and packed to a bulk density of 1.3 g cm^{-3} by adjusting the height of the soil in the cores. The cores were placed individually into 1 L jars with gas-tight lids equipped with septa to allow quantification of headspace CO_2 concentration as described below. The jars were incubated in the dark at 17–21 °C. Soil moisture was maintained at 50% of WHC by checking the water content every few days by weighing cores and adding reverse osmosis (RO) water if necessary. Soil respiration was measured daily from day 1 to 8, then every two days from day 9 to 20 and every four days from day 21 to 48. The longer intervals after day 9 were due to declining respiration rates over time and the lower detection limit of the infrared gas analyser. Cores were destructively sampled on days 16, 32 and 48 for analysis of available N and P, microbial biomass N and P. In a given 16-day period, only the cores to be sampled at the end of the period were placed in the jars. The remaining cores were incubated under the same conditions in large plastic trays covered with aluminium foil. After removal of the cores from the jars for analysis, the cores to be harvested at the next sampling time were placed in the glass jars for respiration measurement.

2.3. Analyses and calculations

Soil analyses were carried out as described in Marschner et al. (2015). Briefly, soil texture was determined by the hydrometer method (Gee and Or, 2002). Soil pH was determined in a 1:5 soil:water extract after 1 h end-over-end shaking at 25 °C (Rayment and Higginson, 1992). Soil water holding capacity was determined using a sintered glass funnel connected to a 1 m water column (matric potential = −10 kPa) (Wilke, 2005). Total organic carbon content of soil and organic materials was determined by wet oxidation and titration (Walkley and Black, 1934). To determine total N and P in soil and organic materials, the material was digested with H_2SO_4 and a mixture of HNO_3 and $HClO_4$, respectively. Total N was measured by a modified Kjeldahl method (Bremner and Mulvaney, 1982). Total P in the digest was measured by the phosphovanado-molybdate method according to Hanson (1950). Available N (ammonium and nitrate) concentration was measured after 1 h end-over-end shaking with 2 M KCl at 1:10 soil extractant ratio. Ammonium-N was determined after Forster (1995). Nitrate-N was determined using a modification of Miranda et al. (2001). Available P was extracted by the anion exchange resin method (Kouno et al., 1995) and the P concentration was determined colorimetrically (Murphy and Riley, 1962).

Microbial biomass N (MBN) was determined by chloroform fumigation-extraction with 0.5 M K_2SO_4 at 1:4 soil to extractant ratio (Moore et al., 2000). Ammonium concentration in the extract was determined as described above for available N. Microbial biomass N was calculated as the difference in NH_4^+ concentration between fumigated and non-fumigated samples divided by 0.57 which is the proportionality factor to convert ammonium to MBN (Moore et al., 2000). Microbial biomass P (MBP) was determined with the anion exchange method (Kouno et al., 1995) using hexanol as fumigant. Microbial biomass P is the difference in P concentration between fumigated and un-fumigated soil (Kouno et al., 1995). No correction factor was used for P because recovery of a P spike in this soil was 98% (Butterly et al., 2010).

Soil respiration was measured by quantifying the CO_2 concentration in the headspace of the jars using a Servomex 1450 infra-red analyser (Servomex Group, Crowborough, UK) (Setia et al., 2011). After each measurement (T0), jars were vented using a fan to refresh the headspace and then resealed followed by another CO_2 measurement (T1). CO_2 produced during this given interval is the difference in CO_2 concentration between T1 and T0 (Setia et al., 2011). Linear regression based on injection of known amounts of CO_2 into empty jars of the same size was used to define the relationship between CO_2 concentration and detector reading.

2.4. Statistical analysis

There were four replicates per treatment and sampling time. Data were analysed by one-way repeated measures ANOVA was carried out in Genstat 15th edition (VSN Int. Ltd., UK) with time as repeated measure. The treatment x time interaction was significant. Then, one-way ANOVA was then carried out for each sampling date separately using Genstat 15th edition (VSN Int. Ltd., UK). Tukey's multiple comparison tests at 95% confidence interval was used to determine significant differences among treatments. One-way ANOVA was also used to compare properties of three organic materials.

3. Results

3.1. Residue properties

In FB and W compared to SM, total organic C was more than four-fold higher, whereas water extractable organic C was about 7 to 11-fold higher (Table 2). Total N was highest in FB, about eight-fold higher than in W and three-fold higher than in SM. Total P was highest in SM, only about 20% higher than in FB but five-fold higher than in W. Water-extractable N and P were highest in FB and lowest in W. C/N and C/P ratios were lower in FB and SM compared to W. FB and SM had similar C/N ratio, but the C/P ratio was about five-fold higher in FB than in SM.

3.2. Cumulative respiration

Cumulative respiration was higher in the first 16 days than the two following 16-day periods and lowest from day 33 to 48 (Fig. 1). Differences in cumulative respiration among treatments were greatest in the first 16 days. Total cumulative respiration was lowest in 100SM. It was similar in 100FB and 100 W where it was about six-fold higher than in 100SM. The organic materials were added at 20 g kg^{-1}, but the organic C content of SM was about four-fold lower that in FB and W. Therefore less organic C was added with 100SM (1.7 g C kg^{-1} soil compared to about 7 g C kg^{-1} soil with 100FB and 100 W). Total cumulative respiration per g C added was about twice as high in 100FB and 100 W than in 100SM (about 1 g CO_2-C g^{-1} C added compared to 0.5 g CO_2-C g^{-1} C in 100SM). In mixes with SM (FB-SM and W-SM),

Table 2
Total organic C, N, P, C/N ratio and C/P ratio, water extractable C, N and P and pH of young faba bean shoot (FB), sheep manure (SM) and mature wheat straw (W) (n = 4). Different letters indicate significant differences between organic materials (P < .05).

Property	FB	SM	W
Total organic C (g kg^{-1})	346.8[b]	83.3[a]	376.3[c]
Total N (g kg^{-1})	38.5[c]	14.4[b]	4.6[a]
Total P (g kg^{-1})	9.2[b]	10.8[c]	2.1[a]
C/N ratio	9[a]	6[a]	82[b]
C/P ratio	38[b]	8[a]	176[c]
Water extractable C (g kg^{-1})	37.8[c]	3.3[a]	23.9[b]
Water extractable N (g kg^{-1})	2.5[c]	1.4[b]	0.2[a]
Water extractable P (g kg^{-1})	3.5[c]	0.9[b]	0.2[a]
pH (1:10)	6.0[b]	6.7[c]	5.6[a]

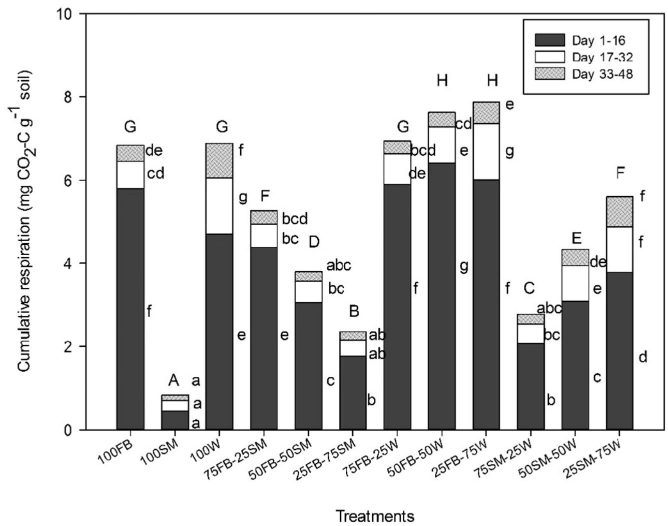

Fig. 1. Cumulative respiration in 16-day intervals (days 0–16, 17–32, 33–48) in soil amended with young faba bean shoot (FB), sheep manure (SM) or mature wheat straw (W) either as individual organic materials (100FB, 100SM, 100 W) or as mixes (FB-W, FB-W, SM-W) with different proportions of the two amendments (n = 4). For treatment details see Table 1. Different lower case letters indicate significant differences in cumulative respiration among treatments for a given interval. Different upper case letters indicate significant differences in total cumulative respiration.

total cumulative respiration decreased with proportion of SM. For example, it was about two-fold higher in 75 W-25SM than in 25 W-75SM. There was little difference in total cumulative respiration among FB-W mixes.

3.3. Microbial biomass N and P

MBN was highest on day 16 and lowest on day 48 (Fig. 2a). On day 16 among treatments with individual organic materials, MBN in 100FB was more than seven-fold higher than in 100SM and about two-fold higher than in 100 W. In the FB-SM mixes, MBN was more than five-fold higher in 75FB-25SM than the treatments with lower proportion of FB. Among FB-W mixes, MBN was about 30% higher in 50FB-50 W than the other two treatments. MBN was similar in FB-W mixes and 100FB. There was no significant difference in MBN among SM-W mixes. On day 32, MBN in 100FB was about four-fold lower than on day 16. In mixes with FB, MBN decreased only by about 30% from day 16 to day 32 but changed little in treatments with SM. MBN was three-fold higher in 100 W than 100SM, but did not differ between 100FB and 100 W. MBN differed little among FB-W mixes. But in SM-W, MBN decreased with increasing proportion of SM. Compared to 100FB, MBN on day 32 was up to two-fold higher in 75FB-25SM and FB-W mixes. On day 48, treatment differences were generally similar as on day 32, but less pronounced. Compared to 100FB, MBN was up to two-fold higher in FB-W mixes.

MBP changed little over time and differed less among treatments than MBN (Fig. 2b). On day 16, MBP was about 30% higher in 100FB than 100SM and 100 W. MBP was about 30% higher in 75FB-25SM than the other two FB-SM mixes, but MBP did not differ among FB-W mixes. In SM-W mixes, MBP was highest in 50SM-50 W. MBP on day 32 differed little among mixes. In treatments with individual organic materials, MBP in 100FB was three-fold higher than in 100SM and 40% higher than 100 W. On day 48, MBP in 100FB was three-fold higher than in 100SM and 30% higher than 100 W. In FB-SM mixes, MBP was 50% lower in 25FB-75SM than in 75FB-25SM, but MBP did not differ among FB-W mixes. In SM-W mixes, MBP was about 30% lower in 75SM-25 W than 50SM-50 W.

3.4. Available N and P

In 100FB, 75FB-25SM and 50FB-50SM, available N was about two-fold higher on days 32 and 48 than on day 16, but it changed little over time in the other treatments (Fig. 3a). On all sampling dates, available N was very low in 100 W. On day 16, available N was about 40% higher in 100FB than 100SM and much higher in both treatments than in 100 W. Available N differed little among FB-SM mixes, but was more than four-fold higher in 75FB-25 W than the other two FB-W mixes. Available N was low in SM-W mixes, but about ten-fold higher in 75SM-25 W than the other two SM-W mixes. On days 32 and 48, available N was about two to three-fold higher in 100FB than 100SM. In FB-SM and FB-W mixes, available N decreased with proportion of FB in mixes, with greater differences among FB-W than FB-SM treatments. Available N was higher in 75SM-25 W than the other two SM-W mixes.

Available P changed little over time (Fig. 3b). It was about five to six-fold higher in 100FB and 100SM than 100 W. In FB-SM mixes, available P was similar to that in 100FB and 100SM and differed little among mixes. In FB-W mixes, available P was about two-fold higher in 75FB-25 W than 25FB-75 W. Available P was two to three-fold higher in 75SM-25 W than 25SM-75 W.

3.5. Measured compared to expected values

In mixes with wheat (FB-W and SM-W), cumulative respiration in the first 16 days was higher than expected, except in 25SM-75 W (Table 3). But from day 33 to 48, cumulative respiration was lower than expected. Measured and expected cumulative respiration matched in FB-SM mixes.

In FB-SM mixes, MBN on day 16 was higher than expected in 75FB-25SM, but lower than expected in the other two mixes. Later, MBN was greater than expected only in 75FB-25SM on day 32. In FB-W mixes, MBN was higher than expected on days 32 and 48 in 75FB-25 W and 25FB-75 W, and on days 16 and 48 in 50FB-50 W. MBN was higher than expected in 75SM-25 W on days 16 and 48, in 50SM-50 W on days 16 and 32, and in 25SM-75 W only on day 48. The relative difference between measured and expected MBN was generally greater in FB-W (up to two-fold) than SM-W mixes (measured MBN was up to about 60% higher than expected).

In FB-SM mixes, differences between measured and expected MBP values were generally small and inconsistent. MBP was greater than expected in FB-W mixes on day 16. In 75SM-25 W, measured MBP was lower than expected on day 16, but higher than expected on day 32. MBP was higher than expected in 50SM-50 W on day 48 and in 25SM-75 W on days 32 and 48.

In 25FB-75SM, measured available N was higher than expected on day 16, but lower than expected on day 32. Measured and expected values matched in the other FB-SM mixes. In FB-W and SM-W mixes, measured available N was always lower than expected values with differences increasing with proportion of W in the mixes. In SM-W mixes for example, measured available N was 20%, 80%, 28-fold lower than expected in 75FB-25 W, 50FB-50 W and 25FB-50 W, respectively. Available P was lower than expected in all FB-W mixes on day 16. But later, only in 75FB-25 W on day 32 and in the other two mixes on day 48. In SM-W mixes, available was lower than expected only in 25SM-75 W on day 48. Measured MBP matched expected values in the FB-SM mixes.

4. Discussion

Based on this study, we can confirm the first hypothesis (mixes of high C/N wheat straw with low C/N faba bean or sheep manure, N availability and microbial biomass N will decrease with proportion of wheat straw) only for available N. We can confirm the second (in mixes with mature wheat straw, the effect of faba bean on N availability and microbial biomass N is greater than with sheep manure) and fourth

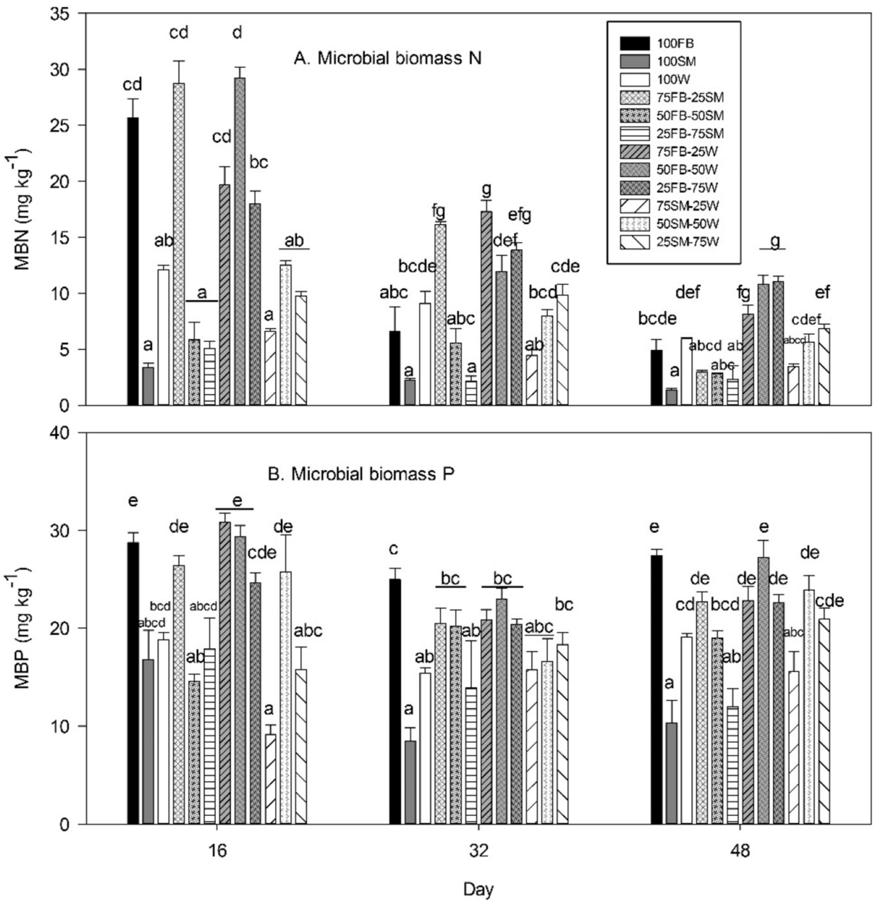

Fig. 2. Microbial biomass N (a) and P (b) on days 16, 32 and 48 in soil amended with young faba bean shoot (FB), sheep manure (SM) or mature wheat straw (W) either as individual organic materials (100FB, 100SM, 100 W) or as mixes (FB-W, FB-W, SM-W) with different proportions of the two amendments (n = 4). For treatment details see Table 1. At a given sampling date, different letters indicate significant differences among treatments.

hypotheses (interactions in mixes depend on ratio of organic materials and that they change over time). The third hypothesis (in mixes of faba bean and sheep manure, N availability and microbial biomass N increase with proportion of faba bean in the mix) can only be confirmed for days 16 and 32.

The following discussion focusses on respiration, available N and MBN because available P and MBP differed little between treatments and over time.

4.1. Single organic materials

The effects of addition of low C/N faba bean and high C/N wheat on N and MBN are consistent with previous studies (Bending and Turner, 1999; Tian et al., 1992). With FB, about 780 mg N were added per g soil, whereas with W it was only 93 mg N g^{-1} soil which can explain the large difference in available N between FB and W. However, MBN on day 16 was only two-fold higher with FB than W, indicating strong N immobilisation with W. With SM, about 290 mg N were added per g soil and correspondingly, available N was about 50% lower with SM than with FB. However, MBN on day 16 was > 10-fold lower with SM than FB indicating very limited N immobilisation with SM.

Soil amended with FB had the highest available N, but cumulative respiration was higher than W only in the first 16 days whereas total cumulative respiration was similar as with W. MBN was highest only on day 16, but later it was similar as with W. This indicates that FB was easily decomposable resulting in high initial respiration rates accompanied with high N release and uptake into the microbial biomass. However, after day 16, the easily decomposable compounds were depleted and a large proportion of microbial N was released resulting in an increase in available N compared to day 16. Available N after day 16 remained high due to low N uptake by microbes which were C limited.

With W, cumulative respiration in the first 16 days was nearly as high as with FB and total cumulative respiration was similar as with FB. MBN was lower than with FB only on day 16, but available N was close to zero throughout the experiment. This N depletion (1–2 mg kg^{-1} from the initial 15 mg kg^{-1}) is consistent with a previous study where mature wheat straw was added to the same soil (Marschner et al., 2015). Thus, microbes depleted available N and were likely N starved towards the end of the experiment. This strong N limitation may have reduced the ability of microbes to decompose W and to grow. The growth limitation is evident in the finding that supply of N by mixing W with FB strongly increased MBN and available N. This is in agreement with previous studies where inorganic N addition to mature cereal straw increased available N and microbial N uptake (e.g., Hadas et al., 2004). Nevertheless, the higher cumulative respiration from day 16 to day 48 indicates that W provided a longer lasting C source for microbes than FB. Turnover of microbial biomass apparently released sufficient N to maintain MBN on days 32 and 48 at concentrations similar to FB. Based on cumulative respiration, about 1 g C was respired per g C added with FB and W. This does not necessarily mean that all added C was respired. A proportion of CO_2-C may also come from soil organic matter or turnover of the microbial biomass.

Soil amended with SM had the lowest cumulative respiration and MBN among single organic materials. This together with little change in MBN and available N over time suggests slow mineralisation of SM which can be explained by its highly decomposed nature. Any feed will have been decomposed in the gut of sheep and possibly during storage of the manure (Hungate, 1966). Some N was mineralised after addition of SM to soil as available N was higher than with W, but microbial uptake was likely limited by C availability.

The amendment rate of the organic materials was relatively high. Therefore it is possible that initially, the size of the microbial biomass

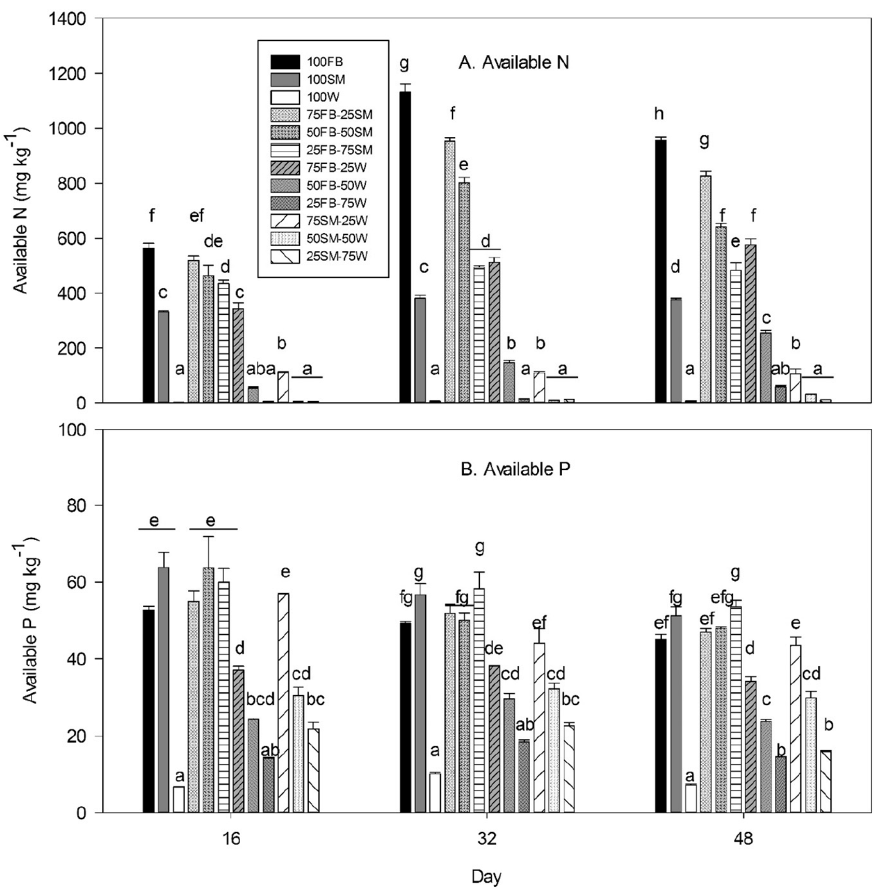

Fig. 3. Available N (a) and P (b) on days 16, 32 and 48 in soil amended with young faba bean shoot (FB), sheep manure (W) or mature wheat straw (W) either as individual organic materials (100FB, 100 W, 100 W) or as mixes (FB-W, FB-W, W-W) with different proportions of the two amendments (n = 4). For treatment details see Table 1. At a given sampling date, different letters indicate significant differences among treatments.

