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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.

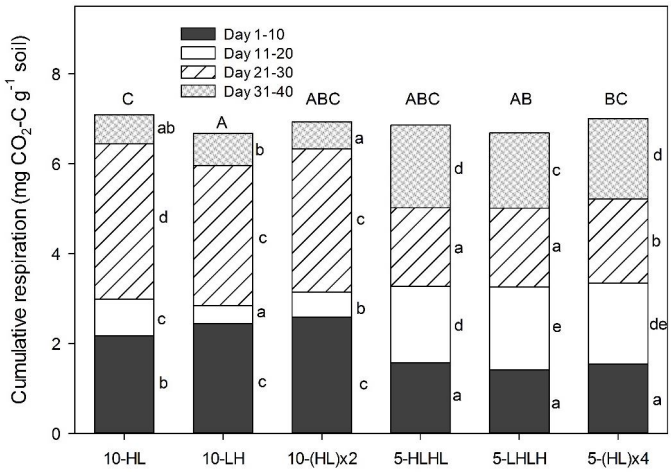

Figure 1. Cumulative respiration in 10-day intervals and total over 40 days in soil amended with high (H) and low C/N (L) residues or their 1:1 mixture at 5 or 10 g kg^{-1} (n=4). Different lower case letters indicate significant differences ($P \leq 0.05$) among treatments for a given 10-day interval.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

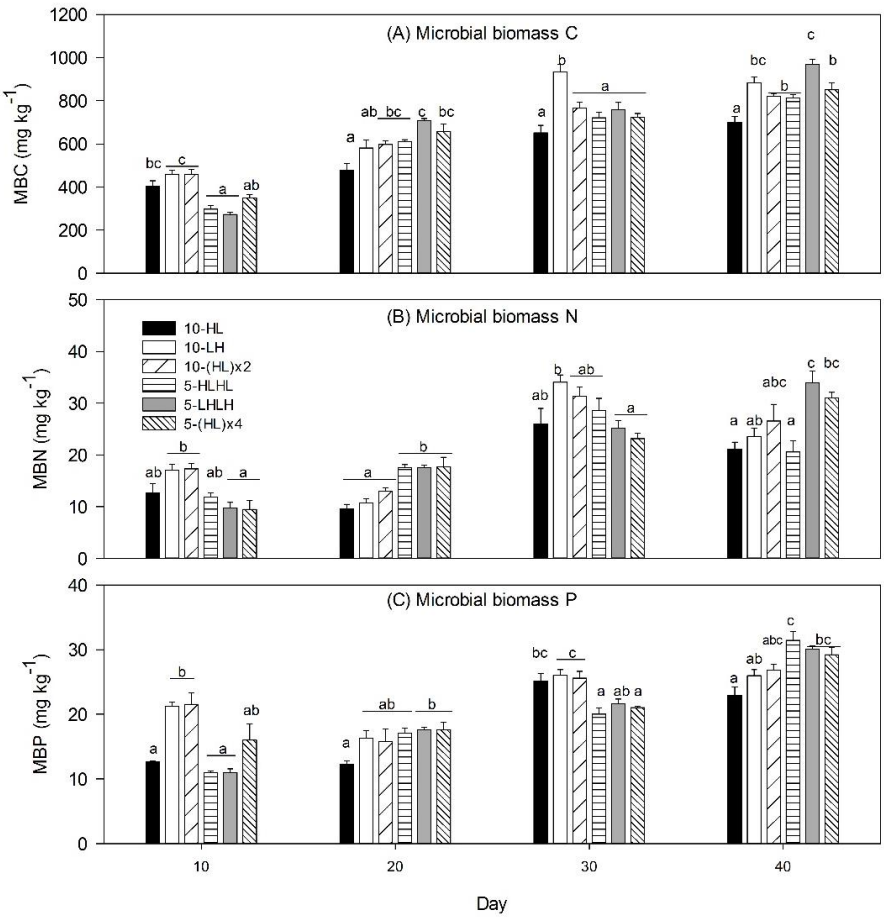

Figure 2. Microbial biomass C (A), N (B) and P (C) on days 10, 20, 30 and 40 in soil amended with high (H) and low C/N (L) residues or their 1:1 mixture (HL) at 5 or 10 g kg^{-1} (n=4, vertical lines indicate standard error). Different letters indicate significant differences ($P \leq 0.05$) among treatments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

