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RESEARCH ARTICLE

Phosphorus and nitrogen in the soil interface between two plant residues differing in C/nutrient ratio: A short-term laboratory incubation study

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ABSTRACT

In studies on the effects of mixing residues with different properties on decomposition rate and nutrient release, the extent of contact between the different residues is not known. In this study, we used an experimental design where crop residues were spatially separated by a layer of soil. Microcosms were set up using young faba bean residue (low carbon (C)/nutrient ratio, L) and mature barley straw (high C/nutrient ratio, H). The microcosms comprised of two caps of PVC tubes, each filled with moist soil. Between the two caps, there were three layers each separated from the others by fine nylon mesh with the middle layer being the moist interface soil. Microcosms had similar (H/H or L/L) or different (L/H) residue types, or only residue type (H/S or L/S) while the other cap had no residue. In treatments with only one residue, measured parameters, except microbial biomass P (MBP), were higher in L/S than H/S. In treatments with two residues, all parameters were lowest in H/H. In L/H compared to L/L after 14 days, available P and microbial biomass N (MBN) were lower, available N was similar and MBP was higher. After 28 days, available P and N were lower in L/H than L/L, but MBP and MBN did not differ. In L/H, measured resin P, MBP and MBN were higher than expected whereas available N was lower. The experimental design used in this study allows assessing the effect of residues on properties of the soil between them.

1 Introduction

The effects of mixing residues with different properties on decomposition rate and nutrient release have been studied extensively. Usually, two or several residues are either placed together in litter bags or mixed into the soil (De Graaff et al., 2011; Shi et al., 2013; Cuchietti et al., 2014; Truong and Marschner, 2018). Then litter mass loss or nutrient release are measured with litter bags or soil properties are measured in residue amended soil. However in such studies, the extent of contact between the different residues is not known. Further, in experiments with residues mixed into the soil, partially decomposed residues may directly contribute to available nitrogen (N) and phosphorus (P) or microbial biomass which could lead to overestimation of the effect of the residues and their interaction. Interactions in 1:1 mixes with two residues are determined by comparing the measured value with expected value, the latter being the average of the two residues incubated separately (De Graaff et al., 2011). In previous experiments with residue mixes, interactions were absent (measured = expected value), positive (measured >

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expected value) or negative (measured < expected value) (Shi et al., 2013; Truong and Marschner, 2018).

Particularly in situations where only small amounts of residues are mixed into soil, direct contact between residues is unlikely. In most cases, residue particles will be separated by a layer of soil. In this study, we used an experimental design that allowed us to sample soil in the interface between two residue layers. Thus, the interface soil could be influenced by both residue layers while the residues remained spatially separated. This allowed us to address the question how the C/nutrient ratio of the two residues influences P pools, available N and microbial biomass in the interface soil. The experiment included four treatments with either only one residue layer or two layers of high or low C/nutrient residue. In another treatment, there was one layer of high C/nutrient residue and one with low C/nutrient residue. The control was unamended soil. The first hypothesis was that P pools, available N and MBN will be higher with low C/nutrient residue on both sides than single low C/nutrient residue whereas they will not differ between high C/nutrient residue on both sides and single high C/nutrient residue. This hypothesis assumes that nutrient release from low C/nutrient residue is high whereas release from high C/nutrient residue is low irrespective of the amount of high C/nutrient residue present. As the residues were separated by the interface soil, we assumed that there would be no interaction between residues with differing C/nutrient ratio. Therefore, the second hypothesis was that in the treatment with both high C/nutrient residue and low C/nutrient residue, measured and expected values will match.

2 Materials and methods

2.1 Soil and residues

As described in Erinle et al. (2018), a loamy sand was collected from 0 to 10 cm on Waite Campus, The University of Adelaide, South Australia (longitude 138°38′E, latitude 35°6′S) which had been under permanent pasture for over 80 years. The study site has a Mediterranean climate and is characterized by cool, wet winters and hot, dry summers occasionally interrupted by short, heavy rainfall events. The soil is a Chromosol in Australian soil classification, and a Rhodoxeralf in US Soil Taxonomy. Soil samples were collected from six different locations and then pooled to a composite sample from which visible plant materials (e.g., roots) were manually removed. Then the soil was dried at 40°C and passed through a 2 mm sieve. It has the following properties: clay 250 g kg^{-1}; sand 370 g kg^{-1}; silt 380 g kg^{-1}; pH (1:5 soil: water) 5.6; EC (1:5) 0.1 dS m^{-1}; total organic C 17 g kg^{-1}; total N 1.5 g kg^{-1}; total P 302 mg kg^{-1}; bulk density 1.3 g cm^{-3}; maximum water-holding capacity (WHC) 349 g kg^{-1}. Detailed properties of the soil are described in Erinle et al. (2019).