Table 3
Measured (bold) and expected values of cumulative respiration, MBN, MBP and available N and P on days 16, 32 and 48 for treatments with organic materials mixes. At a given sampling date, asterisks indicate significant differences between measured and expected values.

Parameter	Day	Values	75FB-25SM	50FB-50SM	25FB-75SM	75FB-25 W	50FB-50 W	25FB-75 W	75SM-25 W	50SM-50 W	25SM-75 W
Cumulative respiration ($mg\ CO_2$-$C\ g^{-1}$ soil)	16	**Measured**	**4.4**	**3.1**	**1.8**	**5.9***	**6.4***	**6.0***	**2.1***	**3.1***	**3.8**
		Expected	4.5	3.1	1.8	5.5	5.3	5.0	1.5	2.6	3.6
	32	**Measured**	**0.6**	**0.5**	**0.4**	**0.7***	**0.9**	**1.3***	**0.5**	**0.9**	**1.1**
		Expected	0.6	0.5	0.4	0.8	1.0	1.2	0.5	0.8	1.1
	48	**Measured**	**0.3**	**0.2**	**0.2**	**0.3***	**0.3***	**0.5***	**0.2***	**0.4***	**0.6***
		Expected	0.3	0.3	0.2	0.5	0.6	0.7	0.3	0.5	0.7
MBN ($mg\ kg^{-1}$)	16	**Measured**	**28.8***	**5.8***	**5.1***	**22.3**	**29.2***	**18.0**	**6.6***	**12.5***	**9.8**
		Expected	20.1	14.5	8.9	19.7	18.9	15.5	5.5	7.7	9.9
	32	**Measured**	**16.1***	**5.6**	**2.2**	**17.3***	**11.9**	**13.9***	**4.5**	**8.0***	**9.8**
		Expected	5.5	4.4	3.3	7.2	7.8	8.5	4.0	5.7	7.4
	48	**Measured**	**2.9**	**2.8**	**2.3**	**8.1***	**10.8***	**11.1***	**3.5***	**5.6**	**6.9***
		Expected	4.0	3.1	2.3	5.2	5.4	5.7	2.5	3.7	4.8
MBP ($mg\ kg^{-1}$)	16	**Measured**	**26.4**	**14.6***	**17.9**	**30.8***	**29.4***	**24.7***	**9.2***	**25.7**	**15.8**
		Expected	25.8	22.8	19.8	26.3	23.8	21.3	17.3	17.8	18.3
	32	**Measured**	**20.5**	**20.2**	**14.0**	**20.9**	**23.0**	**20.4***	**15.7***	**16.6**	**18.3***
		Expected	20.9	16.7	12.6	22.6	20.2	17.8	10.2	12.0	13.7
	48	**Measured**	**22.7**	**19.0**	**12.0**	**22.8**	**27.2**	**22.6**	**15.6**	**23.9***	**20.9***
		Expected	23.2	18.9	14.6	25.3	23.3	21.2	12.5	14.7	16.9
Available N ($mg\ kg^{-1}$)	16	**Measured**	**518.6**	**464.0**	**435.3***	**343.4***	**53.8***	**5.0***	**111.4***	**4.2***	**3.4***
		Expected	505.8	447.6	389.3	423.4	282.7	142.1	248.6	166.2	83.8
	32	**Measured**	**953.5**	**801.2**	**493.7***	**512.0***	**147.2***	**14.6***	**110.3***	**8.7***	**12.2***
		Expected	944.0	756.3	568.7	849.9	568.1	286.4	286.9	192.8	98.7
	48	**Measured**	**826.6**	**641.0**	**482.6**	**575.8***	**254.6***	**58.3***	**104.8***	**30.4***	**10.4***
		Measured	812.3	667.0	521.6	719.8	482.0	244.1	283.7	191.2	98.8
Available P ($mg\ kg^{-1}$)	16	**Measured**	**55.0**	**63.7**	**60.0**	**37.1***	**24.2***	**14.3***	**56.9**	**30.5**	**21.7**
		Expected	55.5	58.3	61.1	41.2	29.7	18.2	49.5	35.2	21.0
	32	**Measured**	**51.8**	**50.1**	**58.2**	**38.1***	**29.6**	**18.4**	**44.0**	**32.2**	**22.6**
		Expected	51.1	52.9	54.8	39.4	29.7	19.9	45.0	33.4	21.7
	48	**Measured**	**46.9**	**48.0**	**53.6**	**34.2**	**23.7***	**14.5***	**43.6**	**29.9**	**15.9***
		Expected	46.6	48.1	49.7	35.6	26.2	16.7	40.2	29.2	18.2

limited decomposition rates. However, MBN increased rapidly after amendment. In a previous study with the same soil, MBN in unamended soil was about 5 mg kg^{-1} (Marschner et al., 2015) and increased about seven-fold (to 35 mg kg^{-1} as in the present study) seven days after addition of 10 g kg^{-1} young Kikuyu grass with a similar C/N ratio as the young faba bean residues used in this experiment. Similarly, De Nobili et al. (2006) showed that microbes grow within a few days after substrate addition to soil. The strong increase in MBN within a few days indicates that this limitation was short-lived. Strong microbial growth after FB addition could induce priming, that is increased decomposition of less decomposable organic materials such as W or SM.

4.2. FB-SM mixes

Measured cumulative respiration matched expected values indicating that presence of one organic material did not influence decomposition of the other. In 75FB-25SM, MBN was greater than expected on days 16 and 32. This indicates that in the early stages of decomposition, microbes were stimulated by easily decomposable compounds in FB and took up N released from FB. However, available N was not lower than expected, likely because available N was high. Thus, the small amount of additional N taken up by microbes did not reduce available N significantly. Another possible explanation is that that FB increased N release from SM. Priming effects have been suggested for mixes where decomposition of low-nutrient materials is stimulated by high-nutrient materials (Chapman et al., 1988; Chapman and Koch, 2007; Wardle et al., 1997). Whereas measured MBN was higher than expected, measured and expected cumulative respiration matched. This suggests that higher MBN was due to greater N uptake per microbial cell, not increased cell number. In 25FB-75SM, available N was greater than expected on day 16. This indicates that initially, microbes stimulated by a small amount of FB, released N from SM. However, available N was lower than expected on day 32. This may be due to the very high expected value due to the high available N with 100FB on that day which was about two-fold higher than on day 16. The large proportion of SM in the mix may have restricted N release from FB at this stage of decomposition, possibly due to inhibitory compounds released by SM (De Marco et al., 2011).

4.3. FB-W mixes

Cumulative respiration in the first 16 days was higher than expected which is in agreement with previous studies using mixes of high and low C/N litter (Cuchietti et al., 2014; Harguindeguy et al., 2008). This suggests that easily decomposable C and N in FB stimulated initial decomposition of W. However cumulative respiration from day 33 to 48 was lower than expected which is likely due to the initially higher respiration and thus more rapid depletion of easily decomposable C compared to FB or W alone. Available N was lower than expected, but MBN was higher, with greatest differences in mixes with ≥50% W. The higher than expected MBN indicates that N released by microbes decomposing FB was taken up by microbes decomposing W which alleviated their N limitation and allowed them to decompose a greater proportion of W. Differences between measured and expected available N were greatest on day 16 which suggests that W initially simulated N release from FB. On the other hand, differences between measured and expected MBN were greatest on day 48 for MBN. Therefore, when sufficient N was available from decomposing FB, W later increased microbial N uptake by providing a more consistent source of C than FB which was rapidly decomposed. Using ^{15}N labelled low C/N litter, Schwendener et al. (2005) showed N transfer from low to high C/N litter.

4.4. SM-W mixes

Presence of W in SM-W mixes stimulated respiration initially as indicated by the higher than expected cumulative respiration in the first 16 days. This is likely due to the supply of more easily decomposable C with W compared to SM. Similar as in FB-W mixes, MBN was higher than expected and available N was lower, probably due to the supply of easily decomposable C by W. However, the poor decomposability of SM resulted in lower respiration and microbial N uptake compared to mixes of W with similar proportion of FB.

5. Conclusion

Although both faba bean residues and sheep manure had low C/N ratio, the effect on respiration, available N and MBN was smaller with sheep manure than faba bean which can be explained by the highly decomposed nature of sheep manure. Mixing wheat with faba bean will provide plants with available nutrients while preventing the rapid release of N after addition of sole faba bean. In mixes with sheep manure, wheat can enhance decomposition, but nutrient release may be too slow to provide rapidly growing plants with sufficient N. Mixes of faba bean with 50% or more sheep manure could allow sustainable maintenance of high N availability compared to sheep manure alone while minimising the potential for N loss of faba bean alone. Longer term experiments with plants are required to better understand the effect of decomposability of low C/N organic materials on plant growth and nutrient uptake.

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CHAPTER 5

PLANT GROWTH AND NUTRIENT UPTAKE IN SOIL AMENDED WITH MIXES OF ORGANIC MATERIALS DIFFERING IN C/N RATIO AND DECOMPOSITION STAGE

Statement of Authorship

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Name of Principal Author (Candidate)	Thi Hoang Ha Truong		
Contribution to the Paper	Performed experiment and analyses of all samples, data analysis and interpretation and manuscript writing. I hereby certify that the statement of contribution is accurate.		
Overall percentage (%)	70%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	22/2/2020

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

x. the candidate's stated contribution to the publication is accurate (as detailed above);

xi. permission is granted for the candidate in include the publication in the thesis; and

xii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Petra Marschner		
Contribution to the Paper	Supervised development of the work, data interpretation and manuscript evaluation and correction. I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.		
Signature		Date	22/02/2020

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ORIGINAL PAPER

Plant Growth and Nutrient Uptake in Soil Amended with Mixes of Organic Materials Differing in C/N Ratio and Decomposition Stage

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Abstract

To determine the effect of low C/N organic amendments differing in decomposability and high C/N residue added to soil separately or as mixes with different ratios on plant growth and nutrient uptake, nutrient availability and microbial biomass over time. A sandy clay loam soil was amended with young faba bean shoot (C/N 9), sheep manure (C/N 6) and mature wheat straw (C/N 82) individually or as mixes of low and high C/N residues at different proportions. Wheat was planted on days 0, 35 and 70 and grown for 35 days. Soil and plants were sampled on days 35, 70 and 105. Shoot and root dry weights were low with high C/N residues alone but similar in the other treatments. Shoot N uptake was 0.3- to 2.5-fold higher than that of the control with low C/N residues alone and their mixes. Available N decreased with the proportion of low C/N residues. Increasing proportion of sheep manure in mixes with faba bean residues reduced shoot N uptake and available N. But in mixes with high C/N wheat straw, the proportion of sheep manure had little effect on shoot N uptake and available N. The effect of slowly decomposing sheep manure on the measured parameters depended on the other organic material in the mix. An increasing proportion of sheep manure increased shoot N uptake under N limiting conditions (mixed with wheat straw), but decreased it with faba bean residues that released large amounts of N.

Keywords C/N ratio · Manure · Microbial biomass N · Mixes · N availability · Shoot dry weight

1 Introduction

It is well-known that the decomposition of organic materials is influenced by their chemical composition (Bending and Turner 1999; Tian et al. 1992). The addition of organic materials with low C/N ratio (C/N < 20) induces net N mineralisation (Hadas et al. 2004), whereas amendment with high C/N organic materials results in net N immobilisation (Moritsuka et al. 2004). The decomposition of organic material mixtures has been studied extensively (Cuchietti et al. 2014; Gartner and Cardon 2004; Mao and Zeng 2012). In such mixes, expected nutrient availability can be calculated based on nutrient availability of organic materials added individually and the percentages of organic materials in mixes. Interactions between components in mixes can be either additive where measured and expected values match or non-additive where measured values are greater or lower than expected (Gartner and Cardon 2004). The spatial separation of microbial communities decomposing different residue types can explain matching expected and measured values (Shi et al. 2013). Greater than expected decomposition may be due to the transfer of nutrients from easily decomposable materials to recalcitrant materials (Blair et al. 1990; Seastedt 1984), changes in decomposer community composition (Blair et al. 1990), niche complementarity or specialisation among decomposer communities (Chapman and Koch 2007) and priming effect in which high-nutrient materials stimulate the decomposition of low-nutrient materials (Chapman et al. 1988; Chapman and Koch 2007; Wardle et al. 1997). Lower than expected values have been explained by diffusion of inhibitory compounds such as phenolics and tannins from slowly decomposing materials to rapidly decomposing materials (Hättenschwiler and Vitousek 2000).

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The influence of organic amendments on plant growth and yield has also been studied extensively (e.g., Bernard et al. 2014; Li et al. 2018) but less is known about the effects of mixes of organic materials differing in C/N and/or decomposability. The effects of mixes of low C/N organic materials with different decomposability were investigated by Zai et al. (2008). Green pea shoot residue (PR) (C/N 16) was used alone or mixed with either dry chicken manure (CM) (C/N 9) or rapeseed oil residue (RR) (C/N 6) at a 5:1 ratio of PR with either CM or RR. The materials were incorporated into the soil which was planted with wheat and then rice. With wheat, N and P availability at harvest were higher in PR + RR than those in PR alone or PR + CM. Wheat shoot N and P concentrations were higher in PR + CM than those in PR alone or PR + RR. The high nutrient uptake with PR + CM was explained by fast N and P release from CM compared with PR and RR, which mitigated slow nutrient release from PR in the PR + CM mix. But in rice, shoot N and P concentrations and most yield components of rice were higher in PR + RR than those in the other treatments, possibly because RR decomposed and released nutrients during rice season. Bunyasi (1997) examined the effects of mixing organic materials differing in the C/N ratio on maize biomass and N uptake. The soil was amended with *Croton macrostachyus* residue (C/N 11) and rice (*Oryza sativa* L.) husks (C/N 52) singly or as 1:1 mix. Maize dry weight 7 weeks after planting was higher with the mix than that with the single amendments and was higher than expected in the mix.

Previous studies focused on the effect of mixes with one mixing ratio on crop biomass at maturity. Less is known about the influence of mixes with a range of mixing ratios on the shoot and root dry weight, microbial and nutrient availability during decomposition. In this study, the soil was amended with young faba bean shoot (C/N 9), sheep manure (C/N 6) and mature wheat straw (C/N 82) individually or as 25:75, 50:50 and 75:25 mixes. Wheat was planted on days 0, 35 and 70 after amendment and grown for 35 days. The selection of these organic materials was based on a preliminary experiment which showed that although young faba bean shoot and sheep manure had a similar low C/N ratio, sheep manure decomposed much more slowly than faba bean. Mature wheat straw was chosen as a high C/N amendment. The aim of this study was to determine the effect of low C/N organic amendments differing in decomposability and high C/N residue added to soil separately or as mixes on plant growth and nutrient uptake, nutrient availability and microbial biomass over time.

The first hypothesis was that in mixes of high C/N wheat straw with low C/N faba bean shoots or sheep manure, shoot and root dry weight, shoot N uptake, N availability and microbial biomass N will decrease with an increasing proportion of wheat straw. The second hypothesis was that the effect of high C/N wheat straw on measured parameters is greater in mixes with slowly decomposing sheep manure than that with rapidly decomposing faba bean shoots. This hypothesis assumes that the larger N release from faba bean will overcompensate N immobilisation by microbes decomposing wheat straw, whereas N release from sheep manure is smaller. The third hypothesis was that the effect of residue mixes on measured parameters will change over time during decomposition with greater differences between mixes initially during rapid decomposition than later when decomposition rates are low.

2 Materials and Methods

2.1 Soil and Organic Materials

The sandy clay loam used in this study was collected from 0 to 10 cm at Waite Campus, The University of Adelaide, Australia (longitude 138° 38′ 3.2″ E, latitude 34° 58′ 0.2″ S). The area is in a semi-arid region and has a Mediterranean climate with cool, wet winters and hot and dry summers. The soil is a Chromosol in Australian soil classification and a Rhodoxeralf according to US Soil Taxonomy. The soil has been managed as permanent pasture for over 80 years. The soil was collected from several randomly selected sites on the plot. At each of these sites, plants and surface litter were removed and five samples were collected. The soil was then air-dried at 40 °C in a fan-forced oven. The drying is not unusual for soils in this area because during summer, topsoil often reaches temperatures of 40–50 °C on hot sunny days. After air-drying, visible plant debris was removed and the soil sieved to < 2 mm. Soil from all sampling sites was pooled and thoroughly mixed before taking soil for the experiment. The soil has the following properties (for methods see below): sand 54%, silt 20% and clay 25%, pH (1:5 soil to water) 6.3, electrical conductivity (EC 1:5 soil to water) 143 μS cm^{-1}, total N 1.5 g kg^{-1} and total P 371 mg kg^{-1}, total organic carbon (TOC) 17 g kg^{-1}, available N 15 mg kg^{-1}, available P 10 mg kg^{-1}, maximum water holding capacity (WHC) 378 g kg^{-1} and bulk density 1.3 g cm^{-3}.

Three types of organic materials were used: young faba bean (*Vicia faba* L., referred to as FB) as fast decomposing low C/N material, sheep manure (referred to as SM) as slowly decomposing low C/N material and mature wheat straw (*Triticum aestivum* L., referred to as W) as high C/N material (Table 1). The plant residues were chosen because they are common crops in Southern Australia and often follow each other in crop rotations. Sheep manure was used because a preliminary experiment showed that sheep manure was slowly decomposing as compared with other low C/N organic materials such as young faba bean, composts, and poultry manure. The organic materials were dried at 40 °C in a fan-forced oven, finely ground and sieved to 0.25–2 mm particle size.

Table 1 Total organic C, N, P, C/N ratio and C/P ratio, water-extractable N and P, water-extractable organic C (WEOC), and pH of young faba bean shoot (FB), sheep manure (SM) and mature wheat straw (W) ($n = 4$). Different letters indicate significant differences between organic materials ($P < 0.05$)

Property	FB	SM	W
Total organic C (g kg^{-1})	346.8^b	83.3^a	376.3^c
Total N (g kg^{-1})	38.5^c	14.4^b	4.6^a
Total P (g kg^{-1})	9.2^b	10.8^c	2.1^a
Water extractable N (g kg^{-1})	2.5^c	1.4^b	0.2^a
Water extractable P (g kg^{-1})	3.5^c	0.9^b	0.2^a
WEOC (g kg^{-1})	37.8^c	3.3^a	23.9^b
C/N ratio	9^a	6^a	82^b
C/P ratio	38^b	8^a	176^c
pH (1:10)	6.0^b	6.7^c	5.6^a

Table 2 Experimental design with treatment names and corresponding details about organic amendments: young faba bean shoot (FB), sheep manure (SM) and mature wheat straw (W) and their mixing proportions

Treatment name	Faba bean % in mix	Sheep manure	Wheat
Control			
100FB	100		
100SM		100	
100W			100
75FB-25SM	75	25	
50FB-50SM	50	50	
25FB-75SM	25	75	
75FB-25W	75		25
50FB-50W	50		50
25FB-75W	25		75
75SM-25W		75	25
50SM-50W		50	50
25SM-75W		25	75

2.2 Experimental Design

Before the start of the experiment, the air-dried soil was incubated for 10 days at 50% of maximum WHC at 20–25 °C in the dark to activate the soil microbes and stabilise soil respiration after rewetting of air-dry soil. This water content was chosen because in previous studies with this soil microbial activity was maximal at 50% WHC (Marschner et al. 2015). The soil was amended with organic materials on day 0 at the rate of 20 g kg^{-1} soil. This rate was high compared with average amounts used in the field, but it could occur in some situations, e.g. in windrows left by harvesting machines, uneven spreading of manure or in nurseries. There were 13 treatments including the control without amendment and 12 treatments in which organic materials were added to the soil singly or in mixes (Table 2). In treatments with single amendment, 100% of FB, SM or W was added to the soil, giving treatments 100FB, 100SM, and 100W. In treatments with mixes, two types of organic materials were mixed at different proportions prior to addition to soil. In the treatment names of the mixes, the value indicates the proportion of each organic material in the mix, giving 75FB-25SM, 50FB-50SM and 25FB-75SM for FB-SM mixes, 75FB-25W, 50FB-5W, and 25FB-75W for FB-W mixes and 75SM-25W, 50SM-50W and 25SM-75W for SM-W mixes. There were four replicates per treatment and sampling time.

