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RESEARCH ARTICLE

# Soil amendment with high and low C/N residue –influence of low soil water content between first and second residue addition on soil respiration, microbial biomass and nutrient availability

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**Abstract**

Soil water content is a major factor influencing organic matter decomposition. In our previous study, we showed that microbial biomass and nutrient availability after the second residue addition is influenced by the C/N ratio of both the first and the second residue (referred to as legacy effect). Different constant soil water content between the first and second residue addition may influence soil respiration, microbial biomass and nutrient availability and also the legacy effect. A loamy soil was unamended (C), or amended with plant residues with either high (mature wheat straw, H) or low C/N ratio (young faba bean, L) on day (d) 0 and d10, giving treatments CH, CL, HH, HL, LL and LH. Between d0 and d10, the soil was maintained at 10, 30 or 50% of water holding capacity (WHC), on d10, before residue addition, soil water content was adjusted to 50% WHC and maintained at this water content until d20. Cumulative respiration from d1 to d10, MBC and MBN on d1 and available N and P on both d1 and d10 were lower at 10% than at 50% WHC. When L was added on d10, cumulative respiration from d11 to d20, microbial biomass C and N on d11 and available N on d20 were higher in soil kept at 10% WHC in the first 10 days than in that maintained at 50% WHC. The previous water content had little effect on respiration and nutrient availability when H was added on d10. Differences in MBC, MBN, MBP and available N on d11 between HL and LL and between LH and HH were greater when the water content in the first period was 10% WHC compared to 50% WHC. It can be concluded that water content between residue additions influences soil respiration and nutrient availability not only directly, but also after rewetting and residue addition.

**Keywords:** Legacy effect, microbial biomass, nutrient availability, residue addition, water content

## 1. Introduction

Organic amendments have been used in agricultural soil to provide nutrients to crops, which is recognised as sustainable farming (Power, 2010; Scotti *et al.*, 2015). The effect of organic amendments on microbial activity and growth and nutrient availability is influenced by the composition of the organic amendment, particularly the C/N ratio (Heal *et al*., 1997). It is well-known that organic amendments, e.g. plant residues, with low C/N are decomposed faster and lead to higher N availability than high C/N residues (Hadas *et al*., 2004). Another factor influencing decomposition is soil water availability through its effect on microbial activity. At low water content, matric potential is more negative because water is bound more tightly to soil particles and held in smaller pores than at high water content (Brady & Weil, 2002). Therefore water is less available for microbes and plants at low compared to high water content and more energy has to be spent to take up water (Schimel *et al*., 2007). Further, water films around soil particles become thinner and disconnected as soils dry which reduces nutrient diffusion (Geisseler *et al*., 2011; Ilstedt *et al*., 2000).
This is particularly important in areas that have long periods with little or no rain, many of which may become drier in the future (Solomon *et al*., 2007).
In previous studies of our group, we showed that microbial biomass and nutrient availability after the second residue addition are influenced by the C/N ratio of both the first and the second residue, which we refer to as legacy effect (Marschner *et al*., 2015; Nguyen *et al*., 2016). For example, nutrient availability was lower in low after high C/N residue than in low after low C/N. This can be explained by microbes decomposing both the previously added residue left in the soil and the freshly added residue. In low after high C/N residue, N mineralised by microbes decomposing low C/N residue can be taken up by microbes decomposing high C/N residue. Zheng & Marschner (2017) varied the amendment rate of the first residue and showed that the legacy effect is smaller at low compared to high amendment rate. This indicated that the legacy effect is influenced by the amount of the initially added residue left in the soil when the second residue is added. Recently, Zhang & Marschner (2016) found that the legacy effect of the first residue added was not influenced by the number of drying and rewetting events between first and second residue addition. However, the amount of the first residue left in the soil when the second residue is added could also be influenced by soil water content between the two residue additions through its effect on microbial activity. The aim of this study was to determine the effect of soil water content between the first and second residue addition on soil respiration, microbial biomass and nutrient availability. We hypothesised that the legacy effect is stronger when the water content after the first amendment is low than when it is high because more of the first residue is left in the soil when the second residue is added.