Two types of crop residues were used: young faba bean shoot (*Vicia faba* L., C/P 38, C/N 9, referred to as L) as a low C/nutrient residue, and mature barley straw (*Hordeum vulgare* L., C/P 255, C/N 95, referred to as H) as high C/nutrient residue (Erinle et al., 2018). These two plant species were used because they are commonly grown in Southern Australia. The residues were dried at 40°C in a fan-forced oven and then ground and sieved to 0.25 to 2 mm using a stack of two sieves with a 2 mm sieve over a 0.25 mm sieve. Material retained on the 0.25 mm sieve was used for the experiment.

2.2 Experimental design

The experimental microcosms comprised of two caps of PVC tubes (20 mm height × 70 mm diameter), with several holes at the bottom of each cap. Caps were filled with 60 g dry soil equivalent at 50% WHC and incubated for 7 days at 25°C in the dark. Then the soil surface on the open end of each PVC cap was covered with fine nylon mesh (mesh size 0.1 mm × 0.8 mm) onto which 1.5 g crop residues was placed and another mesh was used to cover the residues. Before the two caps with residues were brought together, a layer of soil (interface soil; 20 g moist soil) was evenly spread and sandwiched between the meshes on the PVC caps covering the residues. The two caps were held tightly together with rubber bands (Fig. 1).

There were six treatments with three replicates per treatment and sampling time: control without residues between the two layers of mesh in each cap (S/S), one cap with either H (high C/nutrient matured barley straw) or L (low C/nutrient young faba bean residue), the other cap without residues (H/S and L/S), both caps with the same residue (H/H and L/L) or one cap with H and the other with L (L/H). Soil water content was monitored by weighing the microcosms daily and adjusted by adding reverse osmosis water through the perforations at the bottom of the caps. The microcosms were incubated vertically in the dark at 25°C. Sampling of the interface soil was carried out after 14 and 28 days. At each

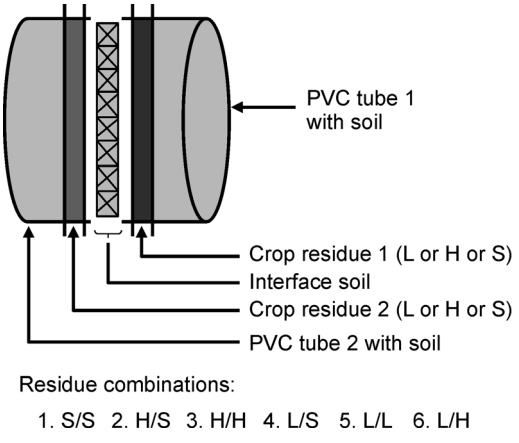

Fig. 1 Schematic diagram of microcosm used for generation of interface (sampled) soil. Microcosms have similar (H/H or L/L) or different (L/H) residue types on either side, or one residue type on only one side (H/S or L/S). L: low C/nutrient faba bean residue; H: high C/nutrient barley straw; S: unamended control soil.

sampling, the two PVC caps were carefully separated from each other, the interface soil removed and used for further analyses. The crop residues remaining on the nylon mesh were dried at 60°C for two days to measure dry weight.

2.3 Analyses

Soil maximum water holding capacity was measured in a sintered glass funnel connected to a 1 m water column (matric potential −10 kPa) (Wilke, 2005). Soil texture was determined by the hydrometer method (Ge and Or, 2002). Soil pH was determined in a 1:5 (w/v) soil to reverse osmosis (RO) water ratio after 1 h end-over-end shaking (Rayment and Higginson, 1992). Total organic C of soil and plant residues was measured by wet oxidation (Walkley and Black, 1934). Total N in soil and plant residues was extracted and determined following the Kjeldahl method (McKenzie and Wallace, 1954). To determine total P, soil and plant residues were digested with a mixture of HNO_3 and $HClO_4$. Total P in the extract was measured by the phosphovanadomolybdate method (Hanson 1950).