The organic materials were thoroughly mixed in 350 g soil (dry weight equivalent) before filling the amended soil into 1-L pots lined with plastic bags. The pots were placed in a glasshouse with natural light in three blocks in a complete randomised block design. Six pre-germinated wheat seeds (*Triticum aestivum* L. variety Axe) were planted in the pots of the first, second and third periods on days 0, 35 and 70, respectively. In each period, wheat plants were harvested 35 days after planting. In a given 35-day period, only pots to be harvested at the end of the period were planted with seeds. The remaining pots were placed under the same conditions in the glasshouse. Soil water content was maintained in all pots at 50% WHC by weight with reverse osmosis water. During wheat growth, pots were watered daily. For pots without plants, moisture loss was smaller than that in planted pots. Soil moisture content could be maintained by adding water to weight every 2–3 days. The temperature in the glasshouse during the experiment ranged from 17 to 32 °C.

2.3 Analyses and Calculations

Soil analyses were carried out as described in Marschner et al. (2015) and Zhang and Marschner (2018). Briefly, soil texture was determined by the hydrometer method (Gee and Or 2002). Soil pH was determined in a 1:5 soil to water extract after 1 h end-over-end shaking at 25 °C (Rayment and Higginson 1992). Soil water holding capacity was determined using a sintered glass funnel connected to a 1-m water column (matric potential = − 10 kPa) (Wilke 2005). The total organic carbon content of soil and organic materials was determined by wet oxidation and titration (Walkley and Black 1934). To determine total N and P in soil, organic materials, and plants, the material was digested with H_2SO_4 (Vickery 1946) and a mixture of HNO_3 and $HClO_4$ (Olsen and Sommers 1982), respectively. Total N was measured by a modified Kjeldahl method (Bremner and Mulvaney 1982). Total P in the digest was measured by the phosphovanado-molybdate method according to Hanson (1950). Shoot N and P concentrations are expressed as mg total N and P per gram shoot dry weight per pot. Available N (ammonium and nitrate) concentration was measured after 1 h end-over-end with 2 M KCl at a 1:10 soil extractant ratio. Shoot N and P concentrations are expressed as mg total N and P per gramme shoot dry weight of a pot. Ammonium N was determined after Forster (1995). Nitrate N was determined using the modification of Miranda et al. (2001). Available P was extracted by the anion exchange resin method (Kouno et al. 1995), and the P concentration was determined colorimetrically (Murphy and Riley 1962).

Microbial biomass N (MBN) was determined by chloroform fumigation-extraction with 0.5 M K_2SO_4 at 1:4 soil to extractant ratio (Moore et al. 2000). Ammonium concentration in the extract was determined as described above for available N. Microbial biomass N was calculated as the difference in NH_4^+ concentration between fumigated and non-fumigated samples divided by 0.57 which is the proportionality factor to convert ammonium to MBN (Moore et al. 2000). Microbial biomass P (MBP) was determined with the anion exchange method (Kouno et al. 1995) using hexanol as fumigant. Microbial biomass P is the difference in P concentration between fumigated and un-fumigated soil (Kouno et al. 1995). No correction factor was used for P because the recovery of a P spike in this soil was 98% (Butterly et al. 2010).

The expected value for the measured parameters in organic material mixes was calculated based on the average concentration of the sole organic amendments (e.g. A and B) according to the following equation (Gartner and Cardon 2004):

Expected value = (proportion of A in mix × concentration in sole A) + (proportion of B in mix × concentration in sole B)

2.4 Statistical Analysis

There were four replicates per treatment and sampling time. After confirming normal distribution by the Shapiro-Wilk test and homogeneity by Bartlett's test, data were analysed by one-way ANOVA for each sampling date separately using GenStat 15th edition (VSN Int. Ltd., UK). One-way ANOVA was also used to compare the properties of three organic materials. Tukey's multiple comparison tests at 95% confidence interval were used to determine significant differences among treatments and materials. The significance of the differences between the measured and expected values was assessed by Student t test ($P < 0.05$).

3 Results

3.1 Shoot and Root Dry Weight and Shoot/Root Ratio

Shoot and root dry weights were lower on day 35 than those on day 105 although plants had been grown for 35 days prior to each harvest (Fig. 1a). On day 35 compared with that of the control, shoot dry weight in 100FB was about 20% higher, similar in 100SM, but 70% lower in 100W. Shoot dry weight did not differ among FB-SM mixes. It was about 20% higher in 75FB-25SM than that in control. In FB-W mixes, shoot dry weight decreased with the proportion of W. Shoot dry weight in 75FB-25W was similar to that in the control and about threefold higher than that in 25FB-75W. In SM-W mixes, shoot dry weight was about 40–50% higher in 75SM-25W than that in the other treatments. On days 70 and 105, treatment differences were similar as those on day 35, except in FB-W mixes where there were no differences among treatments on days 70 and 105.

Root dry weight on day 35 was similar to that of the control, 100FB, 100SM, all FB-SM mixes, FB-W mixes with ≥ 50% FB and in 75SM-25W (Fig. 1b). It was about 40% lower than that in the control in 100W, 25FB-75W and SM-W mixes with ≥ 50% W. Treatment differences in root dry weight on days 70 and 105 were similar as those on day 35, but smaller.

Shoot/root ratio varied among treatments on day 35, but there were no treatment differences on days 70 and 105 where the ratio ranged from 1.5 to 2.5 (Supplementary Table S1). The shoot/root ratio was higher on day 105 than that on day 35. On day 35, the shoot/root ratio was highest in 100FB and lowest in 25FB-75W. Compared with that in the control, the shoot/root ratio was 30% higher in 100FB and 30% lower in 100W and in SM-W treatments with ≥ 50% W. The shoot/root ratio differed only among FB-W mixes where it was higher in 75FB-25W than that in 25FB-75W.

3.2 Shoot N and P Concentrations

Shoot N concentration was generally higher on days 35 and 70 than that on day 105 (Fig. 2a). On day 35, shoot N concentration was highest in 100FB, about 30% higher than that in 100SM and threefold higher than that in 100W. Shoot N concentration was similar in 100SM and in the control and did not differ among the mixes. Compared with that in the control, it was about 30% higher in FB-SM and similar in FB-W and SM-W mixes. Shoot N concentration changed little from day 35 to day 70, except for 100W where it increased twofold. Shoot N concentration was higher than that in the control only in 100FB, 75FB-25SM, 50FB-50SM and 75FB-25W. On day 105, shoot N concentration was lower than that on day 70 in all treatments, but treatment differences were similar to those on day 70.

Shoot P concentration was generally higher on day 70 than that on the other sampling days in most treatments except for the control and 100FB where it was similar on day 70 and day 35 (Fig. 2b). On day 35, shoot P concentration was highest in 100FB and lowest in 100W. Shoot P concentration differed from the control only in 100FB and 100W. On day 70, shoot P concentration was similar among the control, treatments with individual organic materials and FB-W and SM-W mixes. On day 105, shoot P concentration differed little among amended treatments.

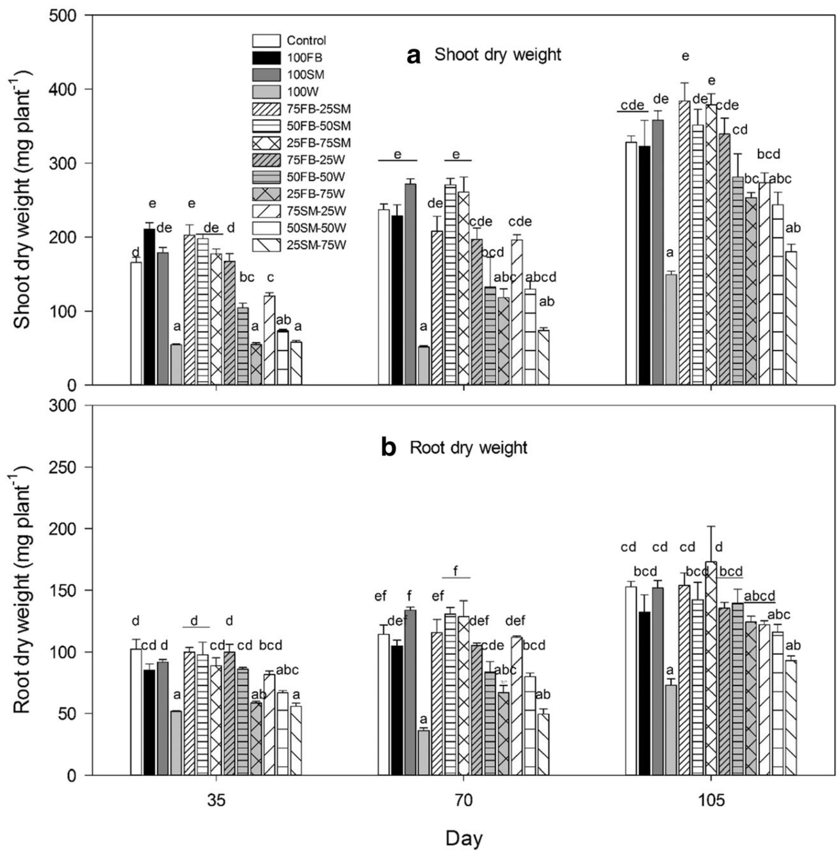

Fig. 1. Shoot dry weight (**a**) and root dry weight (**b**) on days 35, 70 and 105 of wheat plants grown in soil amended with young faba bean shoot (FB), sheep manure (SM) or mature wheat straw (W) either as individual organic materials (100FB, 100SM, 100 W) or as mixes (FB-W, FB-W, SM-W) with different proportions of the two amendments ($n = 4$). For treatment details see Table 2

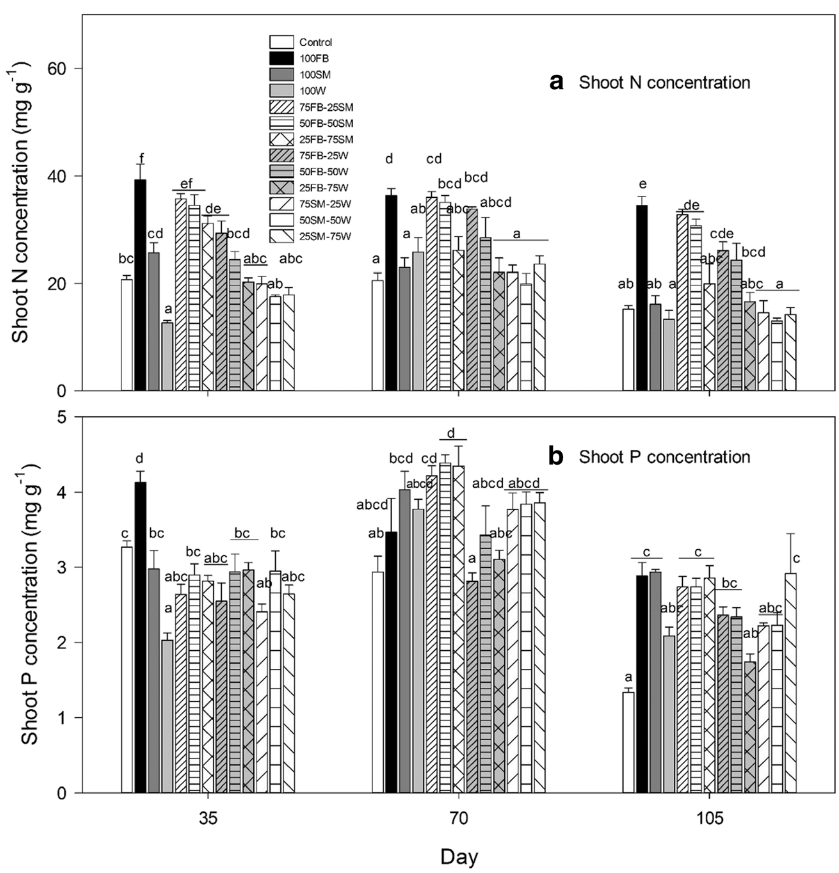

Fig. 2 Shoot N (**a**) and P (**b**) concentration on days 35, 70 and 105 of wheat plants grown in soil amended with young faba bean shoot (FB), sheep manure (SM) or mature wheat straw (W) either as individual organic materials (100FB, 100SM, 100W) or as mixes (FB-W, FB-W, SM-W) with different proportions of the two amendments ($n = 4$). For treatment details see Table 2. At a given sampling date, different letters indicate significant differences among treatments

3.3 Shoot N and P Uptake

Shoot N and P uptake were generally lower on day 35 than those on days 70 and 105. On day 35, among treatments with individual organic materials, shoot N uptake was highest in 100FB, about 40% higher than that in 100SM and ninefold higher than that in 100W (Fig. 3a). Shoot N uptake in 100SM was similar to that in the control, but it was about fourfold lower in 100W. Compared with the control, shoot N uptake was 30% to twofold higher in amended treatments. On days 70 and 105, treatment differences were similar to that on day 35.

Shoot P uptake on day 35 was highest in 100FB where it was about 40% higher than that in the control (Fig. 3b). It was lower than that of the control in 100W, FB-W mixes with ≥ 50% W and all SM-W mixes. Shoot P uptake was about twofold higher in 75FB-25W than that in 25FB-75W and also twofold higher in 75SM-25W than that in 25SM-75W. On day 70, shoot P uptake was about 40% higher than that in the control in 100SM and FB-SM mixes with ≥ 50% SM. It was about 70% lower than that in the control in 100W and 25SM-75W. Shoot P uptake on day 105 was about twofold higher than that in the control in 100FB, 100SM, all FB-SM mixes and in 75FB-25W. There were no differences in shoot P uptake among FB-SM or SM-W mixes. But in FB-W mixes, it was 40% higher in 75FB-25W than that in 25FB-75W.

3.4 Microbial Biomass N and P

MBN was higher on day 35 than that on day 105 (Fig. 4a). On day 35, among treatments with individual organic materials, MBN was lowest in 100SM and in the control. In the FB-SM mixes, MBN was about 30–40% higher in 75FB-25SM than that in the other treatments. MBN was similar among FB-W mixes, 100FB and 75FB-25SM. There was no significant difference among SM-W mixes. On day 70, MBN was higher in the control and 100SM than that in the other treatments. MBN in 100FB was about twofold higher than that in the control and 100SM, but similar in 100W. In FB-SM mixes, MBN in 75FB-25SM was about 25% higher than that in 25FB-75SM. MBN did not differ among FB-W mixes. In SM-W mixes, MBN in 25SM-75W was about 25% higher than that in 75SM-25W. On day 105, MBN was similar among the control and treatments with individual organic materials. MBN did not differ among mixes.

MBP was lowest in the control in all sampling times (Fig. 4b). It was lower on day 35 than that on day 105. On day 35, MBP did not differ among treatments where organic materials were added individually but was about threefold higher in 100FB than that in the control. There was no significant difference in MBP among FB-SM or FB-W mixes. In SM-W mixes, MBP was about twofold higher in 50SM-50W than

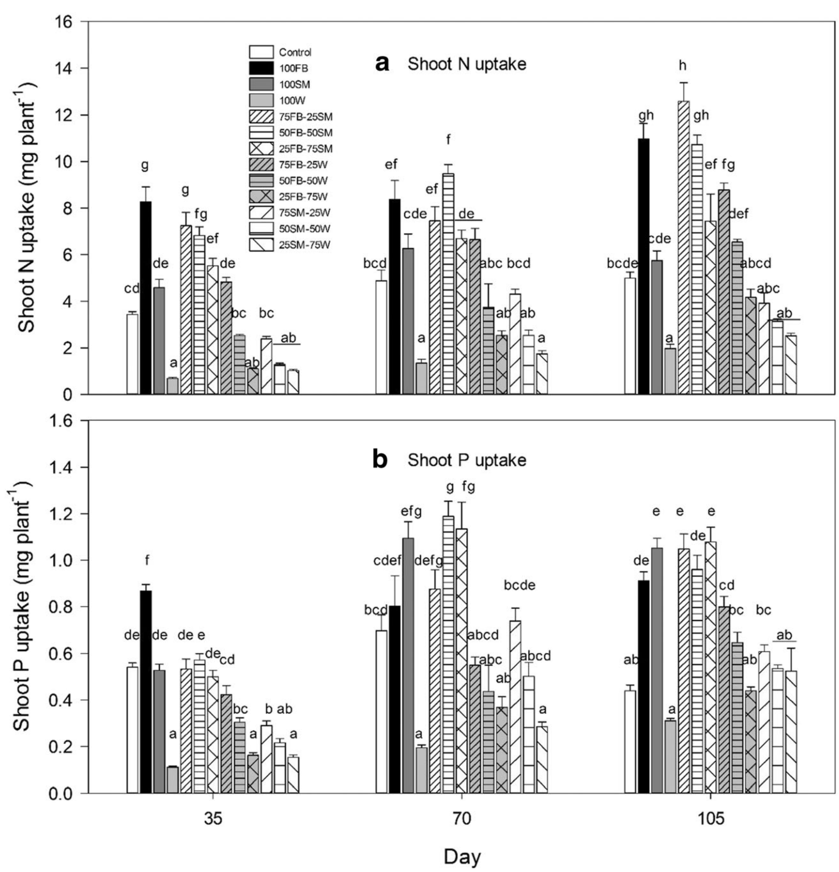

Fig. 3 Shoot N (**a**) and P (**b**) uptake on days 35, 70 and 105 of wheat plants grown in soil amended with young faba bean shoot (FB), sheep manure (SM) or mature wheat straw (W) either as individual organic materials (100FB, 100SM, 100W) or as mixes (FB-W, FB-W, SM-W) with different proportions of the two amendments ($n = 4$). For treatment details see Table 2. At a given sampling date, different letters indicate significant differences among treatments

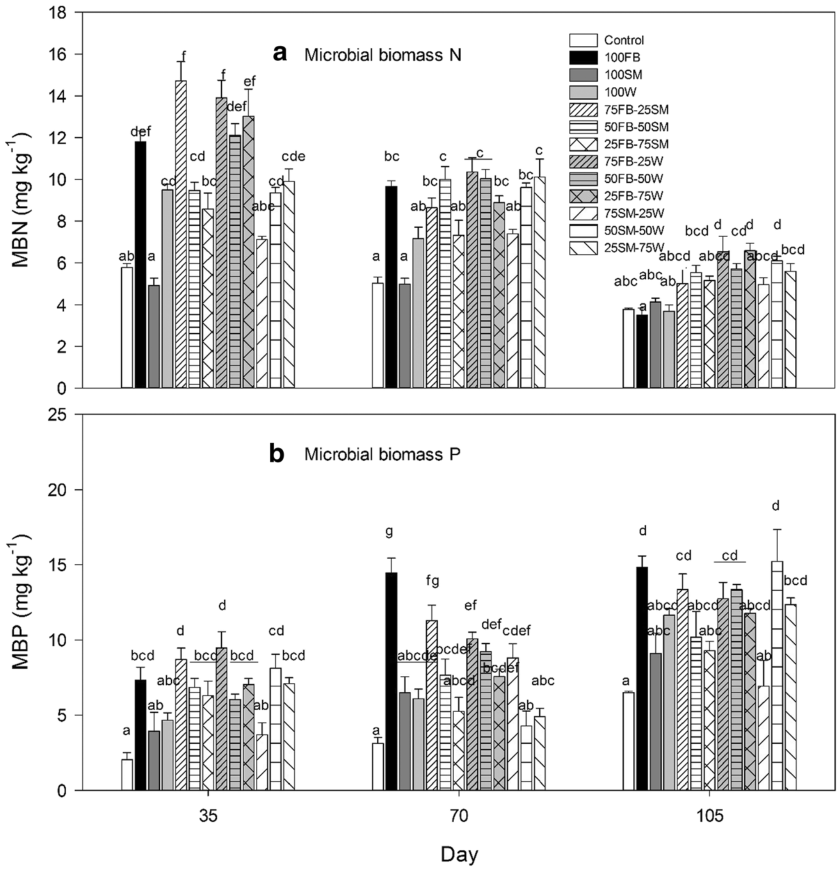

Fig. 4 Microbial biomass N (**a**) and P (**b**) on days 35, 70 and 105 in soil amended with young faba bean shoot (FB), sheep manure (SM) or mature wheat straw (W) either as individual organic materials (100FB, 100SM, 100W) or as mixes (FB-W, FB-W, SM-W) with different proportions of the two amendments ($n = 4$). For treatment details see Table 2. At a given sampling date, different letters indicate significant differences among treatments

that in 75SM-25W. On day 70, MBP was highest in 100FB where it was more than fourfold higher than that in the control. In the FB-SM and FB-W mixes, MBP decreased with increasing proportion of FB in mixes. There were no significant differences among FB-W mixes. But in SM-W mixes, MBP was about twofold higher in 75SM-25W than that in 50SM-50W. On day 105, MBP was about twofold higher in 100FB than that in the control. MBP did not differ among FB-SM and FB-W mixes. In SM-W mixes, MBP was nearly twofold higher in 50SM-50W than that in 75SM-25W.