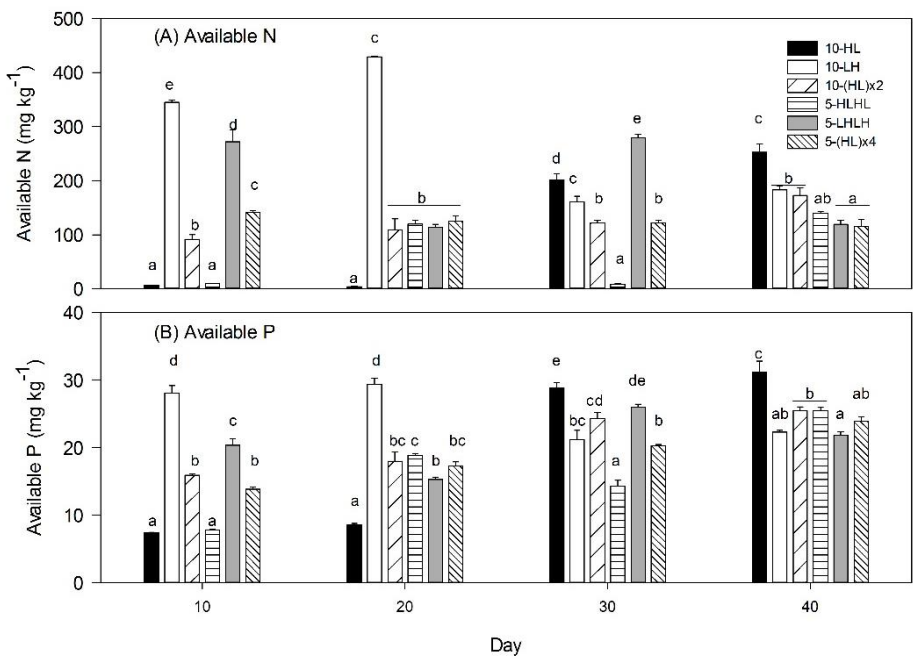

Figure 3. Available N (A) and P (B) on days 10, 20, 30 and 40 in soil amended with high (H) and low C/N (L) residues or their 1:1 mixture (HL) at 5 or 10 g kg^{-1} (n=4, vertical lines indicate standard error). Different letters indicate significant differences ($P \leq 0.05$) among treatments at a given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

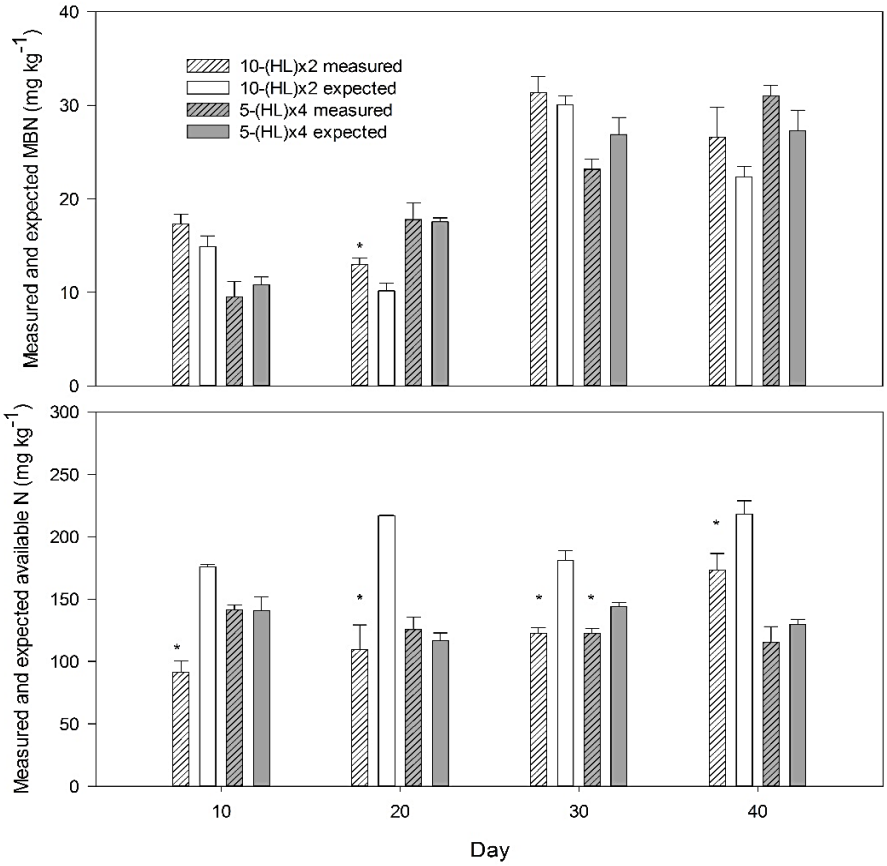

Figure 4. Measured and expected microbial biomass N (MBN) and available N on days 10, 20, 30 and 40 for treatments where 1:1 mixes of H and L were added (10-(HL)x2, 5-(HL)x4). Asterisk indicates significant difference between measured and expected value.

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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