Citrate P and HCl P were measured according to DeLuca et al. (2015). Resin (available) P and microbial biomass P were determined with the anion exchange resin method (Kouno et al., 1995), with hexanol as fumigant. The P concentration in the extracts was determined colorimetrically (630 nm) using the malachite-green method (Ohno and Zibilske, 1991). Available N (NH_4^+ and NO_3^-) was extracted with 2 M KCl in a 1:5 soil extractant ratio. Ammonium and nitrate were determined as described by Willis et al. (1996) and Miranda et al. (2001), respectively. Microbial biomass N was determined by chloroform fumigation-extraction with 0.5 M K_2SO_4 at 1:4 soil to extractant ratio. Microbial biomass N was calculated as the difference in NH_4^+ concentration between fumigated and non-fumigated samples divided by 0.57 as suggested by Moore et al. (2000).

2.4 Statistical analyses

Data of microbial biomass, N and P availability and P pools were log10 transformed to achieve normal distribution. Then the data were analyzed by one-way repeated measures ANOVA with between subjects factor-treatment, within subjects factor-time in SPSS statistics version 25. Tukey's multiple comparison test at 95% confidence interval was used to determine significant differences among treatments at a given sampling time and *t*-test was used for comparison between sampling times. Expected value in the treatment with both high and low C/P residues (L/H) was calculated as average of the measured value of the two residues incubated separately (H/S and L/S) (De Graaff et al., 2011). Significant differences between measured and expected values of P pools, available N and microbial biomass N were determined using *t*-test.

3 Results

Residue dry weight on days 14 and 28 was about twofold higher in barley straw (treatments H/S, H/H and straw in the L/H microcosm (L/H(B)) than in faba bean (treatments L/S, L/L and faba bean in the L/H treatment (L/H(F)) (Fig. 2). On day 14, compared to the initial weight (1.5 g), residue dry weight was about 40% lower barley straw, but about 75% lower in faba bean. On day 28, compared to day 14, residue dry weight was about 15% to 20% lower in H/S, H/H and L/H(B), but did not change in L/S, L/L and L/H(F).

Citrate P, HCl P and resin P were 2- to 6-fold higher in treatments with faba bean (L/S, L/L and L/H) than the unamended control (S/S) and treatments with only barley straw (H/S and H/H) (Fig. 3). At both sampling times in treatments with L compared to L/H, citrate and resin P were 30% higher in L/S and nearly twofold higher in L/L (Fig. 3A, C).

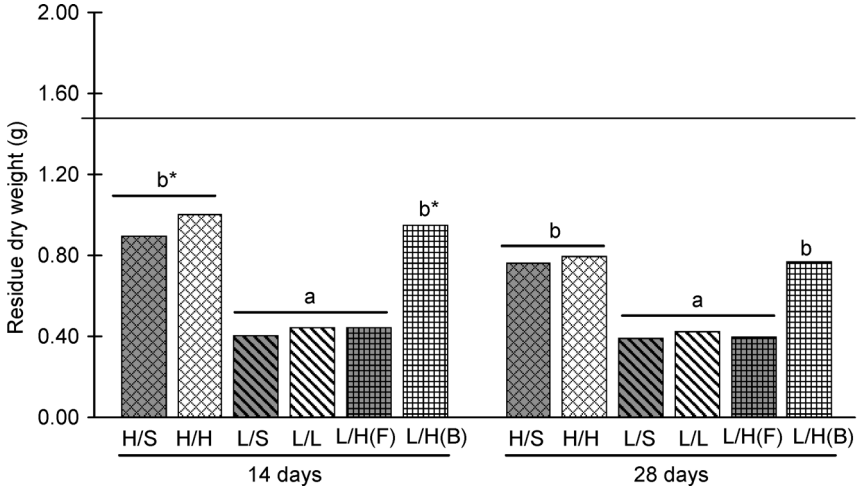

Fig. 2 Dry weight of remaining barley straw and faba bean residue per microcosm after 14 and 28 days incubation. The horizontal line represents the original weight of the crop residues (1.5 g). L/H (F) and L/H (B) represent the faba bean residue and barley straw in the L/H microcosm. At a given sampling date, different letters indicate significant differences between treatments (n = 4; $P \leqslant 0.05$) and an asterisk indicates significantly higher values in a treatment between sampling times.