3.5 Available N and P

In amended soils, available N was highest on day 70 whereas it did not change over time in the control (Fig. 5a). On day 35, available N was higher than that in the control only in 100FB, 75FB-25SM and 50FB-50SM where it was six- to tenfold higher. On day 70, available N was lowest in the control and highest in 100FB. Available N was more than fivefold higher in 100FB than that in 100SM and 100W. Available N was about twofold higher in mixes with 75% FB than that with 25% FB. Available N was similar among SM-W mixes. On day 105, available N was highest in 100FB and low in all other treatments.

Available P was highest on day 105 with similar treatment differences at all three sampling times (Fig. 5b). On day 35, among treatments with single amendments, available P in 100SM was about 40% higher than that in 100FB and sevenfold higher than that in 100W. Available P in 100W was similar to that in the control. Available P did not differ between 100FB and FB-SM treatments. In FB-W mixes, available P was similar to that in the control. In SM-W mixes, available P was about 60% higher in treatments with ≥ 50% SM than that in 25SM-75W. Treatment differences were similar on days 70 and 105.

3.6 Effect of the Proportion of the Organic Materials in the Mixes

Shoot N uptake, available N and MBN increased with the proportion of faba bean in mixes with wheat straw or sheep manure, particularly in the early stages of decomposition (days 35 and 70). The effect of slowly decomposing sheep manure on the measured parameters depended on the other organic material in the mix. Increasing proportion of sheep manure in mixes with faba bean residues reduced shoot N uptake and available N. But in mixes with high C/N wheat straw, the proportion of sheep manure had little effect on shoot N uptake and available N. With increasing proportion of sheep

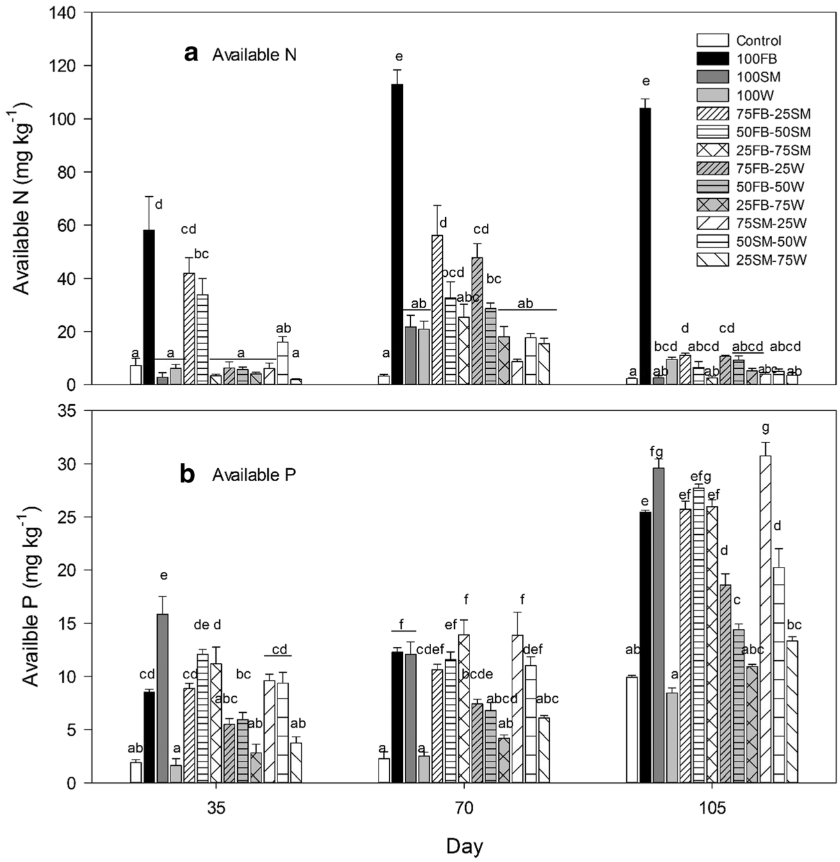

Fig. 5 Available N (**a**) and P (**b**) on days 35, 70 and 105 in soil amended with young faba bean shoot (FB), sheep manure (W) or mature wheat straw (W) either as individual organic materials (100FB, 100W, 100W) or as mixes (FB-W, FB-W, W-W) with different proportions of the two amendments ($n = 4$). For treatment details see Table 2. At a given sampling date, different letters indicate significant differences among treatments

manure microbial biomass N decreased in mixes with faba bean residues, but increased in mixes with wheat straw.

3.7 Measured Compared with Expected Values

In FB-SM mixes, measured and expected shoot dry weight and root dry weight matched throughout the experiment (Table 3). However, the shoot/root ratio was lower than expected on days 35 and 70 in 75FB-25SM. Shoot N uptake was similar as expected on day 35 in all FB-SM mixes, but it was higher than expected in 50FB-50SM on day 70 and in treatments with ≥ 50% FB on day 105. For MBN, on day 35, measured values in mixes with ≤ 50% SM were higher than expected. But on day 70, MBN was higher than expected only in 50FB-50SM. On day 105, it was greater than expected in treatments with ≥ 50% SM. Available N on day 35 was similar as expected in treatments with ≥ 50% FB, but lower than expected in 25FB-75SM. On days 70 and 105, available N was lower than expected in all mixes. On day 70, measured available N was about 50% of expected, but on day 105, measured available N was about sixfold lower than expected.

In FB-W mixes, shoot dry weight was lower than expected values on day 35 in treatments with ≤ 50% FB, but similar as expected on day 70 in all mixes. On day 105, shoot dry weight was greater than expected in 25FB-75W. Compared with expected values, root dry weight was higher in treatments with ≥ 50% FB on day 35, in 75FB-25W on day 70 and in treatments with ≥ 50% W on day 105. On day 35, the shoot/root ratio was smaller than expected in all FB-W mixes, with differences increasing with the proportion of W in the mixes. But later, there was no significant difference between measured and expected shoot/root ratio. Shoot N uptake was lower than expected on day 35, with differences increasing with the proportion of W in the mixes. On days 70 and 105, measured shoot N uptake matched expected values in most treatments except in 25FB-75W on day 70 where measured shoot N uptake was lower than expected. MBN was greater than expected in 75FB-25W on day 35, in 50FB-50W on day 70 and in all treatments on day 105. Available N was lower than expected in all treatments and sampling days, but the differences were greatest on day 105.

In SM-W mixes, shoot dry weight and shoot N uptake on days 35 and 70 were lower than expected in treatments with ≥ 50% W, but matched expected values in 75SM-25W. On day 105, shoot dry weight and shoot N uptake were similar as expected in all SM-W mixes. Measured root dry weight matched expected values in all mixes throughout the experiment. Shoot/root ratio on day 35 was lower than expected in treatments with ≥ 50% SM. But later, measured shoot/root ratio was similar as expected in all SM-W mixes.

Table 3 Measured and expected values of measured parameters on days 35, 70 and 105 for treatments where organic materials were mixed. At a given sampling date, asterisks indicate significant differences (Student's *t* test) between measured and expected values

Parameter	Day	Values	75FB-25SM	50FB-50SM	25FB-75SM	75FB-25W	50FB-50W	25FB-75W	75SM-25W	50SM-50W	25SM-75W
Shoot DW (mg plant^{-1})	35	Measured	202.4	197.7	177.2	167.2	104.5*	54.7*	120.6	73.5*	57.9*
		Expected	202.8	194.8	186.8	171.7	132.7	93.6	147.7	116.7	85.6
	70	Measured	208.0	270.4	260.9	196.7	132.8	118.2	196.0	129.7*	73.8*
		Expected	239.2	249.9	260.7	184.2	140.0	95.8	216.5	161.5	106.6
	105	Measured	384.3	351.4	378.8	339.6	281.0	253.2*	273.6	243.6	180.2
		Expected	331.5	340.4	349.3	279.3	236.0	192.7	306.1	253.8	201.6
Root DW (mg plant^{-1})	35	Measured	99.8	97.8	88.9	99.8*	86.1*	58.5	81.5	66.8	55.8
		Expected	86.9	88.5	90.1	76.8	68.4	60.0	81.7	71.6	61.6
	70	Measured	115.8	130.8	128.6	105.2*	83.8	66.9	112.0	80.0	49.4
		Expected	112.1	119.4	126.7	87.7	70.5	53.3	109.6	85.1	60.6
	105	Measured	154.0	142.2	173.0	135.7	139.8*	124.5*	122.2	116.2	92.9
		Expected	137.3	142.1	147.0	117.5	102.6	87.8	132.1	112.3	92.6
Shoot/root ratio	35	Measured	2.0*	2.1	2.0	1.7*	1.2*	0.9*	1.5*	1.1*	1.1
		Expected	2.4	2.2	2.1	2.1	1.8	1.4	1.7	1.5	1.3
	70	Measured	1.8*	2.1	2.0	1.9	1.7	1.8	1.8	1.6	1.5
		Expected	2.1	2.1	2.1	2.0	1.8	1.6	1.9	1.7	1.6
	105	Measured	2.5	2.5	2.4	2.5	2.0	2.1	2.2	2.1	1.9
		Expected	2.4	2.4	2.4	2.4	2.3	2.2	2.3	2.2	2.2
Shoot N uptake (mg plant^{-1})	35	Measured	7.2	6.8	5.5	4.8*	2.5*	1.1*	2.4	1.3*	1.0*
		Expected	7.3	6.4	5.5	6.4	4.5	2.6	3.6	2.6	1.7
	70	Measured	7.5	9.5*	6.7	6.6	3.7	2.5*	4.3	2.5*	1.7*
		Expected	7.8	7.3	6.8	6.6	4.8	3.1	5.0	3.8	2.6
	105	Measured	12.6*	10.7*	7.4	8.8	6.5	4.2	3.9	3.1	2.5
		Expected	9.7	8.4	7.0	8.7	6.5	4.2	4.8	3.9	2.9
MBN (mg kg^{-1})	35	Measured	14.7*	9.5*	8.6	13.9*	12.1	13.0	7.1*	9.4*	9.9
		Expected	10.1	8.4	6.7	11.2	10.7	10.1	6.1	7.2	8.4
	70	Measured	8.6	10.0*	7.3	10.4	10.0*	8.9	7.4*	9.6*	10.1*
		Expected	8.5	7.3	6.2	9.0	8.4	7.8	5.5	6.1	6.6
	105	Measured	5.0	5.5*	5.2*	6.5*	5.7*	6.6*	5.0*	6.1*	5.6*
		Expected	3.7	3.8	4.0	3.6	3.6	3.6	4.0	3.9	3.8
Available N (mg kg^{-1})	35	Measured	42.0	33.9	3.3*	6.3*	5.7*	4.1*	6.1	16.0*	2.0
		Expected	44.3	30.5	16.7	45.2	32.2	19.2	3.7	4.5	5.3
	70	Measured	56.1*	32.6*	25.4*	47.7*	28.7*	18.0*	8.7*	17.7	15.5
		Expected	90.1	67.3	44.6	89.9	66.9	43.9	21.6	21.4	21.2
	105	Measured	11.1*	6.5*	2.6*	10.7*	9.2*	5.2*	3.9	5.0	3.5*
		Expected	78.7	53.3	28.0	80.4	56.8	33.2	4.3	6.1	7.8

Throughout the experiment, MBN was higher than expected in most of SW-W treatments, except for 25SM-75W on day 35 where measured and expected MBN matched. The difference between measured and expected MBN was greatest in 50SM-50W on all sampling days. Available N in SM-W mixes was higher than expected in 50SM-50W on day 35, but lower than expected in 75SM-25W on day 70 and in 25SM-75W on day 105.

4 Discussion

Based on this study, some of the hypotheses can be confirmed, but not for all measured parameters. In general, the effect of mixes was greater for available N and MBN than for plant growth and N uptake. The first hypothesis (in mixes of high C/N wheat straw with low C/N faba bean shoots or sheep manure, shoot and root dry weight, shoot N uptake, N availability and microbial biomass N will decrease with the proportion of wheat straw) can be confirmed for shoot dry weight and N availability on days 70 and 105 in FB-W mixes. The second hypothesis (the effect of high C/N wheat straw on measured parameters is greater in mixes with slowly decomposing sheep manure than rapidly decomposing faba bean shoots) can be confirmed for N availability and MBN. The third hypothesis (the effect of residue mixes on measured parameters will change over time during decomposition with greater differences initially during rapid decomposition than later) can only be confirmed for available N and MBN in FB-W mixes.

The following discussion will focus on plant biomass (shoot and root dry weight), shoot N uptake (shoot N concentration and content), MBN, and available N. Shoot P uptake, MBP, and available P will not be discussed because treatment differences in shoot P uptake were generally similar as those in shoot N uptake, whereas differences in MBP and available P were less pronounced than those of MBN and available N.

4.1 Individual Amendments

As expected from the low C/N ratio of FB (Zhang and Marschner 2016; Zheng and Marschner 2017), N availability was up to tenfold higher than that in the control and in the other single materials. But despite very high N availability, shoot and root dry weight were similar in 100FB and the control suggesting that other factors limited wheat growth, such as low light intensity as the experiment was conducted during winter and early spring. However, shoot N concentration and N uptake were always higher in 100FB than those in the control and in the other single materials. MBN was higher than that in the control and 100SM on days 35 and 70, but it was similar to that in the control and the treatments with SM and W alone on day 105. This suggests that compared with the control and SM, FB was rapidly decomposed resulting in initially higher N release and uptake into microbial biomass in the first 70 days. But later, N microbial uptake was limited despite high N availability which is likely due to depletion of easily available C.

SM alone did not increase shoot dry weight, N uptake, MBN, and available N compared with the control throughout the experiment although it had a similar low C/N ratio as FB. This is likely due to the low decomposability of manure which is already largely decomposed in the gut of the animals (Hungate 1966).

The addition of W reduced shoot and root dry weight and shoot N uptake compared with that in the control which can be explained by its low N content (high C/N ratio) (Nguyen and Marschner 2016; Zhang and Marschner 2017) which would induce net microbial N immobilisation. However, MBN and available N were similar in the control on two of the three sampling times. The fact that MBN and available N did not differ in the control may be due to plant N uptake and turnover of microbial biomass over the 35-day periods.

The effect of the amendments on plant growth and available N changed little over time, but MBN was lower on day 105 than that on day 35, particularly with FB. This suggests depletion of easily decomposable C due to rapid decomposition of FB whereas the other amendments were decomposed more slowly.

4.2 FB-SM Mixes

FB-SM mixes did not differ in shoot and root dry weight which was similar in the control, 100FB, and 100SM which indicates no interactions between FB and SM with respect to plant growth (Gartner and Cardon 2004). Shoot N concentration in FB-SM was not higher than that in 100FB which is in contrast with Zai et al. (2008) where wheat shoot N concentration was higher in the 5:1 mix of green pea shoot (C/N 16) and chicken manure (C/N 9) than that in green pea shoot alone. The lack of effect of SM may be due to the highly decomposed stage of sheep manure (Hungate 1966) or the relatively short duration of the experiment which meant that FB was decomposed and released nutrients throughout the experiment. Shoot N uptake was greater than that of the control in mixes with ≥ 50% FB on days 70 and 105 and greater than expected on day 105. This occurred despite lower N availability than 100FB which was lower than expected on days 70 and 105 in all FB-SM mixes and higher MBN in 50FB-50SM. A high proportion of FB in the mixes appears to have stimulated N release from SM in the later stages of decomposition (days 70 and 105) which was taken up by both plants and microbes. In this experiment, we cannot determine the source of N in shoots or microbes, but it is well-known that easily available compounds can induce priming that is accelerated decomposition of more recalcitrant compounds (Chapman and Koch 2007; Wardle et al. 1997). Therefore, FB may have stimulated N release from SM. MBN in FB-SM mixes was higher than that in 100SM on day 35 which was also the case for mixes with ≥ 50% FB on day 70 suggesting that easily available compounds in FB stimulated microbial growth and N uptake.

4.3 FB-W Mixes

The proportion of FB in FB-W mixes influenced shoot dry weight only on day 35 where it was highest in 75FB-25W and lowest in 25FB-75W. Shoot dry weight in the mix with the largest proportion of W was higher than 100W only on day 105. This suggests that in the early stages of decomposition, plant roots accessed residue particles depending on their proportion in the mix with little interaction between FB and W. Nevertheless, the shoot/root ratio was lower than expected in all mixes on day 35, indicating that plants allocated more C to roots. Greater root compared with shoot growth is a typical response of plants to low N availability (Brouwer 1962; Van der Werf and Nagel 1996). Shoot N uptake was lowest in 25FB-75W and was lower than expected in all mixes on day 35. This coincided with lower than expected N availability. N availability was also lower than expected on days 70 and 105 but measured and expected N uptake matched. This indicates that the plants were able to access portions of the soil where N availability was high (around FB particles). The lower than expected N availability in the later stages of decomposition could be explained by uptake of N into the microbial biomass. Microbes decomposing W could take up N released during decomposition of FB. N transfer from low C/N to high C/N litter has been demonstrated (Schwendener et al. 2005). However, the difference in MBN between measured and expected was small compared with the difference in measured and expected N availability. This suggests that the lower than expected N availability is due to two processes: uptake by plants and by microbes with the latter being more important in the later stages of decomposition.

4.4 SM-W Mixes

Similar as in FB-W mixes, shoot dry weight in SM-W was lowest in 25SM-75W, but shoot N uptake differed little among mixes. N availability was not consistently lower than expected, but MBN was higher than expected in all mixes and on all sampling dates. This confirms strong immobilisation by microbes decomposing W of N released by SM decomposition. It is possible that the presence of W stimulated SM decomposition by priming because soil respiration rate with W is usually much higher than with SM (Truong unpublished data).

5 Conclusion

The study showed that interactions in residue mixes change over time and influence soil properties such as available N and microbial biomass N to a greater extent than plant growth. This suggests that in mixes of wheat straw with low C/N organic materials, plants can, to some degree, compensate low N availability induced by wheat straw through direct access to particles releasing N thereby minimising competition by microbes. The effect of slowly decomposing sheep manure on the measured parameters depended on the other organic material in the mix. An increasing proportion of sheep manure increased shoot N uptake under N limiting conditions (mixes with wheat straw) but decreased it with faba bean residues that released large amounts of N.

Future experiments could use ^{15}N labelled residues to trace the source of N in soil and plants.

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CHAPTER 6

PRESENCE OF WHEAT STRAW IN SOIL INFLUENCES NUTRIENT AVAILABILITY AND LEACHING IN SOIL MULCHED WITH ORGANIC MATERIALS DIFFERING IN C/N RATIO

Statement of Authorship

Title of Paper	Presence of wheat straw in soil influences nutrient availability and leaching in soil mulched with high or low C/N organic materials.
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Principal Author

Name of Principal Author (Candidate)	Thi Hoang Ha Truong		
Contribution to the Paper	Performed experiment and analyses of all samples, data analysis and interpretation and manuscript writing. I hereby certify that the statement of contribution is accurate.		
Overall percentage (%)	70%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	22/2/2020

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

xiii. the candidate's stated contribution to the publication is accurate (as detailed above);

xiv. permission is granted for the candidate in include the publication in the thesis; and

xv. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Petra Marschner		
Contribution to the Paper	Supervised development of the work, data interpretation and manuscript evaluation and correction. I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.		
Signature		Date	22/02/2020

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Presence of wheat straw in soil influences nutrient availability and leaching in soil mulched with high or low C/N organic materials

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ABSTRACT
The study aimed to determine nutrient availability in soil mulched with organic materials and how this is influenced by the presence of high C/N residue in the soil. Three organic materials were used: mature wheat straw (W, C/N 81), young faba bean shoots (FB, C/N 7) and sheep manure (SM, C/N 8). There were eight treatments: unamended control, soil containing W, soil containing W and mulched with W, FB or SM and soil only with W, FB or SM mulch. Soils were leached on days 4, 12, 20 and 28 and sampled 8 days after each leaching. Cumulative respiration over 36 days was influenced by mulch type: FB > W > SM and higher in soil mixed with W and mulched than in soil with only mulch. Microbial biomass N was two-fold higher in soil mixed with wheat and mulched than in soil with only mulch. Available N was between 0.2 and two-fold higher in soil with only mulch than soil with mulch and mixed with W. Available N increased over time with FB mulch but changed little with SM or W mulch. In conclusion, W in the soil can enhance N immobilisation and thus reduce N leaching from FB mulch.