## 2. Materials and Methods

### *2.1. Soil and plant residues*

A loamy soil was collected in spring 2015 from 0 to 10 cm depth in Urrbrae, South Australia (Longitude 138°38´3.2″ E, Latitude 34°58´0.2″S) from an area that had been under pasture for more than 80 years. This site is in a semi-arid area and has a Mediterraneanmclimate with cool, wet winters and hot, dry summers. The soil is a Red-brown Earth according to Australian soil classification (Isbell, 2002) and classified as Rhodoxeralf in US Soil Taxonomy. Soil was collected along a randomly selected central transec

in three 2 x 2 m plots which were at least 10 m apart. In each sampling plot, after removal of plants and surface litter five samples of the topsoil (0–10 cm) were taken and sieved to less than 2 mm followed by air-drying in a fan-forced oven at 40 °C. Soil from all sampling points were mixed before starting the experiment. The soil properties are 22% sand, 60% silt, 18% clay, maximum water capacity (WHC) 371 g $kg^{-1}$, pH (1:5) 5.6, EC (1:5) 0.1 dS $m^{-1}$, total organic C 17 g $kg^{-1}$, total N 1.5 g $kg^{-1}$, bulk density 1.3 g $cm^{-1}$, available P 10 mg P $kg^{-1}$ and available N 15 mg N $kg^{-1}$. Two types of plant residues were used: young faba bean (*Vicia faba* L.) as low C/N ratio residue (L), and mature wheat straw (*Triticum aestivum* L.) as high C/N ratio residue (H) (Table 1). These two plant species are typical crops in Southern Australia and often follow each other in crop rotations. The residues were dried at 40 °C in a fan-forced oven, finely ground and sieved to 0.25-2 mm particle size. Low C/N ratio residue had 5 to 10 times higher total N, total P, available N and P and two-fold higher water extractable C concentration, but lower C/N ratio and C/P ratios than H (Table 1). The residues had a similar pH and total organic C content.

**Table 1.** Total organic C, total N, total P, C/N ratio and C/P ratio, available N and P, water-extractable C, pH and electrical conductivity (EC) of low C/N (young faba bean) and high C/N (mature wheat straw) residues (n=4). ($P \leq 0.05$).

| Property | Low C/N | High C/N |
|---|---|---|
| Total organic C (g $kg^{-1}$) | 374 | 418 |
| Total N (g $kg^{-1}$) | $22.9^{b}$ | $4.9^{a}$ |
| Total P (g $kg^{-1}$) | $6.5^{b}$ | $0.7^{a}$ |
| C/N ratio | $16^{a}$ | $86^{b}$ |
| C/P ratio | $58^{a}$ | $643^{b}$ |
| Available N (mg $kg^{-1}$) | $487^{b}$ | $87^{a}$ |
| Available P (mg $kg^{-1}$) | $247^{b}$ | $30^{a}$ |
| Water extractable C (g $kg^{-1}$) | $92^{b}$ | $54^{a}$ |
| pH (1:10) | 6.2 | 6.3 |
| EC (1:10) (mS $m^{-1}$) | 10.2 | 5.6 |

*2.2. Experimental design*

Before the start of the experiment, the air-dried soil was incubated for 10 days at 21-23 °C in the dark at 50% of maximum WHC to activate the soil microbes and to stabilise soil respiration. This water content was selected based on previous studies that showed that microbial activity is maximal at 50% WHC in this soil (Marschner *et al*., 2015)

After pre-incubation, the soil was either kept at 50% or dried in a fan-forced oven at 40 °C to 30% or 10% of WHC in 2-4 h. Water contents of 50, 30 and 10% of WHC correspond to water potentials of -0.078, -0.32 and -1.7 Mpa. These water contents were used because they gave large differences in soil respiration in this soil in another experiment (Xue *et al.*, 2016). After reaching the target water content, soil was left unamended (C) or amended with L or H residues at a rate of 10 g $kg^{-1}$.

The water content was maintained at 10, 30 or 50% WHC from day (d) 0 to d10. On d10, the soil water content of all treatments was adjusted to 50% WHC and then residues were added at 10 g $kg^{-1}$ to give six residue treatments (CH, CL, HH, HL, LL, and LH). The soil was kept at 50% WHC from d10 until the end of the experiment (d20).