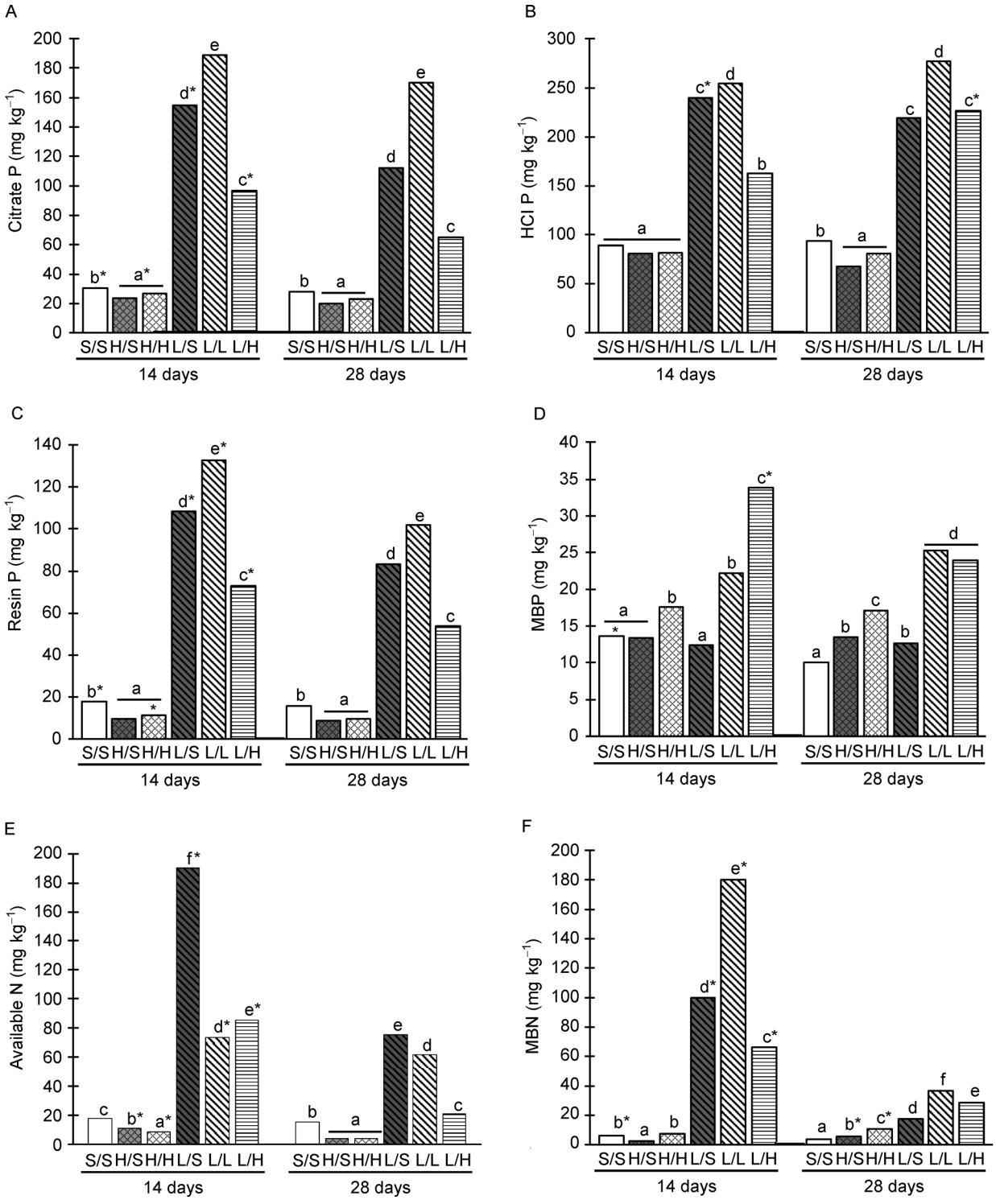

Fig. 3 Citrate P (A) HCl P (B), resin P (C), microbial biomass P (D), available N (E) and microbial biomass N (F) (mg kg^{-1}) in unamended control soil (S/S), interface of high C/nutrient barley (H/S or H/H), low C/nutrient faba bean (L/S or L/L), and faba bean-barley (L/H) 14 and 28 days after incubation. At a given sampling date, different letters indicate significant differences between treatments (n = 4; $P \leqslant 0.05$) and an asterisk indicates significantly higher values in a treatment between sampling times.