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Leaching; low C/N; mulch; manure; wheat straw

Introduction

In many agricultural systems, residues may be present in the soil when the mulch is applied, for example, when mulch is applied after harvest of a crop. But studies on the effect of plant residues in the soil on nutrient availability after mulching are scarce. Mulching with organic materials improves soil quality and productivity through their beneficial effects on soil properties (Tu et al. 2006; Mulumba and Lal 2008; Marinari et al. 2015). Application of organic mulches increases soil organic C content (Havlin et al. 1990; Saroa and Lal 2003), microbial biomass and activity (Tu et al. 2006) and soil moisture storage (Ji and Unger 2001), and reduces nutrient loss (Rees et al. 1999) and fluctuations in soil temperature (Naeini and Cook 2000). Decomposition of organic mulches and leaching after rain or irrigation can add nutrients to the soil (Tu et al. 2006; Halde and Entz 2016; Ibrahim et al. 2018).

Nutrients are released from organic amendments through physical processes (e.g. leaching) or biological processes (decomposition by microbes). Chemical properties of organic amendments such as C/N ratio and N, lignin and polyphenol content influence decomposition rate and nutrient availability (Bending and Turner 1999; Palm et al. 2001). For example, application of high C/N organic materials induces net immobilisation (Moritsuka et al. 2004) whereas low C/N amendments (C/N < 20) lead to net mineralisation (Hadas et al. 2004). Nitrogen and P release from organic materials are positively related to their N and P concentrations (Halde and Entz 2016; Ibrahim et al. 2018). Particularly in organic materials with low C/N ratio, N release is greatest initially due to leaching of water-soluble compounds (Rosolem et al. 2005) which is the bases for the first hypothesis of this study.

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A number of studies investigated the effects of mulching on soil respiration and microbial biomass in field experiments. Tu et al. (2006) reported that mulching with low C/N organic materials increased microbial biomass C and N and soil respiration compared to the control with synthetic fertilisers over a two-year field experiment. Tu et al. (2006) explained that mulching improved soil organic C and water availability, thus mitigating the negative effect of low soil water content, especially in the dry season. Marinari et al. (2015) found that the soil microbial C/N ratio after mulching with legume cover crops was higher than the control without which they explained by a greater abundance of fungi. Microbial biomass C was higher in soil mulched with wheat straw than unamended soil, particularly at the high mulch rate (Wang et al. 2018). In another study, microbial biomass P was higher in soil mulched with green water hyacinth than unamended soil because the high content of rapidly decomposable compounds in the mulch induced microbial growth and nutrient uptake (Balasubramanian et al. 2013). Studies on mulching with organic materials focused on nutrient release from mulches which were usually fresh or dried plant residues. There is limited understanding about mulching with manures which may occur when livestock is present or after manure spreading. Manures are products of microbial decomposition in the digestive system of animals (Janssen 1996). Easily decomposable compounds in the feed are digested during passage through the animal's digestive tract thereby concentrating more recalcitrant compounds such as lignin in manures. This may result in lower decomposability of manures compared to plant residues with similar low C/N ratio (Janssen 1996). Further, most previous studies on effects of mulching on soil properties were conducted over long periods (e.g. from 250 days in Halde and Entz (2016) to 9 years in Wang et al. (2018)). Less is known about the effect of mulching shortly after mulch application which may be important for the early stages of crop growth.

This study aimed to determine the effects of mulching with organic materials differing in C/N ratio and the presence of high C/N residues in the soil on soil respiration, microbial biomass N and P and available N and P within the first 5 weeks after amendment.

The first hypothesis was that the effects of mulching on soil respiration and nutrient availability will decrease over time because most nutrients in mulches will be released in the very early decomposition stages when the decomposition rate is high. The second hypothesis was that faba bean will have greater effects on soil respiration, microbial biomass N and N availability than sheep manure because faba bean is decomposed more quickly than sheep manure. The third hypothesis was that nutrient availability in mulched soil will be lower in soil that contains wheat straw than without straw because microbes decomposing wheat straw will immobilise nutrients leached from mulches.

Materials and methods

Soil and organic materials

The study used the same soil as in Truong and Marschner (2018). The sandy clay loam was collected from 0 to 10 cm at Waite Campus, The University of Adelaide (Longitude 138°38′3.2″ E, Latitude 34°58′0.2″ S). Collection and soil properties have been described before (Truong and Marschner 2018). Briefly, the area has a Mediterranean climate. The soil is classified as Chromosol (Australian soil classification) and Rhodoxeralf (US Soil Taxonomy). The soil has the following properties (for methods see below): sand 54%, silt 20% and clay 25%, pH (1:5 soil:water) 6.3, electrical conductivity (EC 1:5 soil:water) 143 µS cm^{-1}, total N 1.5 g kg^{-1} and total P 371 mg kg^{-1}, total organic carbon (TOC) 17 g kg^{-1}, available N (inorganic N including ammonium and nitrate N) 15 mg kg^{-1}, available P (inorganic P) 10 mg kg^{-1}, maximum water-holding capacity (WHC) 378 g kg^{-1} and bulk density 1.3 g cm^{-3}. After collection in the field, the soil was air-dried and then sieved to < 2 mm.

Selection and preparation of organic materials in this study were similar as in Truong and Marschner (2018). The organic materials used were young faba bean shoots (*Vicia faba* L., C/N 7, referred to as FB) as fast decomposing low C/N material, sheep manure (C/N 8 referred to as SM) as slow decomposing low C/N material and mature wheat straw (*Triticum aestivum* L., C/N 81, referred to as W) as high C/N material. The organic materials were dried at 40 °C in a fan-forced oven and then ground and sieved to different particle sizes (see below). Compared to SM, total organic C was about three-fold higher in W and FB (Table 1). Total N and P were 3–9 times higher in FB and SM than in W. The C/N ratio was similar in FB and SM, where it was about ten-fold lower than in W. Compared to FB, the C/P ratio was about three-fold lower in SM but four-fold higher in W.

The experiment was designed based on two experiments which were conducted to investigate the effect of mulch rate and particle size (Experiment 1) and leaching frequency (Experiment 2) on the measured parameters (for methodology and results, see supplementary file). The organic materials were ground and sieved to particle size 0.25–2 mm. The small particle size was used to maximize contact of organic materials with the soil and thus increase their rate of decomposition in soil and mulches compared to larger particles. To determine nutrient leaching from mulches, 0.54 g of W, FB or SM was moistened and spread evenly on fabric pieces which were placed into empty PVC cores similar to those used in the experiments (see below). The moistened mulches were leached on day 4 with 5 mL of reverse osmosis (RO) water. Organic C and inorganic N and P in leachates were measured as described below.

Experimental design

The soil was pre-incubated for 10 days at 50% of maximum WHC and then packed into cores following the same procedure as in Truong and Marschner (2018). In this soil, microbial activity was maximal at 50% of WHC (Marschner et al. 2015). Soil was either left unamended or amended with organic materials by mixing and/or mulching. Soil mixed with residues (see below for details) or left unamended was filled into PVC cores (diameter 3.7 cm, height 5 cm with nylon net base of 7.5 μm pore size). Then, the soil was compacted to bulk density of 1.3 g cm $^{-3}$. In treatments with mulch, a piece of fabric (mesh size 0.1 mm) was placed between the soil surface and the organic materials to minimize movement of mulch particles into the soil. The mulch materials were maintained moist throughout the experiment to enhance decomposition.

There were eight treatments differing in amendment type (mulching or mixing with W or both) and mulching materials (W, FB or SM) and two controls. In treatments that were mulched only, referred to as m-treatments, W, FB or SM were placed on the soil surface as mulch, giving treatments: Wm, FBm and SMm. In m/s-treatments, W was mixed into the soil. Then, W, FB or SM were placed on the soil surface as mulch, giving treatments Wm/Ws, FBm/Ws and SMm/Ws. Two other treatments included an unamended control (control) and soil mixed with W (Ws). Of the organic materials, only W was mixed into the soil because it was expected that W leads to immobilisation of nutrients leached from the mulch. In Ws and m/s-treatments, 30 g soil (dry weight equivalent) was mixed with mature wheat straw at 10 g kg^{-1} soil in a small plastic bag. With this straw addition rate, the amount of C added was about 4 g kg^{-1} which is within the range used in other studies from our group [e.g. from 3 g kg^{-1} in Shi et al. (2013) to 7 g kg^{-1} in Nguyen and Marschner (2016)]. Then, the amended soil was filled into PVC cores. For mulch treatments, organic materials were placed on the soil surface at 0.5 kg m^{-2}. The amount of organic materials in

Table 1. Total organic C, N, P, C/N ratio and C/P ratio, water-extractable C and pH of mature wheat straw (W), young faba bean shoot (FB) and sheep manure (SM) (n = 4). Within rows, means followed by different letters are significantly different (p ≤ 0.05).

	W	FB	SM
Total organic C (g kg^{-1})	399.85^{c}	318.43^{b}	116.43^{a}
Total N (g kg^{-1})	5.13^{a}	47.07^{c}	15.19^{b}
Total P (g kg^{-1})	3.31^{a}	9.51^{b}	10.35^{c}
C/N	80.65^{b}	6.79^{a}	7.71^{a}
C/P	120.61^{c}	33.54^{b}	11.27^{a}
pH (1:10)	5.72^{b}	5.40^{a}	6.55^{c}

mulches (0.54 g per core) was nearly two-fold higher than in soil (0.3 g per core). The mulch amount was used to give a thin and even layer of mulch. The lower amount in soil was chosen to keep the total amount per core low but allow even distribution of W in the soil. Then, the mulches were moistened and left undisturbed throughout the experiment.

Leaching was carried out on days 4, 12, 20 and 28. For leaching, 5 mL of RO water which simulated a rainfall event of 5 mm was added in 1 mL aliquots. Between additions, the water was allowed to drain from the mulch surface before the next aliquot was added. This amount of water was used to leach nutrients from the mulches but prevent leaching from the bottom of the soil-filled cores. After leaching, cores were dried at 30 °C in a fan-forced oven for about 12 h until the soil water content was 50% WHC as determined by weight. The mulches dried in the oven and were moistened again by spraying with RO water. Soils were destructively sampled 8 days after each leaching event (i.e. on days 12, 20, 28 and 36) for measurement of available N and P and microbial biomass N and P. Respiration was measured over 36 days, except for the time in which the soils were dried. There were four replicates per treatment and sampling time.

After the application of organic materials and bulk density adjustment, single cores were placed into 1 L glass jars with a gas-tight lid that had a septum to allow sampling of headspace CO_2 concentration as described below. In a given 8-day period, only the cores to be sampled at the end of the period were placed back into the jars after leaching. The remaining cores were leached, dried and then incubated in large plastic trays covered with aluminium foil. After removal of the cores from the jars for analysis, the cores to be harvested at the next sampling time were placed in the glass jars for respiration measurement. The jars and trays with cores were incubated in the dark at 19–22 °C. Between leaching events, soil water content was maintained at 50% of WHC and mulch were kept moist by checking the water content every few days by weighing cores and adding RO water if necessary. Soil respiration was measured daily from day 1 to 4, then every 2 days from day 5 to day 36.

Analyses and calculations

Soil analyses were carried out as described in Marschner et al. (2015) and Truong and Marschner (2018). Briefly, soil texture was determined after Gee and Or (2002), soil pH was measured in a 1:5 soil:water extract (Rayment and Higginson 1992). Soil water-holding capacity was determined at matric potential = −10 kPa (Wilke 2005). The total organic carbon content of soil and organic materials was determined according to Walkley and Black (1934). For total N in soil and organic materials, the material was digested with H_2SO_4 and H_2O_2. Total N was measured by a modified Kjeldahl method (Bremner and Mulvaney 1982). Total P in soil and organic materials was determined after digestion with a mixture of HNO_3 with HCl and H_2O_2, respectively. Total P in soil was measured after Murphy and Riley (1962), total P in organic materials according to Hanson (1950). Available N (ammonium and nitrate) was measured after shaking with 2 M KCl at a 1:10 soil extractant ratio. Ammonium-N was determined after Forster (1995), nitrate-N after Miranda et al. (2001). The anion exchange resin method (Kouno et al. 1995) was used to extract available P, and P was measured by the Murphy and Riley (1962) method.

Chloroform fumigation-extraction with 0.5 M K_2SO_4 at 1:4 soil to extractant ratio was used for extraction of microbial biomass N (MBN) (Moore et al. 2000). The ammonium concentration in the extract was determined as described above for available N. Microbial biomass N is the difference in NH_4^+ concentration between fumigated and non-fumigated samples divided by 0.57 as described in Moore et al. (2000). Microbial biomass P (MBP) was determined with the anion exchange method (Kouno et al. 1995) using hexanol as a fumigant. Microbial biomass P was calculated by subtracting the P concentration of the unfumigated soil by that of the fumigated soils (Kouno et al. 1995).

Inorganic N and P in the leachate from mulches were determined using the same colorimetric methods as for available N and P. Leached organic C was determined by oxidizing with K_2Cr_2O7 and H_2SO_4 and titration as described in Anderson and Ingram (1993).

Soil respiration was measured by quantifying the CO_2 concentration in the headspace of the jars with an infra-red analyser as described in Setia et al. (2011).

Statistical analysis

Prior to the analysis, data were checked for normality using residual plots in Genstat 15th edition (VSN Int. Ltd, UK). Data were then analysed by one-way repeated measures ANOVA in Genstat 15th edition with time as a repeated measure which showed that the treatment x time interaction was significant for all parameters. Data of each sampling time were analysed by one-way ANOVA. Organic material properties data were analysed using one-way ANOVA. Significant differences among treatments and organic material properties were determined using Tukey's multiple comparison tests at 95% confidence interval.

Results

Nutrients leached from mulches in the first leaching event (day 4)

Leached organic C was highest in FB mulch, about three-fold higher than in W mulch and seven-fold higher than in SM mulch (Table 3). Leached inorganic N was lowest in W which was about 50-fold lower than in FB and SM. Inorganic P in the leachate was lowest in W; it was two-fold higher in FB and about 20% higher in SM.

Cumulative respiration

Cumulative respiration was lowest in the control throughout the experiment (Figure 1). Differences in cumulative respiration among treatments were greatest in the first 12 days. Compared to Ws, cumulative respiration in Wm was about 20% lower from day 1 to 12, but 20% higher in the later periods. From day 1 to 12, cumulative respiration in FBm/Ws was about three-fold higher than in Ws, two-fold higher than in Wm/Ws and 60% higher than in SMm/Ws. Among m-treatments, cumulative respiration from day 1 to 12 in Wm was about 60% lower than in FBm but three-fold higher than in SMm. Cumulative respiration was about 20–70% higher in m/s-treatments than in m-treatments that were mulched with the same organic materials. In the following 8-day periods (day 13 to 20, day 21 to 28 and day 29 to 36), cumulative respiration was up to two-fold higher in m/s- than in corresponding m-treatments. Total cumulative respiration at the end of the experiment was up to 60% higher in m/s-treatments than in Ws and corresponding m-treatments. Total cumulative respiration was about 20% higher in SMm than in the control, and more than 20% lower in Ws than in Wm.

Microbial biomass N and P

From day 1 to 36, MBN decreased in the treatments with FB (FBm/Ws and FBm) but changed little in the other treatments (Figure 2(a)). On day 12, MBN was about two-fold higher in FBm/Ws than in the other treatments with W (Ws, Wm/Ws and SMm/Ws). MBN did not differ between the control and m-treatments (Wm, FBm and SMm), where it was 60–70% lower than in the m/s-treatments with the same mulches. From day 12 to day 20, MBN decreased by about 50% in FBm/Ws and by 30% in FBm. On day 20, MBN was similar among treatments with W in soil (Ws and m/s-treatments) where it was two- to three-fold higher than the control. MBN was about two- to four-fold higher in m/s-treatments than in the m-treatments with the same mulches. MBN on days 28 and 36 did not differ among control, FBm/Ws and m-treatments (Wm, FBm and SMm), but was about up to four-fold higher in Ws, Wm/Ws and SMm/Ws. MBN in m/s-treatments was about 60% to four-fold higher than in corresponding m-treatments.

Microbial biomass P changed little throughout the experiment (Figure 2(b)) and differed little between control and m-treatments or among m-treatments. It was about 30–40% lower in m-treatments than the corresponding m/s-treatments. Compared to the control, MBP was up to two-fold higher in m/s-treatments. There was little difference between Ws and m/s-treatments except on day 20 where MBP was about 30% higher in FBm/Ws and SMm/Ws than Ws.

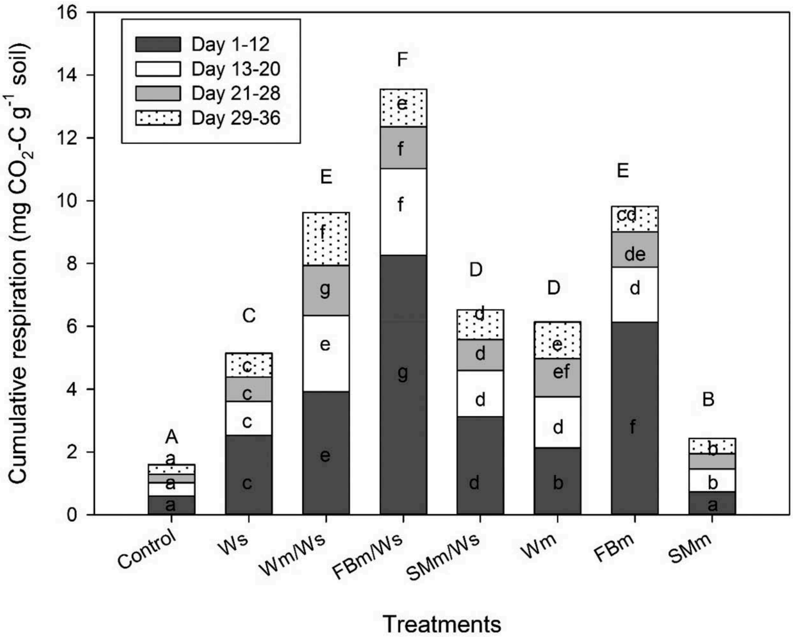

Figure 1. Cumulative respiration in mg CO_2-C g^{-1} soil in periods: days 1–12, 13–20, 21–28 and 29–36 in soil alone, or soil either mulched only or mulched and amended with mature wheat straw (W), young faba bean shoot (FB) or sheep manure (SM) and then leached. For treatment details see Table 2. Different lower-case letters indicate significant differences in cumulative respiration among treatments for a given interval. Different upper-case letters indicate significant differences in total cumulative respiration.

Table 2. Experimental design with treatment names and corresponding details about organic materials as well as mulching and amendment rates.

Treatment names	Mulch types and mulching rate (kg m^{-2})	Organic materials in soil and amendment rate (g kg^{-1})
Control	Nil	Nil
Ws	Nil	W-10
Wm/Ws	W-0.5	W-10
FBm/Ws	FB-0.5	W-10
SMm/Ws	SM-0.5	W-10
Wm	W-0.5	Nil
FBm	FB-0.5	Nil
SMm	SM-0.5	Nil

Table 3. Leached organic carbon and inorganic N and P (mg per kg mulch) leached from W, FB and SM mulches in the first leaching event (day 4) (n = 4). Within columns, means followed by different letters are significantly different ($p \le 0.05$).