After each residue amendment, 30 g dry soil equivalent was filled into PVC cores with 1.85 cm radius, 5 cm height and a nylon mesh base (7.5 µm, Australian Filter Specialist) and packed to a bulk density of 1.3 g $cm^{-1}$. The cores were placed individually into 1 L jars with gas tight lids equipped with septa to allow quantification of the headspace $CO_2$ concentration as described below. The jars were incubated in the dark at 21–23 °C. Soil moisture was maintained by checking the water content every few days by weight and adding reverse osmosis (RO) water if necessary. Soil respiration was measured daily. Four cores per treatment were destructively sampled on d1 (one day after the first residue addition), d10 (end of first period), d11 (one day after water content was adjusted to 50% WHC in all treatments and second residue addition) and d20 and analysed for available N and P, microbial biomass C, N and P, giving a total of 216 cores.

The extent of the legacy effect in the second period was calculated by comparing the measured parameters in LH with those in HH and in LL with those in HL for each water content in the first period. To be specific, if the second residue was L, then the extent of the legacy effect in the second period was (LL-HL)/HL; while if the second residue was H, then the extent of the legacy effect in the second period was (LH-HH)/HH.

### *2.3. Measurements*

Soil texture was determined according to the rapid textural analysis (Chaudhari *et al*., 2008; Kettler *et al*., 2001). Maximum soil water holding capacity was measured in a sintered glass funnel connected to a 100 mm water column (ψm = -10 kPa). Soil was placed in rings in the sintered glass funnel, thoroughly wetted and allowed to drain for 48 h. Dry weight of the soil was determined after oven drying at 105 °C for 24 h.

Soil pH and EC were measured in a 1:5 (w/v) soil to reverse osmosis (RO) water ratio after 1 hour shaking at 25 °C. Total organic carbon of soil and plant residues was measured according to Walkley & Black (1934) and total nitrogen was measured using the Kjeldahl method followed by colorimetric measurement as described in Bremmer & Mulvaney (1982).

Soil and plant residues were digested with a mixture of $HNO_3$ and $HClO_4$, to determine total P. Total P in the extract was measured by the phosphovanadomolydate method. Water extractable organic carbon was determined by shaking 1 g residue with 30 ml RO water for 1 hour. Then the extract was centrifuged at 3000 rpm for 10 min and filtered through a Whatman# 42 filter paper. The organic C in the extract was determined after $K_2Cr_2O_7$ and $H_2SO_4$ oxidation by titration with acidified $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$.

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). Jars were vented using a fan to refresh the headspace daily after each measurement (t1) and then resealed followed by another $CO_2$ measurement (t0). The $CO_2$ produced during this given interval is the difference in $CO_2$ concentration between t 1 and t0 (Setia *et al*., 2011). Linear regression based on injection of known amounts of $CO_2$ into empty jars of similar size was used to define the relationship between $CO_2$ concentration and detector reading.

Available N (ammonium and nitrate) concentration was measured after 1 hour end-over-end shaking with 2M KCl at a 1:5 soil to extractant ratio. Ammonium-N was measured after Willis *et al*. (1996). Nitrate-N was

determined as described in Cavagnaro *et al*. (2006). Available P was extracted by the anion exchange resin method (Kouno *et al.*, 1995), the P concentration was determined colorimetrically (Murphy & Riley, 1962). Microbial biomass C (MBC) and N (MBN) were determined by chloroform fumigation-extraction with 0.5 M $K_2SO_4$ at 1: 4 soil to extractant ratio (Vance *et al*., 1987). Organic C concentration in the extract was measured by titration with 0.033 M acidified $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ after dichromate oxidation. Themchloroform-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 differences in $NH_4^+$ concentration between fumigated and non-fumigated samples divided by 0.57 which is the proportionality factor to convert ammonium to MBN suggested by Moore *et al.* (2000). Microbial biomass P (MBP) was determined with the anion exchange method as described by 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).

### *2.4. Statistical analysis*

There were four replicate cores for each treatment and sampling time. Data was tested for homogeneity and equal variance. For measurements carried out repeatedly during the experiment, two-way analysis of variance (ANOVA) was carried out in SPSS (IBM NY, USA). The interaction between residue treatment and water content treatment was significant ($p$<0.05). Therefore Tukey's multiple comparison test at 95% confidence interval was used for each sampling time separately One way ANOVA was also used to compare cumulative respiration in the two 10-day intervals as well as for the properties of two plant residues.