Citrate and resin P were generally about 20% higher on day 14 than day 28. HCl P in L treatments was highest in L/L (Fig. 3B). On day 14, HCl P in L/L was about 10% higher than L/S and 40% higher than L/H. HCl P on day 28 was 25% higher in L/L than the other two faba bean treatments. HCl P changed from day 14 to day 28 only in L/S and L/H, decreasing by 15% in L/S, but increasing by 20% in L/H. On day 14, MBP was similar in S/S and treatments with a single residue layer (H/S and L/S) (Fig. 3D). Compared to S/S, MBP was 30%–50% higher in H/H and L/L and more than twofold higher in L/H. MBP did not change from day 14 to day 28 in most treatments except in S/S and L/H where it decreased by 15% and 30%, respectively. On day 28, MBP was lowest in S/S. Compared to S/S, MBP was 20% higher in H/S and L/S, 50% higher in H/H and more than twofold higher in L/L and L/H.

At both sampling times, available N was about 50% lower in treatments with only H (H/S and H/H) than S/S (Fig. 3E). Compared to S/S, available N on day 14 was 10-fold higher in L/S and 4- to 5-fold higher in L/L and L/H. Available N decreased from day 14 to day 28 in all treatments with residues; by 50% in treatments with only H, more than 50% in L/S, 10% in L/L and by 75% in L/H. Compared to S/S, available N on day 28 was 5-fold higher in L/S and about 4-fold higher in L/L and only 75% higher in L/H. MBN in day 14 was similar in S/S and H/H, but 50% lower in H/S (Fig. 3F). It was higher than S/S in all treatments with L, 10-fold higher in L/H, 30-fold higher in L/L and 15-fold higher in L/S. MBN decreased strongly from day 14 to day 28 in treatments with L. Compared to day 14, MBN on day 28 was about 80% lower in L/S and L/L and 50% lower in L/H. It was also 50% lower on day 28 than day 14 in S/S. On day 28, compared to S/S, MBN was about 30% higher in H/S, twofold higher in H/H, 5-fold higher in L/S and 10-fold higher in L/L and L/H.

In the interface soil of low and high C/P residue, measured values on day 14 were higher than expected values for resin P (20% higher), MBP (threefold higher) and MBN (25% higher). But measured available N was 50% lower than the expected value (Table 1). On day 28 measured values were higher than expected values for HCl P (35% higher), resin P (15% higher) and MBP and MBN (twofold higher), whereas; but measured available N was 50% lower than expected.

4 Discussion

This study showed that P pools, available N and MBN in the interface soil between two residues are influenced by the properties of both residues. The effects of the residues on interface soil are likely due to nutrient diffusion from the residues into the soil and possibly transfer of nutrients from the soil into the residues. Direct effects of residues, e.g., particles that passed the mesh and entered the soil cannot be excluded, but are likely to be small.

In agreement with previous studies, the low C/nutrient ratio residue decomposed faster and released more N and P than high C/nutrient ratio straw (Alamgir et al., 2012; Chen et al., 2014). The higher citrate P and HCl P in treatments with L shows that P released from L is to a large extent bound to soil particles which is in agreement with our previous study with similar microcosms where we used only one residue layer (Erinle et al., 2019). The first part of the first hypothesis (P pools, available N and MBN will be higher with low C/nutrient residue on both sides than single low C/nutrient residue) can be accepted, except for available N. The higher P pools and available N in L/L than L/S are likely due to the greater N and P release from the twofold higher L amount in L/L. The unexpected higher available N in L/S than L/L on day 14 can be explained by the lower microbial N uptake in L/S which suggests that microbes in L/S were C-limited (Mooshammer et al., 2014). In L/L, larger amounts of C than in L/S seemed to have been available in the first 14 days as MBN was much higher which reduced available N. Both available N and MBN in L treatments were lower on day 28 than day 14 which could be due to denitrification. Microbial P immobilisation was also higher in L/L than L/S, but citrate P, HCl P and resin P were still higher in L/L. The likely reason for this is that MBP was a small pool compared to the other P pools which would therefore be little affected by an increase in MBP.