	Organic C	Inorganic N	Inorganic P
Mulch types	mg kg^{-1}		
W	606^b	24^a	93^a
FB	2122^c	1142^c	225^c
SM	324^a	925^b	108^b

Available N and P

Available N was higher on day 36 than on day 12 in treatments with FB mulch (FBm/Ws and FBm) but changed little throughout the experiment in the other treatments (Figure 3(a)). Available N was between

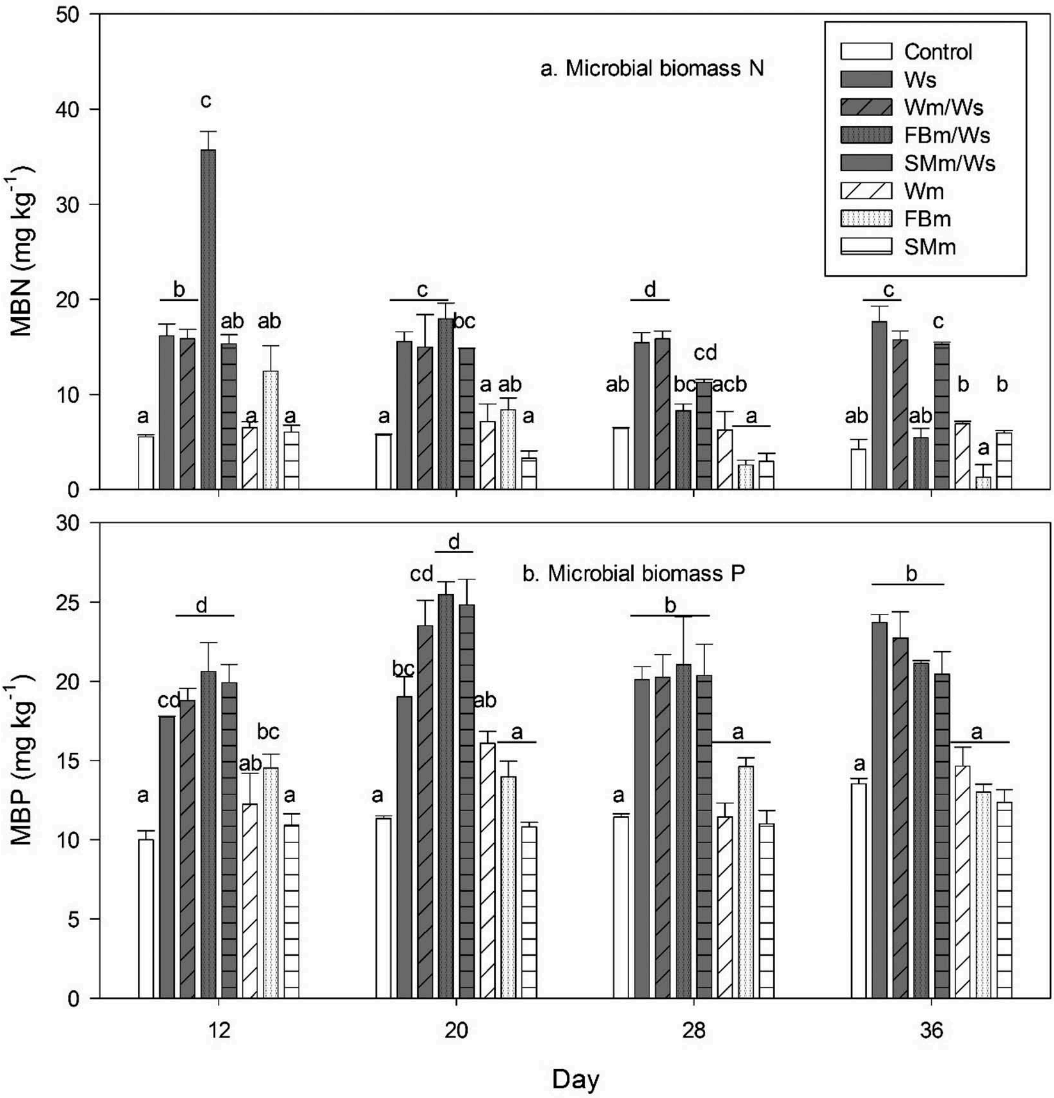

Figure 2. Microbial biomass N (a) and P (b) on days 12, 20, 28 and 36 in soil alone, or soil either mulched only or mulched and amended with mature wheat straw (W), young faba bean shoot (FB) or sheep manure (SM) and then leached. For treatment details see Table 2. At a given sampling date, different letters indicate significant differences among treatments.

30% and two-fold higher in FBm than the control with greater differences towards the end of the experiment than on day 12. It was 10–50% higher than the control in FBm/Ws except on day 12 where it was 20% lower. Available N did not differ among Ws, Wm/Ws and SMm/Ws where it was about 15-fold lower than the control. Available N was similar in the control and Wm on days 12 and 20, but it was about 20% lower in Wm than the control on days 28 and 36. Compared to the control, available N in SMm/Ws was about 20% higher on days 12 and 20, but similar on the last two sampling times.

Available P did not differ between control and Ws where it remained stable throughout the experiment (Figure 3(b)). It was between 30% and 50% higher in m/s-treatments than the control. Available P differed little among m/s-treatments where it was higher on day 36 than day 12. In m-treatments, available P was between 10% and 20% higher than the control with greater differences to the control on days 20 and 28 than the other sampling times. Available P did not differ among m-treatments on days 12 and 36 but was about 10% higher in SMm than Wm on days 20 and 28.

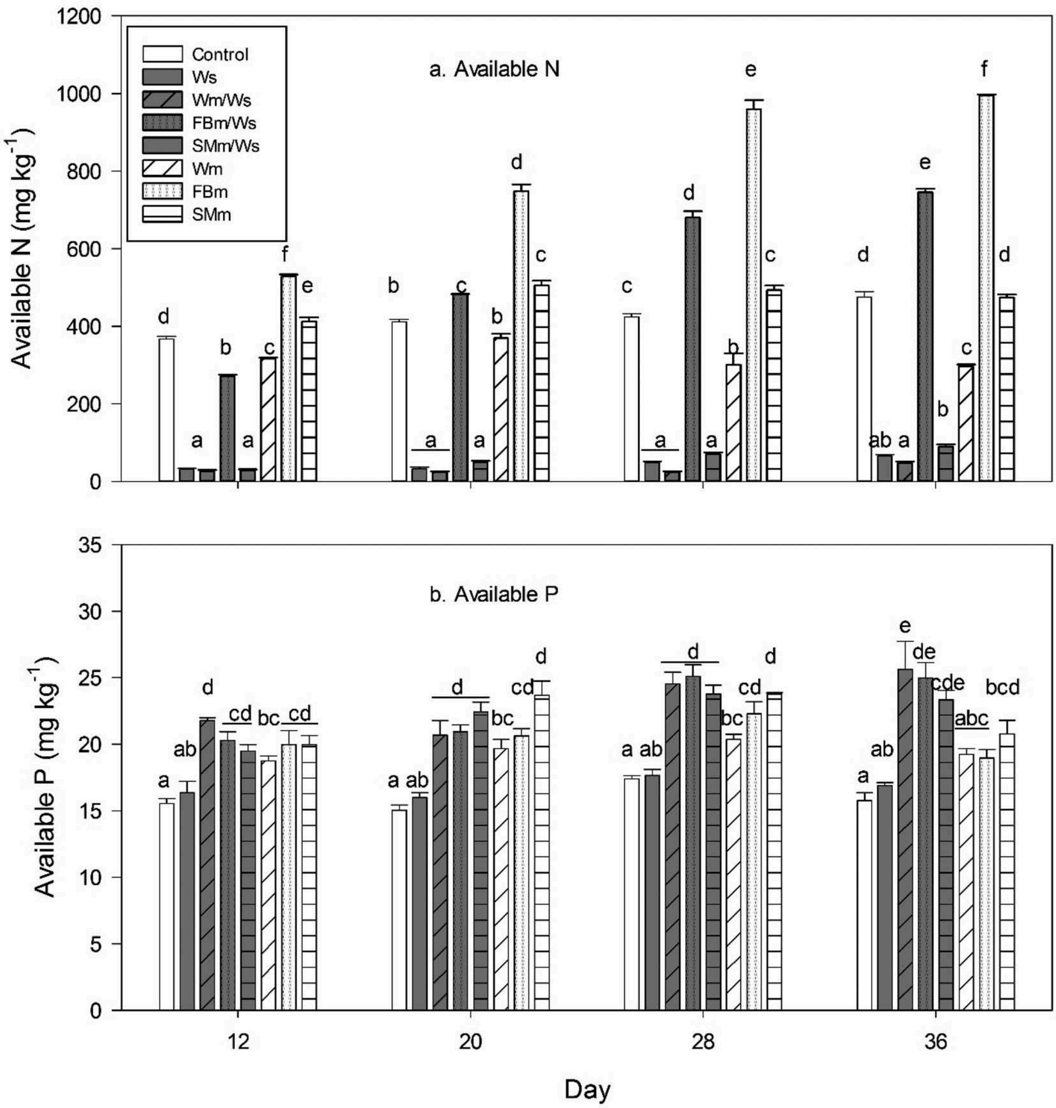

Figure 3. Available N (a) and P (b) on days 12, 20, 28 and 36 in soil alone, or soil either mulched only or mulched and amended with mature wheat straw (W), young faba bean shoot (FB) or sheep manure (SM) and then leached. For treatment details see Table 2. At a given sampling date, different letters indicate significant differences among treatments.

Discussion

The study showed that soil incorporation of W significantly reduced N availability from FB mulch. Further, wheat in the soil generally increased microbial biomass N after mulching with W or manure which reduced plant N availability but also N leaching.

The following discussion will focus on soil respiration, MBN and available N because differences in MBP were similar to those in MBN, whereas available P differed little among treatments and over time. The first hypothesis (effects of mulching on soil respiration and nutrient availability will decrease over time) can be confirmed for soil respiration (Figure 1), but not for available N. Available N increased over time with faba bean mulch and remained stable with wheat and sheep manure mulch (Figure 3 (a)). This suggests that throughout the 5 weeks, N release from faba bean remained high whereas that from wheat and sheep manure was low and changed little over time.

Mulching only

The second hypothesis (faba bean will have greater effects on soil respiration, microbial biomass N and N availability than sheep manure) can be confirmed. Among treatments with only mulch, FBm had the highest available N (Figure 3(a)). On the other hand, cumulative respiration was higher than Wm only in the first 12 days and then differed little between W and FBm (Figure 1). With FBm, MBN decreased from day 12, when it was similar as in Wm, to day 36, when it was lower (Figure 2(a)). This indicates that via leaching, FB mulch supplied easily decomposable compounds resulting in high initial respiration rates, accompanied by high N release and uptake into the microbial biomass. However, after day 12, easily decomposable compounds were probably depleted which reduced cumulative respiration and microbial N uptake. N availability increased from day 12 to 28 and remained high thereafter likely due to low N uptake by microbes which were C-limited. The lower available N/MBN ratio at the later sampling times also indicates that microbial N use efficiency in FBm decreased over time (Shi et al. 2006).

Soil mulched with SM had the lowest cumulative respiration among m-treatments throughout the experiment (Figure 1) which was likely due to low leachate organic C from SM mulch. This together with the small changes in MBN and available N throughout the experiment suggests slow mineralisation of SM mulch which can be explained by its chemical composition. Manures are decomposed during gut passage in animals (Hungate 1966; Janssen 1996). Thus, SM contained relatively recalcitrant compounds such as lignin which are decomposed slowly (Palm et al. 2001; Nhamo et al. 2003). Some N in SM mulch was released and then leached into the soil as indicated by higher available N than the control on days 12 and 20, but microbial N uptake did not increase which is likely because microbes were limited by the low organic C release from SM mulch.

Total cumulative respiration and cumulative respiration in Wm were lower than in FBm until day 28, but from day 29 to 36 cumulative respiration in Wm was higher than in FBm (Figure 1). This suggests that W mulch supplied the soil with small amounts of organic C, but this was longer lasting than with FB mulch. The higher MBN in Ws than Wm is probably due to the more intimate contact of the microbes to the straw in Ws than in Wm. In Wm only microbes adjacent to the mulch had direct contact whereas microbes deeper in the soil only received leached organic C. However, MBN was only 10 mg kg^{-1} higher in Ws than Wm whereas available N was about 300 mg kg^{-1} lower (Figure 2(a)). This suggests that N mineralisation was very low in Ws possibly because the presence of the straw limited access of microbes to soil N. The low N availability may be due to binding of ammonium to the straw (Makkar and Singh 1987) and its high lignin content (Del Río et al. 2012).

Mulching on soil mixed with W

Throughout the experiment, cumulative respiration per g soil was higher in m/s-treatments than the corresponding m-treatments, Ws and the control (Figure 1). This can be explained by the greater amount of C in m/s-treatments than in the m-treatments and Ws which is in agreement with previous studies using organic amendments (Tu et al. 2006; Duong et al. 2009). The higher cumulative respiration in m/s-treatments than in corresponding m-treatments throughout the experiment indicates that in m/s-treatments, W in soil supplied microbes with a more abundant and longer lasting source of C. In contrast, in m-treatments, mulches supplied the soil with mainly soluble C which was respired quickly. Only microbes close to the soil surface had direct contact with the organic materials in the mulches.

The third hypothesis (nutrient availability in mulched soil will be lower in soil that contains wheat straw than without straw because microbes decomposing wheat will immobilise nutrients leached from mulches) can be confirmed for available N. Available N was lower in Wm/Ws, FBm/Ws and SMm/Ws than in corresponding m-treatments whereas MBN was higher.

In Wm/Ws, available N was up to ten-fold lower than the control and Wm (Figure 3(a)), but MBN was only about 70% higher (Figure 2(a)). This is likely due to net immobilisation, microbial

N limitation and binding of ammonium to straw (Makkar and Singh 1987) in Wm/Ws and Ws. Low N availability after soil amendment with high C/N residues is consistent with previous studies where wheat straw was mixed in the same soil (Marschner et al. 2015) or in soil mulched with millet straw that had high C/N ratio (Ibrahim et al. 2018).

In FBm/Ws on day 12, available N was only 20% lower than in the control but MBN was five-fold higher, indicating net immobilisation. Microbes decomposing W mixed in soil likely took up a proportion of N leached from FB mulch. On day 12, microbial N uptake in FBm/Ws was about three-fold higher than in FBm, but on day 36 it was similar (Figure 3(a)). After day 12, MBN decreased in FBm/Ws, whereas available N increased, indicating that N release was greater than microbial N uptake. Some of the available N could have come from microbial biomass turnover. Compared to FBm, available N in FBm/Ws was lower throughout experiment likely because in FBm/Ws, a proportion of N leached from FB mulch was taken up by microbes decomposing W. In FBm on the other hand, N release far exceeded N uptake by microbes which became C-limited in the late stages of decomposition. This indicates higher microbial N use efficiency in FBm/Ws than FBm.

In SMm/Ws, available N was about ten-fold lower than in the control and SMm, but MBN was up to 70% higher, indicating net immobilisation. It is possible that the small amount of N released from SM mulch limited microbial N uptake. This is indicated by the fact that MBN in SMm/Ws was similar as in the control and changed little over time, although microbes were supplied with abundant C from W mixed in the soil. Higher N availability in SMm/Ws on day 36 than on day 12 could be explained by a turnover of microbial biomass.

Conclusion

Sheep manure and faba bean had similar low C/N ratio. However, the increase in available N, MBN and soil respiration was greater with faba bean than with sheep manure mulch which can be attributed to the lower decomposability of sheep manure. Mulching with low C/N organic materials alone increased available N and decreased MBN compared to mulch over soil mixed with wheat straw. Application of faba bean mulch on soil mixed with wheat straw will provide higher plant-available nutrients than unamended soils, but reduce nutrient availability compared to faba bean mulch only. The reduction in nutrient availability could limit nutrient uptake by crops in the short term. With manure, wheat in the soil resulted in very low available N concentrations which could limit plant N uptake. But by enhancing microbial N uptake, wheat straw in soil under faba bean or sheep manure mulch can reduce N loss via leaching compared to only mulch. The results of this experiment provide information about plant nutrient availability in the first weeks after amendment which is useful for the early growth of crops planted immediately after amendment. Longer term studies with plants are necessary to assess the effect high C/N mulch and low C/N mulches differing in decomposability on soil with and without wheat straw mixed on crop productivity.

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CHAPTER 7

INFLUENCE OF MULCH C/N RATIO AND DECOMPOSITION STAGE ON PLANT N UPTAKE AND N AVAILABILITY IN SOIL WITH OR WITHOUT WHEAT STRAW

Statement of Authorship

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Name of Principal Author (Candidate)	Thi Hoang Ha Truong		
Contribution to the Paper	Performed experiment and analyses of all samples, data analysis and interpretation and manuscript writing. I hereby certify that the statement of contribution is accurate.		
Overall percentage (%)	60%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	22/2/2020

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

xvi. the candidate's stated contribution to the publication is accurate (as detailed above);

xvii. permission is granted for the candidate in include the publication in the thesis; and

xviii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Petra Marschner		
Contribution to the Paper	Supervised development of the work, data interpretation and manuscript evaluation and correction. I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.		
Signature		Date	22/02/2020

Name of Co-Author	Paul Kristiansen		
Contribution to the Paper	Contributed to discussion and proof-reading and revision of manuscript. I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.		
Signature		Date	8/2/2020

Influence of mulch C/N ratio and decomposition stage on plant N uptake and N availability in soil with or without wheat straw

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Abstract

Mulches can improve soil properties, but little is known about nutrient availability in mulched soil that contains plant residues and the effect of mulching with manures. The aim of this study was to determine the effects of mulching with high or low C/N organic materials, in which low C/N materials differed in decomposability, and the presence of wheat straw in the soil on plant growth and N uptake, soil N availability and microbial biomass N within about four months after mulching. Three organic materials were used: mature wheat straw (W, C/N 80), young faba bean shoots (FB, C/N 7), and sheep manure (SM, C/N 8). There were eight treatments differing in amendment methods (mulching or mixing with W or both) and mulching materials (W, FB or SM). Treatments that were only mulched with W, FB or SM are referred to as m-treatments. In m/s-treatments, after W was mixed into the soil, W, FB or SM were placed on the soil surface as mulch. Two other treatments included an unamended control and soil mixed with W. Wheat was planted 0, 35 or 70 days after mulching (referred to as 0, 35, and 70 DAM) and grown for 35 days. Faba bean mulch increased shoot dry weight, shoot N uptake and available N compared to wheat or sheep manure mulch, particularly in the m-treatments. Shoot dry weight was higher in m-treatments than corresponding m/s-treatments with the same mulch type. Shoot N uptake was higher in 70 DAM than in 0 DAM in all treatments and 0.3 to three-fold higher in m-treatments than the corresponding m/s-treatments. Microbial biomass N was higher in 0 DAM than in 35 and 70 DAM in most treatments and up to two-fold higher in m/s-treatments than the corresponding m-treatments. Available N in m/s-treatments was two to six-fold higher than m-treatments in 0 DAM, but differed little in older mulch ages of W and SM. It can be concluded that compared to soil with only mulch, mixing of wheat straw into soil reduced plant growth and N uptake, particularly in the early stages of mulching (0 and 35 DAM). However, the presence of wheat in mulched soil may provide a longer lasting source of N for plants and reduce the risk of N leaching from rapidly decomposing low C/N mulch due to greater microbial biomass N uptake than only soil with mulch.

Key words: C/N ratio / manure / mulch / microbial biomass N / N availability / shoot dry weight / wheat straw

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1 Introduction

Mulching with organic materials improves soil quality and productivity through their beneficial effects on soil properties (*Tu* et al., 2006; *Mulumba* and *Lal*, 2008; *Marinari* et al., 2015). The application of organic mulches increases soil organic C content (*Havlin* et al., 1990; *Duiker* and *Lal*, 1999; *Saroa* and *Lal*, 2003), reduces nutrient loss (*Rees* et al., 1999), increases soil microbial biomass and activity (*Tu* et al., 2006), enhances soil moisture storage (*Ji* and *Unger*, 2001), and buffers changes in soil temperature (*Pinamonti*, 1998; *Naeini* and *Cook*, 2000). Decomposition of organic mulches and leaching can add nutrients to the soil (*Tu* et al., 2006; *Halde* and *Entz*, 2016; *Ibrahim* et al., 2018). As a result, mulching with organic materials can increase crop growth and yield (*Sinkevičienė* et al., 2009; *Kumar* et al., 2014; *Ibrahim* et al., 2015).

The effect of organic amendments on soil properties and plant growth is influenced by their chemical composition which determines decomposition rate and nutrient availability (*Tian* et al., 1992; *Bending* and *Turner*, 1999). Key properties of organic materials are nitrogen (N) concentration and carbon/nitrogen (C/N) ratio (*Hadas* et al., 2004). The influence of organic mulches on crop growth, yield and nutrient uptake also depends on their C/N ratio. Higher crop yields with low C/N mulches (C/N < 20) compared to higher C/N mulches (C/N > 20) have been shown in a number of studies. For example, *Radicetti* et al. (2016) planted eggplants in soil mulched with residues of hairy vetch (C/N 13), oat (C/N 46) and oilseed rape (C/N 26). Hairy vetch increased marketable yield and dry matter of eggplants compared to soils without mulch or mulched with the other two residues. *Ibewiro* et al.

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(2000) examined the effect of mulching with shoot and root residues of two legumes (C/N 9 and 11) and leaf and root residues of a grass (C/N 58 and 71) on maize dry weight and N uptake in two cropping seasons. Legume mulches decomposed much more quickly and resulted in higher maize yield and N uptake than grass leaf and root mulches. However, *Ibrahim* et al. (2015) used mulches with C/N ratios between 20 and 64 and found that all mulches increased millet growth by 30–50% compared to the control. They explained this by the higher water retention capacity and efficient use of available rainfall in mulched soil compared to the control.

Previous studies on the effect of organic mulch on crop growth and/or nutrient uptake focused on comparing several types of organic mulches with a wide range of C/N ratios. There is limited understanding about the effect of mulching on crop N uptake in soil that contains plant residues, which may occur in the field. For example, after harvest, plant residues such as cereal stubble may remain undecomposed in soil until the following crop is planted. The presence of high C/N crop stubbles can lead to net N immobilization and thus limited plant N uptake and may reduce crop yield (*Gupta* et al., 2018). Further, little is known about the effect of mulching with manures on plant N uptake which may occur in fields where livestock is present or after manure spreading. Manures can have a low C/N ratio, but they are already strongly decomposed while passing through the digestive system of animals and may be further decomposed during storage (*Hungate*, 1966). Therefore, they are at a late stage of decomposition and after addition to soil, may result in lower soil respiration and N availability than plant residues with similar C/N ratio. The decomposition stage, *i.e.*, time since mulch application, may also influence nutrient release as easily decomposable compounds are depleted over time (*Berg* and *Laskowski*, 2005; *Ibrahim* et al., 2018).