## 3. Results

### *3.1. Cumulative respiration*

In the first 10 days, cumulative respiration was about two-fold higher in L than H and about three-fold higher in H than in unamended soil (Figure 1a). It was 2 to 3-fold higher at 50% WHC than at 10% WHC. From d11 to d20 (after residue addition and adjusting the water content in all treatments to 50%, Figure 1b), cumulative respiration was higher in HL, LL and LH than in treatments that had received only H (CH and HH).

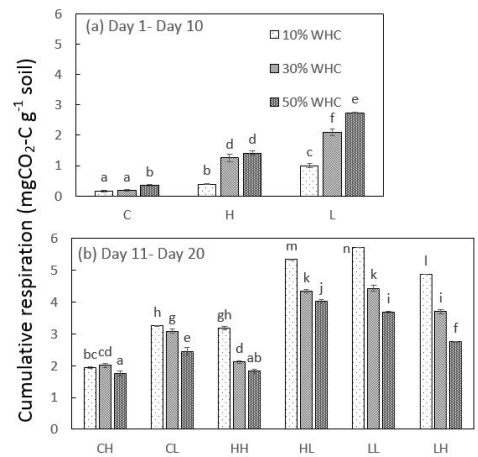


**Figure 1**. Cumulative respiration from day 0 to day 10 and from day 11 to day 20 in soil with different water contents (10%, 30% or 50% WHC) in the first 10 days and at 50% WHC from day 10 to day 20. The soil was unamended (C) or amended with high (H) or low (L) C/N ratio residue on day 0 followed by addition of H or L on day 10 (n=4, means ± SE). For each 10-day period (day 1-10, day 11-20), bars with different letters indicate significant differences among treatments ($P \leq 0.05$).

It was 20-40% higher in soil previously maintained at 10% WHC than that kept at 30 or 50% WHC with a smaller difference in CH and CL, which had not been amended in the first period, than in treatments with residue addition on d0. In the previously amended soils, cumulative respiration in the second period in soil maintained at 30% in the first period was only slightly (5-10%) higher than at 50%. In all moisture treatments, cumulative respiration from d11 to d20 was 50-75% higher in LH than HH and similar in HL and LL.

*3.2. Microbial biomass*

On d1, MBC was highest in L and lowest in unamended soil and decreased with soil water content (Figure 2a). Differences in MBC between residue treatments were greater at 50 and 30% than at 10% WHC. On d10, MBC was lower than on d1 at 30 and 50% WHC, but similar as on d1 at 10% WHC (Figure 2b).

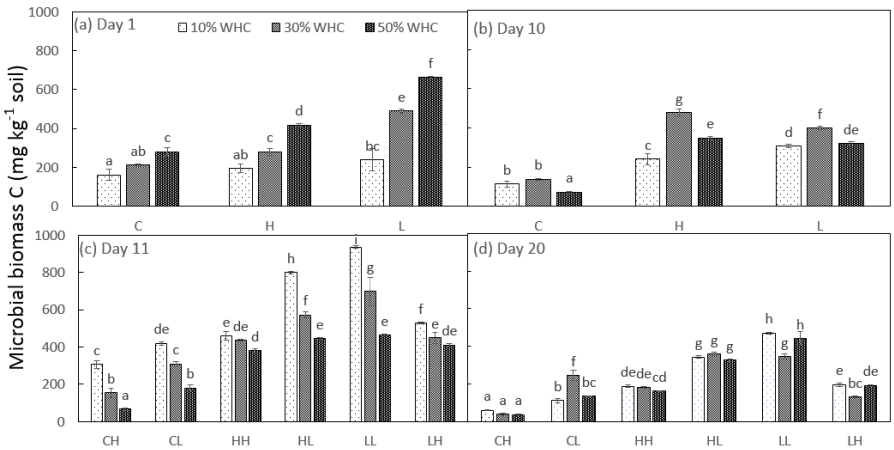


**Figure 2.** Microbial biomass C concentration on days 1 (a), 10 (b), 11 (c) and 20 (d) in soil with different water contents (10%, 30% or 50% WHC) in the first 10 days and at 50% WHC from day 10 to day 20. The soil was unamended (C) or amended with high (H) or low (L) C/N ratio residue on day 0 followed by addition of H or L on day 10 (n=4, means ± SE). For each sampling time, bars with different letters indicate significant differences among treatments ($P \leq 0.05$).