The second part of the first hypothesis (P pools, available N and MBN will not differ between high C/nutrient residue on both sides and single high C/nutrient residue) cannot be accepted for MBN and MBP which were higher in H/H than H/S. The higher microbial N and P in H/H suggest that the twofold greater residue amount compared to H/S supplied not only more C, but also more N and P. Despite this increase, MBN and MBP in treatments with only H differed little from the unamended control which shows that nutrient release from H was small.

The higher P pools, available N and MBN in L/H compared to treatments with H only can be explained by the greater N and P release from L (Yadvinder-Singh et al., 2005). As

Table 1 Measured and expected values of P pools, available N and microbial biomass N and P (mg kg^{-1}) on days 14 and 28 ($P \leqslant 0.05$, $n = 4$) for treatments with faba bean and barley (1:1) mixes (L/H).

Days	Values	Citrate P	HCl P	Resin P(mg kg^{-1})	MBP	Available N	MBN
14	Expected	89.03	160.39	59.07	12.94	246.86*	51.37
	Measured	96.87	162.81	72.97*	33.81*	179.72	66.34*
28	Expected	66.09	143.46	46.01	13.05	39.93*	11.61
	Measured	65.11	227.04*	53.79*	23.90*	20.59	28.50*

At a given sampling date. Asterisks indicate significant differences between measured and expected values.

expected from previous studies with mixes of low and high C/nutrient residues, citrate P, HCl P and resin P were lower in L/H than treatments with only L (Cuchietti et al., 2014; Truong and Marschner, 2018). The lower resin P in L/H than treatments with only L on day 14 could be due to greater MBP. But on day 28, MBP was similar in L/H and L/L whereas resin P was greater in L/L than L/H. Further, MBN in L/H was lower than in L/L on day 14 and did not differ from that in L/L and L/S on day 28. The lower citrate P, HCl P, resin P and available N in L/H could be due to N and P transfer to H through the mesh by fungal hyphae (Frey et al., 2003) followed by microbial immobilization in the residue layer. This apparent N and P transfer did not influence decomposition of H as mass loss of H in L/H was similar that of H in treatments with H only, possibly because the amount of transferred N and P was small.

The second hypothesis (in the treatment with both high C/nutrient residue and low C/nutrient residue, measured and expected values will match) cannot be accepted. This hypothesis was based on the assumption that in this experiment, the residues were spatially separated by the interface soil which would minimize interactions. However, measured resin P, MBP and MBN were higher than expected whereas available N was lower. This suggests that available P in the interface was predominately influenced by L. In our previous study (Erinle et al. 2018), we showed that the low C/nutrient residue decomposed more quickly than the high C/nutrient residue. Therefore, it is likely that in the first 14 days, L supplied microbes with organic C, N and P which increased microbial N and P uptake. However, in the second half of the experiment, when L had been largely decomposed, the more slowly decomposing H was the organic C source for microbes which took up N previously released from L. The lower than expected available N indicates N transfer by fungal hyphae into H (Frey et al., 2003). The interaction between L and H in this study differs from our previous study with microcosms where H and L were applied one after the other (Erinle et al., 2019). In that study, either H or L where applied at the start of the incubation, and then replaced on day 14 by the residue with contrasting C/P ratio giving treatments H followed by L and L followed by H. On day 28, the measured parameters were predominantly influenced by the second residue. That is, P pools, available N and MBN were higher in H followed by L than in L followed by H. This is likely because very little of the residue added first remained on day 28, thus detritusphere properties were influenced by the second residue. In the present study, where residues were present at the same time, L mainly influenced available P in the interface whereas H provided organic C which enhanced microbial N and P uptake.

5 Conclusion

This study showed that the soil between two residues with different C/nutrient ratio is influenced by the interactions between the two residues although they are spatially separated. In the field different types of residues may be spatially separated if, for example, legume residues are applied to soil after cereal harvest. Our results indicate that the interface soil is initially mainly influenced by N and P release from the legume residue, but after the first two weeks the presence of the cereal residue increased net N and P immobilization. Thus, a crop sown shortly after residue addition may have sufficient available N and P initially, but may later experience N and P deficiency. The advantage of the experimental design used in this study is that it allows assessing the effect of residues on properties of the soil between them. Future experiments could use ^{13}C or ^{15}N labeled residues to follow C and N from one residue into the interface soil and the adjacent residue.

Conflict of interest

The authors declare no conflict of interest.

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