The aim of this study was to determine the effects of mulching with high or low C/N organic materials, in which low C/N materials differed in decomposability, and the presence of high C/N residues in the soil on plant growth and N uptake, soil N availability and microbial biomass within about four months after mulching. The organic materials (*i.e.*, wheat straw, faba bean shoots, sheep manure) were selected based on a preliminary experiment which showed that although young faba bean shoot and sheep manure had a similar low C/N ratio, soil amendment with sheep manure resulted in lower soil respiration and N availability than faba bean. Mature wheat straw was chosen as high C/N amendment. The effects of presence of high C/N material in the soil and mulch decomposition stage were investigated by placing mulch on soil that was unamended or mixed with mature wheat straw followed by incubation for 0, 35, and 70 days before planting wheat.

The first hypothesis was that the effect of mulching on shoot dry weight, shoot N concentration, shoot N uptake, microbial biomass N and available N will increase with mulch age because nutrients will be released over time and, in absence of leaching or plant uptake, accumulate in the soil. The second hypothesis was that in soils with only mulch, faba bean mulch will increase the measured parameters compared to wheat or sheep manure mulch due to its higher water extractable nutrients. Based on differences in decomposability of faba bean and sheep manure, the third hypothesis was that faba bean will have greater effects on shoot dry weight, shoot N concentration and uptake, microbial biomass N and available N than sheep manure. The fourth hypothesis was that, compared to soil with only mulch, shoot dry weight and shoot N uptake will be lower in mulched soil that contains wheat straw, but microbial biomass N will be higher because microbes decomposing straw will immobilize N leached from mulches.

2 Material and methods

2.1 Soil and organic materials

The sandy clay loam used in this study was collected from 0 to 10 cm at Waite Campus, The University of Adelaide (Longitude 138°38′3.2′′ E, Latitude 34°58′0.2′′ S). Collection and soil properties have been described before (*Truong* and *Marschner*, 2018). Briefly, the soil is a Chromosol in Australian soil classification (*Isbell*, 2002) and a Rhodoxeralf according to US Soil Taxonomy (*USDA*, 1999). It has been managed as permanent pasture for over 80 years. The soil has the following properties (for methods see below): sand 54%, silt 20%, and clay 26%, pH (1:5 soil : water) 6.3, electrical conductivity (EC 1:5 soil : water) 143 μS cm^{-1}, total N 1.5 g kg^{-1}, and total P 371 mg kg^{-1}, total organic carbon (TOC) 17 g kg^{-1}, available N 15 mg kg^{-1}, available P 10 mg kg^{-1}, maximum water holding capacity (WHC) 378 g kg^{-1}, and bulk density 1.3 g cm^{-3}. After collection, the soil was dried at 40°C in a fan-forced oven. Then, visible plant debris was removed and the soil sieved to < 2 mm.

The organic materials were dried at 40°C in a fan-forced oven and then finely ground and sieved to 0.25–2 mm. The small particle size was used to maximize contact of organic materials with the soil and thus increase their decomposition in soil and mulches. Three types of organic materials were used: young faba bean shoots (*Vicia faba* L., referred to as FB, C/N 7) as fast decomposing low C/N material, sheep manure (referred to as SM, C/N 8) as slow decomposing low C/N material, and mature wheat straw (*Triticum aestivum* L., referred to as W, C/N 80) as high C/N material (Tab. 1). Compared to SM, total organic C was about three-fold higher in W and FB, while water extractable organic C was about nine-fold higher in W and twenty-two-fold higher in FB. Total N was 3–9 times higher in FB and SM than in W. The C/N ratio was similar in FB and SM, where it was more than tenfold lower than in W. Water extractable N was 5–11 times higher in FB and SM than in W.

2.2 Experimental design

Before the start of the experiment, the soil was incubated for 10 days at 50% of maximum WHC at 20–25°C in the dark to activate the soil microbes and stabilize soil respiration after rewetting of air-dry soil. In previous studies with this soil, microbial activity was maximal at 50% WHC (*Marschner* et al., 2015). Soil was either left unamended or amended with

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Table 1: Total organic C, N and C/N ratio, water extractable C and N and pH of mature wheat straw (W), young faba bean shoot (FB) and sheep manure (SM) (n = 4). Within rows, means followed by different letters are significantly different ($p \leq 0.05$).

	W	FB	SM
Total organic C (g kg^{-1})	405.85^{c}	318.43^{b}	116.43^{a}
Total N (g kg^{-1})	5.13^{a}	47.07^{c}	15.19^{b}
C/N	79.70^{b}	6.79^{a}	7.71^{a}
Water extractable C (g kg^{-1})	25.18^{b}	63.55^{c}	2.80^{a}
Water extractable N (g kg^{-1})	0.15^{a}	0.72^{b}	1.63^{c}
pH (1:10)	5.44^{a}	5.40^{a}	6.55^{b}

organic materials by mixing and/or mulching. There were eight treatments differing in amendment methods (mulching or mixing with W or both) and mulching materials (W, FB or SM). In treatments that were mulched only, referred to as m-treatments, W, FB or SM were mulched, giving treatments Wm, FBm, and SMm. In m/s-treatments, after W was mixed into the soil, W, FB or SM were placed on the soil surface as mulch, named treatments Wm/Ws, FBm/Ws, and SMm/Ws. Two other treatments included an unamended control (control) and soil mixed with W (Ws). W was mixed into the soil because it was expected that W leads to immobilization of nutrients leached from the mulch. In Ws and m/s-treatments, wheat straw was mixed at a rate of 10 g kg^{-1} soil [4 g mature wheat straw was thoroughly mixed in 396 g soil (dry weight equivalent) in a plastic bag]. With this addition rate of wheat straw, the amount of C added into the soil was about 4 g kg^{-1} which was within the range used in other studies from our group [*e.g.*, from 3 g kg^{-1} in *Shi* et al. (2013) to 7 g kg^{-1} in *Nguyen* and *Marschner* (2016)]. Then the amended soil was filled into 500-mL pots lined with plastic bags. For mulch treatments, organic materials were placed on the soil surface at 0.5 kg m^{-2} (3.6 g organic materials per pot). The mulching rate of 0.5 kg m^{-2} was chosen because it was used in other studies (*e.g.*, *Halde* and *Entz*, 2016). Further, our preliminary experiments showed that there were little differences in N availability among four different mulching rates: 0.25, 0.5, 0.75, and 1 kg m^{-2}. Mulches were moistened and spread evenly on the soil surface. In m/s-treatments, the amount of organic materials in mulches (3.6 g per pot) was close to that in soil (4 g per pot).

The treatments were prepared so that they were all planted with wheat on the same day (70 days after preparation of the first batch), but differed in mulch age at planting. Wheat was planted either immediately after mulching or 35 or 70 days after mulching (mulch age 0, 35, and 70, referred to as 0 DAM, 35 DAM, 70 DAM). There were four replicate pots per treatment. Before planting, pots for 35 DAM and 70 DAM were covered with aluminium foil and incubated at room temperature. During incubation, soil water content at 50% of WHC and mulch moisture were maintained by checking the water content every few days by weighing the pots and spraying with reverse osmosis (RO) water if necessary. All pots were placed at the same time in a glasshouse with natural light in a randomized block design with three blocks, one for each mulch age. Ten pre-germinated wheat seeds (*Triticum aestivum* L. variety Axe) were planted in the pots. Wheat plants were harvested 35 days after planting. During plant growth, pots were watered daily by weight to maintain soil water content at 50% of WHC. The temperature in the glasshouse during the experiment ranged from 17 to 25°C.

2.3 Analyzes and calculations

Soil and organic material properties were analyzed prior to the experiment. Soil analyzes were carried out as described in *Marschner* et al. (2015). Briefly, soil texture was determined by the hydrometer method (*Gee* and *Or*, 2002). Soil pH was determined in a 1:5 soil : water extract after 1 h end-over-end shaking at 25°C (*Rayment* and *Higginson*, 1992). Soil water holding capacity was determined using a sintered glass funnel connected to a 1 m water column (matric potential = –10 kPa) (*Wilke*, 2005). Total organic carbon content of soil and organic materials was determined by wet oxidation and titration (*Walkley* and *Black*, 1934). To determine total N in soil and organic materials, the material was digested with H_2SO_4 and H_2O_2. Total N was measured by a modified Kjeldahl method (*Bremner* and *Mulvaney*, 1982). Available N (ammonium and nitrate) concentration was measured after 1 h end-over-end shaking with 2 M KCl at 1:10 soil extractant ratio. Ammonium-N was determined after *Forster* (1995). Nitrate-N was measured as described in *Miranda* et al. (2001). Microbial biomass N (MBN) was determined by chloroform fumigation-extraction with 0.5 M K_2SO_4 at 1:4 soil to extractant ratio (*Moore* et al., 2000). Ammonium concentration in the extract was determined as described above for available N. Microbial biomass N is the difference in NH_4^+ concentration between fumigated and non-fumigated samples divided by 0.57 which is the proportionality factor to convert ammonium to MBN (*Moore* et al., 2000).

2.4 Statistical analysis

One-way analysis of variance (ANOVA) was used to compare initial properties of three organic materials. There were four replicates per treatment and mulch age (block). Prior to the analysis, normal distribution of the residuals was checked using residual *versus* fitted plots in Genstat 18th edition (VSN Int. Ltd., UK). Data were analyzed by one-way ANOVA for the first week sampling and for each mulch age separately using Genstat 18th edition. To determine the effect of mulch age, the data were also analyzed by one-way ANOVA for each treatment separately. Tukey's multiple comparison tests at 95% confidence interval was used to determine significant differences among treatments or mulch ages.

3 Results

3.1 Microbial biomass N and N availability 7 days after mulching

Compared to the control, MBN was three-fold higher in FBm/Ws and two-fold higher in Ws, Wm/Ws, SMm/Ws, and FBm (Tab. 2). But MBN did not differ between the control and

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Table 2: Microbial biomass N (MBN) and available N measured on day 7 after daily leaching from day 1 ($n = 4$). For a given parameter, different letters indicate significant differences among treatments ($p \leq 0.05$).

Treatments	MBN ($mg\ kg^{-1}$)	Available N ($mg\ kg^{-1}$)
Control	7.0^{a}	230.4^{c}
Ws	14.6^{b}	17.6^{a}
Wm/Ws	15.9^{b}	5.1^{a}
FBm/Ws	22.8^{c}	63.6^{b}
SMm/Ws	15.8^{b}	43.7^{b}
Wm	6.8^{a}	224.0^{c}
FBm	14.3^{b}	297.8^{e}
SMm	6.5^{a}	256.8^{d}

Wm and SMm. Compared to the control and Wm, available N was 45 times lower in Ws and between four and 10 times lower in m/s-treatments. Available N was about 20% higher than the control in FBm and SMm.

3.2 Shoot and root dry weight, shoot and root ratio

Shoot dry weight increased with mulch age in Ws and m/s-treatments, but differed little among mulch ages in the control and m-treatments (Tab. 3). At all mulch ages, shoot dry weight did not differ between the control and Wm and SMm (Fig. 1a). Compared to the control, shoot dry weight was about 20% higher in FBm and 10–15% higher in FBm/Ws in 35 DAM and 70 DAM. However, shoot dry weight was 30–50% lower than the control in Ws, Wm/Ws, and SMm/Ws. Shoot dry weight was generally higher in m-treatments than corresponding m/s-treatments with the same mulch type.

Root dry weight increased with mulch age in Ws but changed little in the other treatments (Tab. 3). In 0 DAM, root dry weight was similar in the control and m-treatments (Fig. 1b). But it was more than 50% lower in Ws, Wm/Ws, and SMm/Ws and 30% lower in FBm/Ws. Root dry weight in 35 DAM was similar as in the control in most treatments except Ws, Wm/Ws, and FBm. Root dry weight differed little among treatments in 75 DAM. It was higher in m-treatments than corresponding m/s-treatments only in 0 DAM.

The shoot/root ratio was higher in 70 DAM than 0 DAM in most treatments except Ws where shoot/root ratio was not influenced by mulch age (Tab. 3, Fig. 1c). The shoot/root ratio was similar among treatments in 0 DAM. In 35 and 70 DAM, the shoot/root ratio was 20% lower than the control in Ws, Wm, SMm/Ws, but 20% higher in FBm/Ws, FBm and SMm.

3.3 Shoot N concentration and N uptake

Shoot N concentration was higher in 70 DAM than 0 DAM in most treatments except the control, Wm/Ws and FBm/Ws where it differed little among mulch ages (Tab. 3). At all mulch ages, shoot N concentration was between 20% and 30% higher than the control in FBm (Fig. 2a). In 0 DAM and 70 DAM, shoot N concentration was 20% lower than the control in Ws and Wm/Ws. It did not differ between the control and the other treatments.

Shoot N uptake was higher in 70 DAM than in 0 DAM in all treatments (Tab. 3). In all mulch ages, shoot N uptake was highest in FBm where it was 50% to two-fold higher than in the control, Wm and SMm (Fig. 2b). Shoot N uptake was 30–75% lower than the control in Ws, Wm/Ws, and SMm/Ws. In 35 DAM and 70 DAM, shoot N uptake in FBm/Ws was 30–75% higher than the control. Shoot N uptake was 30% to three-fold higher in m-treatments than the corresponding m/s-treatments.

3.4 Microbial biomass N

MBN was higher in 0 DAM than in 35 and 70 DAM in most treatments except the control where it changed little with mulch age (Tab. 3). In 0 DAM, MBN compared to the control was up to two-fold higher in Ws and m/s-treatments and 60% higher in Wm and FBm (Fig. 3a). It did not differ between the control and SMm. MBN in 35 DAM and 70 DAM was 40–80% higher than the control in Ws, Wm/Ws, and SMm/Ws. It differed little between the control and m-treatments. In 70 DAM,

Table 3: Comparison of measured parameters for different mulch ages for each treatment separately ($n = 4$). For a given treatment, letters indicate differences between mulch ages ($p \leq 0.05$).

Treatments	Mulch ages (days after mulching)	Shoot DW ($mg\ plant^{-1}$)	Root DW ($mg\ plant^{-1}$)	Shoot/root ratio	Shoot N concentration ($mg\ g^{-1}$)	Shoot N uptake ($mg\ plant^{-1}$)	MBN ($mg\ kg^{-1}$)	Available N ($mg\ kg^{-1}$)
Control	0	1.1^{a}	1.1^{b}	1.0^{a}	30.8	3.5^{a}	8.4^{a}	13.1^{b}
	35	1.2^{ab}	0.9^{a}	1.3^{b}	35.0	4.3^{ab}	11.7^{b}	6.7^{a}
	70	1.3^{b}	0.8^{a}	1.5^{c}	38.0	4.8^{b}	8.1^{a}	40.0^{c}
Ws	0	0.4^{a}	0.5^{a}	0.9	20.7^{a}	0.9^{a}	18.3^{c}	32.6^{b}
	35	0.6^{b}	0.7^{b}	0.9	26.9^{b}	1.6^{b}	15.9^{b}	17.2^{a}
	70	0.9^{c}	0.9^{c}	1.0	25.8^{b}	2.3^{c}	12.8^{a}	29.7^{b}

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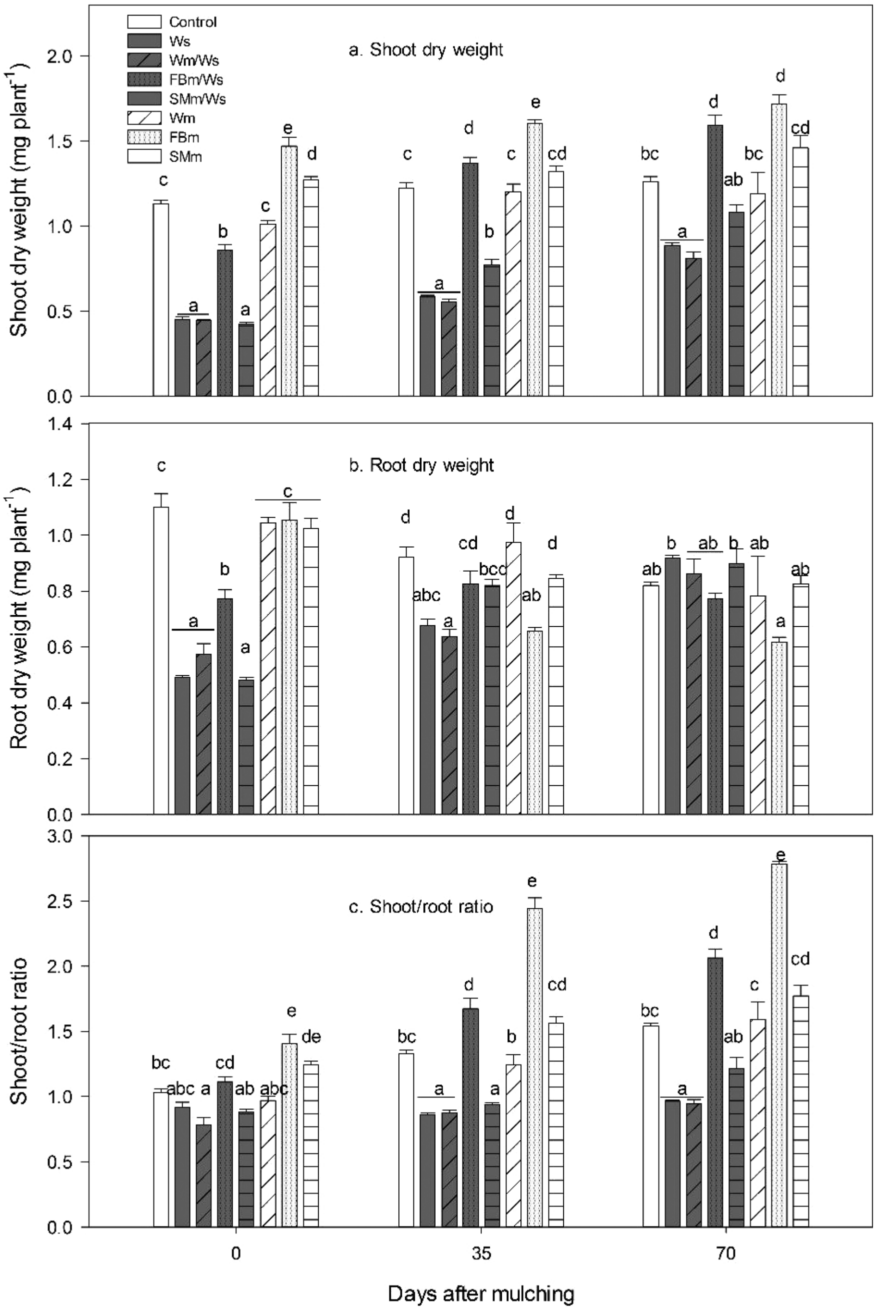

Figure 1: Shoot dry weight (a), root dry weight (b), and shoot/root ratio (c) of 5 week old wheat planted in soil 0, 35, and 70 days after mulching in unamended soil or soil either mulched only with mature wheat straw (W), young faba bean shoot (FB) or sheep manure (SM) or mulched and with mature wheat straw mixed into the soil and then leached daily (n = 4 ± standard error). At a given mulch age, different letters indicate significant differences among treatments.

MBN in FBm/Ws was 40% higher than the control and was up to two-fold higher in m/s-treatments than the corresponding m-treatments.

3.5 Available N

Available N increased with mulch age in m-treatments, while it was lower in 35 DAM than other mulch ages in the control, Ws and SMm/Ws. It differed little among mulch ages in Wm/Ws and FBm/Ws (Tab. 3). In 0 DAM, available N was up to two-fold higher in Ws and m/s-treatments than the control (Fig. 3b). Compared to the control, available N in 35 DAM was two-fold higher in Ws, Wm/Ws, and Wm, but four-fold higher in FBm/Ws and more than 10-fold higher in FBm. Available N in 70 DAM differed little between the control and most treatments except for Wm/Ws and SMm/Ws where it was 50% lower than the control and in FBm where it was six-fold higher. Available N was two to six-fold higher in m/s-treatments than corresponding m-treatments in 0 DAM. But in older mulches, it differed little between the corresponding treatments except with faba bean mulch where it was up to six-fold higher in FBm than FBm/Ws.

4 Discussion

The study showed that mulching only with FB resulted in higher shoot dry weight and N uptake than unamended soil and other mulching treatments. However, in the late stages of decomposition, release of N from FB mulch exceeded plant and microbial N uptake which could lead to N loss *via* leaching into deeper soil layers. In the later stages of decomposition, the presence of W in soil mulched with FB resulted in lower shoot N uptake than FB mulch alone, but shoot dry weight and N uptake were higher than the control. Further, W in soil mulched with FB increased microbial biomass compared to the control and soil mulched only with FB. This could reduce N loss and increase potential N availability after microbial biomass turnover.