In amended soil (L and H), MBC on d10 was highest at 30% WHC, but in unamended soil it was similar at 10 and 30% WHC, both being higher than 50% WHC. On d 11, one day after adjustment of the water content to 50% WHC and residue addition, MBC was up to threefold higher than on d10, with a greatest increase in soil previously maintained at 10% and the smallest increase in soil kept at 50% WHC in the first period (Figure 2c).

In all residue treatments, MBC on d11 was highest in soil kept at 10% WHC in the first period and lowest in soil maintained at 50% WHC. Microbial biomass C was up to two-fold higher at 10% than 50% WHC in treatments that were amended with L in the second period (CL, HL, LL). In soils amended with H on d10, MBC on d11 was 1-20% higher in HH and LH at previous 10% WHC than at 50%, but three-fold higher in CH.

On d11, MBC was 14% higher in LH than HH at 10% WHC in the first period, but only 7% higher at 50% WHC. Microbial biomass C was about 20% higher in LL than HL at 10% WHC, but similar in both treatments at 50% WHC in the first period (d1-10). In general, MBC on d20 was lower than on d11 (Figure 2d). It was two to four-fold higher in soils amended with L on d10 (CL, HL, LL) than those amended with H (CH, HH, LH). The previous water content had no consistent effect on MBC on d20 and also had no consistent effect on differences between HL and LL or LH and HH.

Microbial biomass N on d1 (Figure 3a) was highest in soil amended with L. Within each residue treatment, MBN was higher at 50% than at 10% WHC with greater differences in residue amended soils (about two-fold higher at 50% than at 10%) than in unamended soil (20% higher at 50% WHC). Microbial biomass N did not change from d1 to d10 in unamended soil or soil with H. With L, MBN did not change from d1 to d10 at 50% WHC, but decreased about two-fold at the lower water contents (Figure 3b).

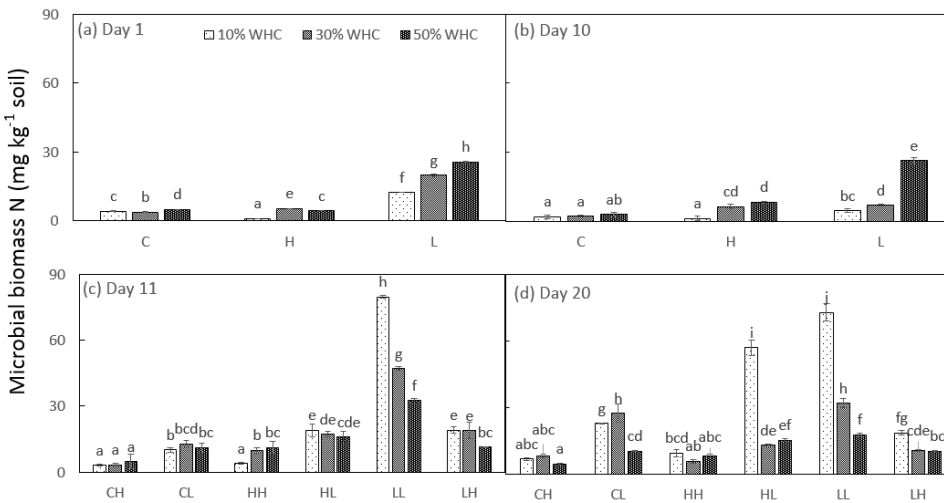


**Figure 3.** Microbial biomass N concentration on days 1 (a), 10 (b), 11 (c) and 20 (d) in soil with different water contents (10%, 30% or 50% WHC) in the first 10 days and at 50% WHC from day 10 to day 20. The soil was unamended (C) or amended with high (H) or low (L) C/N ratio residue on day 0 followed by addition of H or L on day 10 (n=4, means ± SE). For each sampling time, bars with different letters indicate significant differences among treatments ($P \leq 0.05$).

Differences between residue treatments in MBN on d10 were greater at 50% WHC than 30 or 10%. On d11 (one day after adjustment to 50% WHC and residue amendment), MBN was up to eight-fold higher than on d10 with the greatest increase in LL previously kept at 10% WHC (Figure 3c). Microbial biomass N on d11 was highest in LL and lowest in CH. The previous water content did not influence MBN in CH, CL and HL. In HH, MBN on d11 was lowest in soil previously maintained at 10% WHC whereas in LL and LH it was lowest in soil maintained at 50% WHC. The difference between 10 and 50% was greater in LL (two-fold higher in 10% WHC) than in LH (20% higher). At 10% WHC in the first 10 days, MBN on d11 was nearly four-fold higher in LH than HH, whereas it was only 7% higher at 50% WHC. The difference in MBN between LL and HL was also greater at 10% WHC in the first period (three-fold higher in LL) than at 50% (two-fold higher). Microbial biomass N changed little from d11 to d20 in CH, HH, LL and LH, but increased in CL and HL in soil previously kept at 10% WHC where it was up to two-fold higher on d20 than d11 (Figure 3d). In soil amended at least once with L (CL, HL, LL and LH), MBN on d20 was two to three-fold higher with previous 10% WHC than 50% WHC, but MBN was not influenced by the previous water content in soils only amended with H (CH and HH). The difference in MBN on d20 between LH and HH was greater at 10% WHC in he first period (about two-fold higher in HL) than at 50% (25% higher in HL). The difference between LL and HL was greatest at 30% WHC in the first period (1.5-fold higher in LL), followed by 10% WHC (30% higher in LL) and smallest in soil maintained at 50% WHC (15% higher in LL). Microbial biomass P on d1 was five to ten-fold higher in soil amended with L than with H and unamended soil (Table 2).

**Table 2.** Microbial biomass P concentration (mg $kg^{-1}$ soil) on days 1, 10, 11 and 20 in soil with different water contents (10%, 30% or 50% WHC) in the first 10 days and at 50% WHC from day 10 to day 20. The soil was unamended (C) or amended with high (H) or low (L) C/N ratio residue on day 0 followed by addition of H or L on day 10 (n=4, means ± SE). For each sampling time, values with different letters are significantly different ($P \leq 0.05$).

| Residue treatment | 10% WHC | 30% WHC | 50% WHC | 10% WHC | 30% WHC | 50% WHC |
|---|---|---|---|---|---|---|
| | Day 1 | | | Day 10 | | |
| C | 7.3±0.9$^{ab}$ | 2.8±1.5$^{ab}$ | 5.5±2.5$^{ab}$ | 2.6±0.4$^{a}$ | 3.4±1.0$^{a}$ | 5.3±1.6$^{abc}$ |
| H | 10.4±0.1$^{b}$ | 2.4±0.5$^{a}$ | 5.9±1.6$^{ab}$ | 5.2±0.9$^{ab}$ | 10.4±1.0$^{cd}$ | 10.3±1.9$^{cd}$ |
| L | 45.6±1.5$^{c}$ | 53.3±2.3$^{d}$ | 58.4±1.5$^{d}$ | 10.0±3.5$^{bcd}$ | 15.5±4.0$^{e}$ | 16.3±1.4$^{e}$ |
| | Day 11 | | | Day 20 | | |
| CH | 7.2±1.1$^{a}$ | 8.9±1.4$^{ab}$ | 8.4±2.0$^{ab}$ | 22.3±0.7$^{b}$ | 11.4±0.2$^{a}$ | 14.4±3.0$^{a}$ |
| CL | 25.9±3.7$^{fg}$ | 17.2±2.4$^{cde}$ | 14.0±1.7$^{abcd}$ | 24.8±0.3$^{b}$ | 20.8±1.9$^{b}$ | 21.4±3.1$^{b}$ |
| HH | 9.4±3.5$^{abc}$ | 16.9±2.2$^{cde}$ | 13.6±3.9$^{abcd}$ | 26.3±0.9$^{bc}$ | 13.0±1.7$^{a}$ | 12.7±2.3$^{a}$ |
| HL | 16.1±1.7$^{bcde}$ | 30.3±3.7$^{gh}$ | 38.2±8.8$^{hi}$ | 32.5±1.0$^{de}$ | 25.9±1.1$^{bc}$ | 21.±3.7$^{b}$ |
| LL | 45.9±1.4$^{i}$ | 43.7±0.5$^{i}$ | 42.6±2.7$^{i}$ | 46.7±1.4$^{f}$ | 44.5±3.4$^{f}$ | 37.3±2.2$^{e}$ |
| LH | 20.8±1.0$^{def}$ | 27.9±1.3$^{fg}$ | 22.7±2.0$^{efg}$ | 30.8±2.3$^{cd}$ | 30.6±2.2$^{cd}$ | 25.5±3.1$^{bc}$ |