The first hypothesis (the effect of mulching on shoot dry weight, shoot N concentration, shoot N uptake, microbial biomass N, and available N will increase with mulch age) was only confirmed for shoot dry weight in m/s-treatments, available N in m-treatments, and shoot N uptake for some m/s- and m-treatments. This suggests that in m/s-treatments, N released from mulches were taken up by plants and microbes and could therefore not accumulate as available N in the soil. In Wm/Ws this is likely due to the low nutrient release from W throughout the decomposition. In FBm/Ws and SMm/Ws, shoot N uptake increased with mulch age but available N did not. This indicates that the plants were able to access more N with older mulches and prevented an increase in N availability. Some of the N taken up by the plants may have also come

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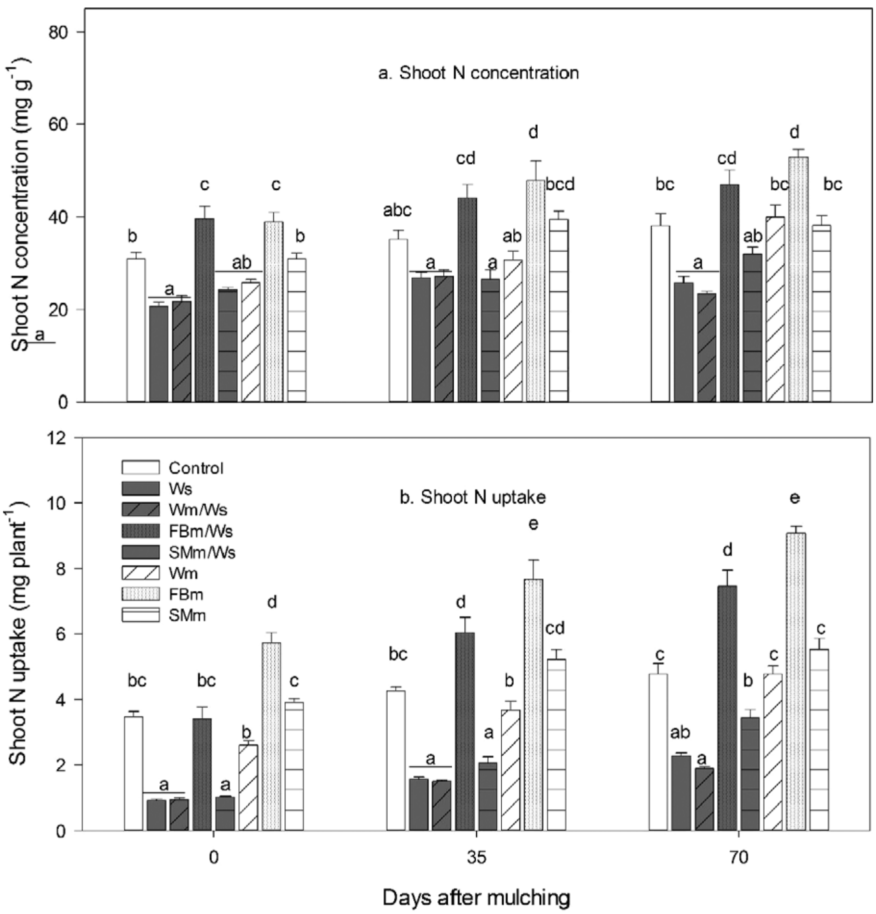

Figure 2: Shoot N (a) concentration and N (b) uptake of 5 week old wheat planted in soil 0, 35, and 70 days after mulching in unamended soil, or soil either mulched only with mature wheat straw (W), young faba bean shoot (FB) or sheep manure (SM) or mulched and with mature wheat straw mixed into the soil and then leached daily (n = 4 ± standard error). At a given mulch age, different letters indicate significant differences among treatments.

from microbial biomass turnover as MBN decreased with mulch age.

4.1 Mulching only

The second hypothesis (in soils with only mulch, faba bean mulch will increase the measured parameters compared to wheat or sheep manure mulch) can be confirmed for shoot dry weight, shoot N concentration, shoot N uptake, and available N. Compared to the control, Wm and SMm, FBm had highest shoot dry weight, shoot/root ratio, shoot N concentration and uptake. Thus, decomposition of FB mulch resulted in high N release and high N uptake irrespective of mulch age which can be explained by its high N concentration (*Moritsuka* et al., 2004). FBm had higher MBN than the control only in 0 DAM where it was 40% higher. But in the later stages of decomposition (35 and 70 DAM), MBN was similar as in the control although available N was up to ten-fold higher in FBm. This indicates that most easily available C was released from FB mulch within the first weeks and that C availability limited microbial N uptake later. The accumulation of available N in the later stages of decomposition suggests that mineralised N also exceeded plant N uptake.

Mulching only with W did not increase shoot dry weight, shoot N concentration and uptake and available N compared to the control. This can be explained by the low N concentration and high C/N ratio of W and thus small net N release (*Nguyen* and *Marschner*, 2016; *Zhang* and *Marschner*, 2017). In Wm, MBN in 0 DAM was about 50% higher than the control, but available N was three-fold lower, indicating net immobilization. But in 35 and 70 DAM, MBN and available N differed little between Wm and the control, suggesting that immobilization was lower than initially due to depletion of easily available C. In Ws compared to Wm, MBN was higher, but shoot dry weight and N uptake were lower. The higher MBN in Ws was likely due to greater access of microbes to W than in Wm where only microbes close to the W mulch had direct contact. Therefore, in Ws, plants were likely outcompeted by microbes for N which resulted in lower shoot dry weight and N uptake than in Wm.

The third hypothesis (faba bean will have greater effects on shoot dry weight, shoot N concentration and uptake, MBN and available N than sheep manure) was confirmed for most measured parameters, except MBN which did not differ between FBm and SMm. Shoot dry weight, shoot N concentration and N uptake in soil mulched with SM were lower than in FBm and differed little from the control. Further, MBN in SMm was similar to the control and changed little over time, whereas available N increased with mulch age. This suggests slow mineralization of SM mulch compared to FB which can be explained by its highly decomposed state. Manures are already decomposed in the digestive system of animals and may be further decomposed during storage (*Hungate*, 1966). Thus, manures contain relatively recalcitrant compounds and have low C/N ratios. Some water extractable N was initially leached from SM mulch, as indicated by higher available N than the control 7 days after mulching, but MBN was similar as in the control which was likely due to low release of organic C from SM mulch and/or N uptake by the plants.

4.2 Mulching on soil mixed with W

The fourth hypothesis (compared to soil with only mulch, shoot dry weight and shoot N uptake will be lower in the mulched soil that contains wheat straw, but MBN will be higher) can be confirmed for soil mixed with W and mulched with W or SM, but not for Wm/Ws. Shortly after mulching (7 days) and in the different mulch ages (0, 35, and 70 DAM), MBN in m/s-treatments was higher than in the control and corre-

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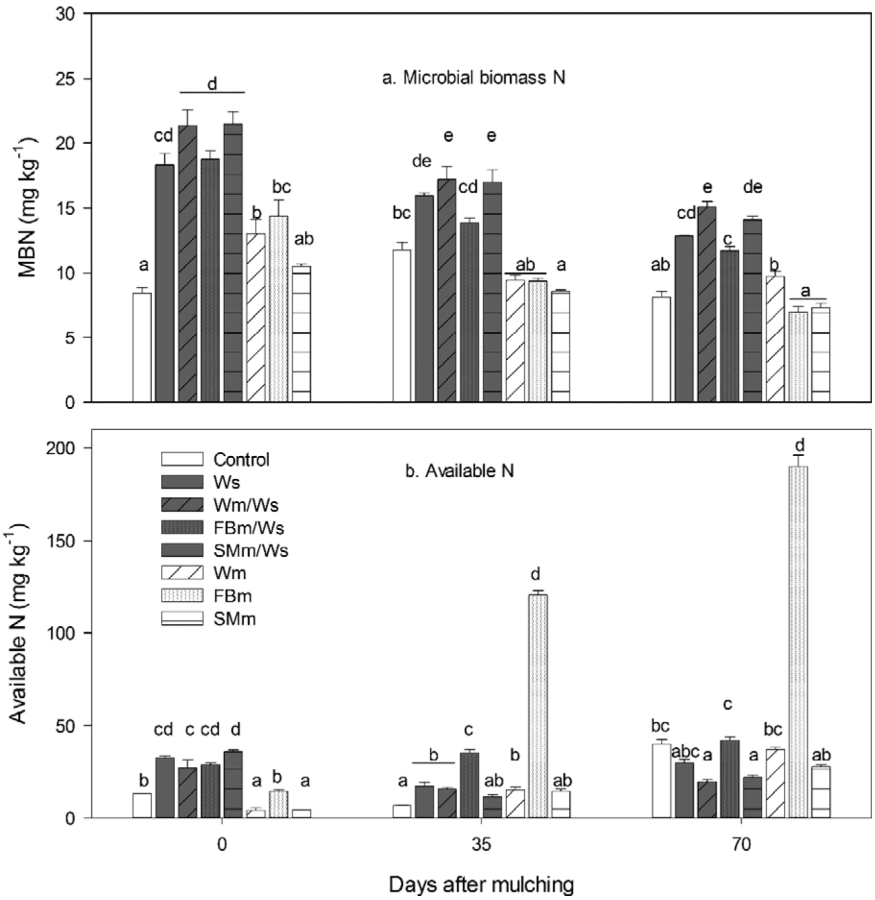

Figure 3: Microbial biomass N (a) and available N (b) at plant harvest, 5 weeks after planting in soil 0, 35, and 70 days after mulching in unamended soil, or soil either mulched only with mature wheat straw (W), young faba bean shoot (FB) or sheep manure (SM) or mulched and with mature wheat straw mixed into the soil and then leached daily ($n = 4 \pm$ standard error). At a given mulch age, different letters indicate significant differences among treatments.

sponding m-treatments. But available N in m/s-treatments differed little from the control. This indicates net immobilization in m/s-treatments which was likely due to the presence of W in soil. Net N immobilization also occurs in soils where high C/N crop stubbles are retained in soil after harvest (*Gupta* et al., 2018; *Hart* et al., 1993). Shoot dry weight and N uptake in Wm/Ws and SMm/Ws was always lower than in the corresponding m-treatments indicating that plants were limited by N. But in FBm/Ws, shoot dry weight and N uptake were lower than FBm only in 0 DAM. This suggests that N release from FB mulch exceeded microbial N demand in the later stages of decomposition due to depletion of easily decomposable C.

Compared to FBm, available N was much lower in FBm/Ws at all mulch ages. Shoot N uptake was also lower in FBm/Ws than FBm, but the difference between the two treatments became smaller with increasing mulch age. This is likely due to the smaller microbial N uptake in 75 DAM compared to 0 DAM which allowed plants in FBm/Ws to take up a greater proportion of N released from FB.

In Wm/Ws compared to Wm, available N was similar, but shoot N uptake was about 60% lower and MBN was 30–40% higher at all mulch ages. The greater amount of W in Wm/Ws compared to Wm may have resulted in greater N release possibly from native soil organic matter as indicated by the higher MBN. However, the greater N immobilization limited plant N uptake compared to Wm.

In SMm/Ws, shoot dry weight and N uptake increased with mulch age, but MBN was lower in 35 and 70 DAM than 0 DAM, whereas available N changed little with mulch age. This suggests that N from microbial turnover was taken up by plants. In SMm/Ws compared to SMm, MBN was about two-fold higher at all mulch ages, but shoot N uptake was lower with smaller differences towards older mulch ages. Thus, in SMm/Ws compared to SMm, plant N uptake may have been limited due to N uptake by microbes decomposing W in soil. However, in contrast to FBm/Ws, the low N availability in SMm/Ws limited plant N uptake which was lower than the control and SMm.

5 Conclusion

This study showed that presence of wheat straw in the soil can reduce the risk of N leaching after application of an easily decomposable low C/N residue due to a greater microbial N uptake. The N taken up by microbes may provide a longer lasting N source for plants. The results suggest that application of easily decomposable low C/N mulch after harvest of cereals is a suitable management option to reduce N loss. However, with application of low C/N manure, available N was low even in absence of wheat straw and the presence of straw reduced plant N uptake compared to manure alone. This indicates that if manure is used as N fertilizer, it should be applied to soil that contains little high C/N organic materials. The study also showed that mulches increase plant growth and N uptake particularly when they are applied at least 4 weeks before planting to allow nutrient release from the mulches and nutrient release from the microbial biomass.

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CHAPTER 8

CONCLUSION AND FUTURE RESEARCH

8.1 Conclusion

In this thesis, the influence of the following factors on soil nutrient availability, microbial activity and plant growth were studied: i) addition rate, order and frequency; ii) incorporation of organic materials differing in C/N ratio and decomposition stage; iii) mulching of organic materials differing in C/N ratio and decomposition stage without or with plant residues incorporated into soil.

In Chapters 2 and 3, effects of amendment rate, order and frequency on soil nutrient availability and microbial biomass were investigated. The total amount of residue added was the same in all treatments (20 g kg^{-1}), added between two and eight times as low C/N (L), high C/N (H) or 1:1 mix of L and H. In the experiment described in Chapter 2, with repeated addition of H and L, N availability and microbial biomass N were influenced by residue rate and order. Differences between measured and expected values depended on residue addition frequency. When the 1:1 mix of H and L was added four times at 5 g kg^{-1}, the difference between measured and expected microbial biomass N and available N changed over time, possibly because the proximity of microbes to the residues changed. In this experiment, the interaction between H and L in a 1:1 mix of could not be elucidated. In the 48 day experiment described in Chapter 3, H and L were added either sequentially in different order (H then L or L then H), or as 1:1 mix (HL) two, four and eight times at 10, 5 and 2.5 g kg^{-1} soil. The results confirmed that amendment frequency influenced interactions in mixes regarding N availability and microbial biomass N. Frequent addition of small amounts of residues (eight times at 2.5 g kg^{-1}) limited microbial N uptake initially compared to higher amendment rates, but resulted in similar available N irrespective of the C/N ratio of the residue added. Less frequent addition of larger amounts of residues (two and four times at 10 and 5 g kg^{-1}) on the other hand resulted in large changes in available N and microbial biomass N depending on the C/N ratio of both the freshly added and the previously added residue. It can be concluded that N availability remains stable with frequent addition of residues with different C/N ratio whereas it strongly fluctuates when large amounts of residues with different C/N ratio are added.

The experiments in Chapter 4 and 5 were conducted to elucidate the influence of mixing organic materials differing in C/N ratio and decomposition stage on soil nutrient availability, microbial activity and plant growth. In these experiments, soil was amended with young faba bean shoots (C/N 9), sheep manure (C/N 6) and mature wheat straw (C/N 82) either individually or as 25:75, 50:50 and 75:25 mixes. Chapter 4 was an incubation experiment in which soil was incubated for 48 days. Chapter 5 was a pot experiment, in which wheat was planted 0, 35 and 70 days after organic amendment and grown for 35 days.

Although faba bean and sheep manure have similar low C/N ratio, the effect on N availability, microbial biomass N and respiration was smaller with sheep manure than with faba bean which can be explained by the highly decomposed state of sheep manure (Chapter 4). Mixing sheep manure with 50% or more faba bean maintained higher N availability than sheep manure alone while minimizing the potential for N loss compared to sole faba bean. Mixing wheat with 50% or more faba bean resulted in higher N availability than wheat alone, but prevented the rapid release of N that occurred after addition of sole faba bean. In mixes of wheat and sheep manure, wheat stimulated decomposition of sheep manure, but N availability remained low. It can be concluded that mixes with a high proportion of faba bean may increase plant N uptake whereas mixes of wheat and sheep manure are unlikely to provide sufficient N for plants.

To understand the influence of incorporation of mixes of organic materials differing in C/N ratio and decomposition stage on plant growth and nutrient uptake, the experiment described in Chapter 5 was conducted. Wheat was planted 0, 35 and 70 days after amendment and grown for 35 days. Shoot and root dry weight were low with wheat alone and in mixes with

75% wheat, but did not differ among the other treatments. Shoot N uptake was higher than the control in treatments with faba bean alone and in mixes of faba bean with ≤50% sheep manure. In mixes of faba bean with wheat and sheep manure, available N decreased with proportion of faba bean. However, in mixes of faba bean with sheep manure or faba bean with 25% wheat, plant growth and N uptake were similar or higher than the control. It can be concluded that in these mixes, plants can access directly organic particles releasing N and thereby compensate low N availability.

In the previous studies, organic amendments were mixed into the soil. However, organic materials can also be applied as mulch on the soil surface. In Chapters 6 and 7, the effects of mulching organic materials differing in C/N ratio and decomposition stage without or with high C/N residues mixed into the soil on soil nutrient availability, microbial activity and plant growth were studied. In these experiments, soil was i) left unamended, ii) mixed with mature wheat straw (C/N 80), iii) mixed with mature wheat straw and mulched with mature wheat straw, young faba bean shoot (C/N 7) or sheep manure (C/N 8) and iv) mulched only with mature wheat straw, young faba bean shoot or sheep manure. In the experiment described in Chapter 6, the effect of sheep manure mulch on soil respiration, available N and microbial biomass N was smaller than that of faba bean mulch. This confirms the results of Chapter 4 and can be attributed to the low decomposability of sheep manure. Particularly with faba bean mulch, the presence of wheat straw in mulched soil reduced N availability but increased microbial biomass N compared to soil with mulch only. Therefore, wheat mixed in mulched soil can reduce N loss via leaching from rapidly decomposing low C/N mulch and increase potential N availability through microbial biomass turnover.

In Chapter 7, the same treatments as in Chapter 6 were used, but wheat was planted 0, 35 and 70 days after organic amendment and grown for 35 days. Shoot dry weight and nutrient uptake were higher with faba bean mulch than with sheep manure mulch, irrespective mulch age, likely because faba bean is rapidly decomposing whereas sheep manure decomposes slowly. Compared to soil with only mulch, mixing of wheat straw into soil reduced plant growth and nutrient uptake in the early stages of mulching (0 and 35 days after mulching). However, the greater microbial biomass N with wheat mixed into the soil compared to soil without wheat may provide a longer lasting source of N for plants due to turnover of microbial biomass more than 70 days after residue addition. Further, the presence of wheat in soil with rapidly decomposing low C/N mulch can reduce the risk of N leaching from the mulch due to greater microbial N uptake compared to mulch alone. It can be concluded that mulching rapidly decomposing low C/N residues on soil mixed with high C/N residues and incubating for several weeks (5-10 weeks) may be a suitable management option to enhance plant growth and yield.

In summary, the experiments showed that with repeated addition of high and low C/N residues, N availability and microbial biomass N were influenced by addition rate and order. Amendment frequency affected the interactions regarding N availability and microbial biomass N between high and low C/N residues in mixes. Incorporation of mixes of rapidly decomposing low C/N residues with ≥ 50% slowly decomposing low C/N sheep manure maintained high N availability while reducing the risk of N leaching from rapidly decomposing residues alone. Further, it was found that the presence of high C/N wheat straw in soil mulched with low C/N organic materials resulted in higher microbial N uptake than without wheat straw in the soil. The higher microbial biomass N uptake could prevent N loss via leaching from rapidly decomposing low C/N residues and be a long-lasting source of available N upon turnover of microbial biomass.

8.2 Future research

The study provided new knowledge about factors influencing nutrient availability, microbial activity and plant growth in soil amended with organic materials. However, the study also revealed some research gaps which could be investigated in the future.

1. In this research, only C/N and decomposition stage were considered as factors influencing decomposition and nutrient release of organic materials. However, decomposition and nutrient release of organic materials are also influenced by other properties such as N content, lignin and polyphenol concentration (Vigil and Kissel, 1991; Wang et al., 2004). Therefore it would be important to assess effect of other properties of organic materials on nutrient release. Nutrient release could be related to chemical properties of organic amendments determined by ^{13}C CPMAS NMR (Bonanomi et al., 2019), fourier-transform infrared (FTIR) spectroscopy (Parolo et al., 2017) or gas chromatography - mass spectrometry (GC-MS) pyrolysis (Buurman et al., 2007).

2. In all experiments, only a small particle size (0.25-2 mm) of organic materials was used. Small particle sizes were used to stimulate decomposition of organic materials. However, applied organic materials in the field are usually coarser and the effect of organic materials on nutrient availability, microbial activity and plant growth may vary with particle size. Therefore it would be important to undertake experiments in which particle size of organic materials is larger (e.g 1 cm in Seneviratne et al. (1997) or the whole wheat shoot in Saroa and Lal (2003) and Tu et al. (2006)).

3. In the incubation experiments (Chapters 2, 3, 4 and 6), the contribution of each organic material to CO_2 respired, available N and P and microbial biomass N and P could not be determined. To investigate the source of C, ^{13}C labelled residues or ^{13}C natural abundance could be used (Schweizer et al., 1999; Blagodatskaya et al., 2011). The source of N and P could be determined by using ^{15}N and ^{32}P-labelled residues combined with unlabelled organic materials (Hundal, 1992; Schwendener et al., 2005).

4. In the plant experiments (Chapter 5 and 7), the source of N in soil and plants was not identified. The source of N in soil and plants could be traced using ^{15}N labelled residues (Blair et al., 2005). Further, plant growth duration in these experiments was only five weeks. The duration could be longer to understand the long-term effect of organic amendment on plant growth and yield.

5. Changes in nutrient availability and microbial biomass could also be influenced by changes in microbial community structure over time which were not investigated in our study. Microbial community structure may influence plant growth indirectly by nutrient release or directly by production of plant-growth promoting or suppressing compounds (Peyraud et al., 2017; Singh et al., 2018). Changes in microbial community structure could be assessed by whole genome sequencing (Dai et al., 2017), next generation sequencing (Cesarano et al., 2017) or expression of functional genes (Kaurin et al., 2018).

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