In soil with L, MBP increased with soil water content and was about 25% higher at 50% WHC than at 10% WHC. Water content had little effect on MBP on d1 in unamended soil and with H. With L, MBP decreased by 70% from d1 to d10, but changed little in the other two amendment treatments. In both H and L amended soil, MBP on d10 was 30-50% higher at the two higher water contents than at 10% WHC. At all water contents, MBP was highest in soil with L. Microbial biomass P increased from d10 to d11 (one day after residue addition and adjusting the water content to 50% WHC), ranging from a two-fold increase in CH to a five-fold increase in LL. On d11 MBP was highest in LL and lowest in CH. In soils amended with H in the second period (CH, HH, LH), the previous water content did not affect MBP on d11. In CL and LL, MBP was higher in soil kept at 10% WHC in the first 10 days than at 50% WHC, but in HL the reverse was true. The difference in MBP between LH and HH on d11 was greater in soil kept at 10% WHC previously (LH two-fold higher than HH) than in soil maintained at 50% WHC (LH 60% higher than HH). Similarly, MPB on d11 was nearly three-fold higher in LL than HL in soil kept at 10% WHC in the first 10 days whereas it was only 11% higher in soil maintained at 50%. Microbial biomass P remained stable from d11 to d20 in LL and CL in all previous water contents. In the other residue treatments, MBP in soil kept at 10% in the first 10 days increased up to twofold from d11 to d20, but changed little with the other previous water contents. The difference in MBP on d20 between LH and HH was greater in soil kept at 50% WHC previously (LH two-fold higher than HH) than in soil maintained at 10% WHC (LH about 20% higher than HH). Microbial biomass P on d20 was about 80% higher in LL than HL in soil with 50% WHC in the first 10 days whereas it was only 40% higher in soil maintained at 10%.

### *3.3. Available N and P*

On d1 and d10, available N was three to six-fold higher in soil with L than with H or unamended soil (Figure 4a, b).

Available N on d1 increased with water content in soil with L, but was not influenced by water content in the other two residue treatments. Available N on d10 was highest at 10% WHC in all residue treatments. In treatments that were amended with L on d10 (CL, LL and HL), available N increased about two-fold from d10 to d11 whereas it remained unchanged when H was added on d10 (CH, HH, LH) (Figure 4c). Soil water content in the first 10 days did not influence available N on d11 in CH and HH. In LL and LH, available N was 10-20% higher in soil kept at 10% previously than soil maintained at 50% WHC. But in CL and HL, available N was two-fold higher in soil maintained at 50% WHC than soil kept at 10% WHC in the first 10 days. Available N on d11 was about ten-fold higher in LH than HH at all previous water contents. But the difference in available N on d11 between LL and HL was greater in soil previously kept at 10% WHC (three-fold higher in LL than HL) than soil maintained at 50% (two-fold higher in LL). Available N increased about two-fold in most residue treatments (except LH) from d11 to d20 in soil that was kept at 10% WHC previously whereas it changed little over time in soil maintained at 50% WHC (Figure 4d). In all residue treatments except HH, available N was about two-fold higher in soil kept at 10% WHC previously compared to that kept at 50% WHC. Available N on d20 was threefold higher in LH than HH in soil that was kept at 10% WHC previously, but only 14% higher in soil maintained at 50% WHC. It was about two-fold higher in LL than HL at all previous water contents.

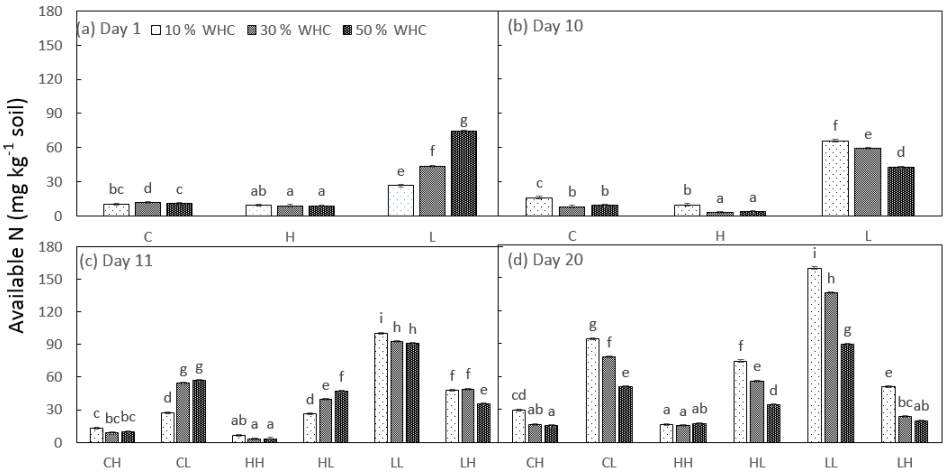


**Figure 4.** Available N concentration on days 1 (a), 10 (b), 11 (c) and 20 (d) in soil with different water contents (10%, 30% or 50% WHC) in the first 10 days and at 50% WHC from day 10 to day 20. The soil was unamended (C) or amended with high (H) or low (L) C/N ratio residue on day 0 followed by addition of H or L on day 10 (n=4, means ± SE). For each sampling time, bars with different letters indicate significant differences among treatments ($P \leq 0.05$).

Available P on d1 was lower at 10% WHC than at 50% in unamended soil and when amended with L, but was not influenced by water content in soil with H (Figure 5a).

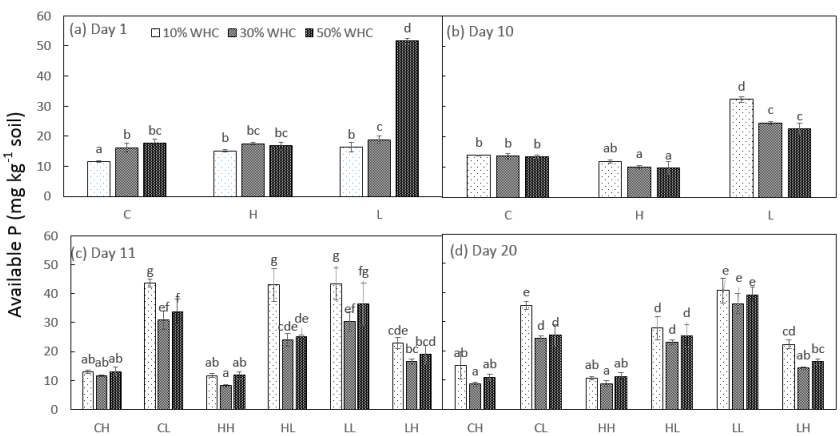


**Figure 5**. Available P concentration on days 1 (a), 10 (b), 11 (c) and 20 (d) in soil with different water contents (10%, 30% or 50% WHC) in the first 10 days and at 50% WHC from day 10 to day 20. The soil was unamended (C) or amended with high (H) or low (L) C/N ratio residue on day 0 followed by addition of H or L on day 10 (n=4, means ± SE). For each sampling time, bars with different letters indicate significant differences among treatments ($P \leq 0.05$).

At 50% WHC, available P on d1 was about two-fold higher with L than unamended soil or with H, but residue treatments differed little in available P at the lower water contents. In unamended soil and with H, available P did not change from d1 to d10 and available P on d10 was not influenced by soil water content (Figure 5b). With L at 10% WHC available P increased from d1 to d10 about two-fold whereas it remained unchanged at 30% WHC and decreased two-fold at 50% WHC from d1 to d10. With L, available P on d10 was about a third higher at 10% WHC than at 50% WHC. Available P did not change from d10 to d11 in soil amended with H on d10 (CH, HH, LH), but increased about two-fold in soil amended with L (CL, HL, LL) (Figure 5c). In LL, the increase was similar at all previous water contents, but in CL and HL, the increase was 20-30% greater in soil kept at 10% WHC previously than at the two higher water contents. In LL, available P in soil at 10% WHC in the first 10 days was only about 20% higher than at the higher water contents. Available P on d11 was lower in CH and HH than the other residue treatments. It was about two-fold higher in LH than HH soil kept at 10 and 30% WHC previously, but only 60% higher in soil maintained at 50% WHC. Available P on d11 did not differ between LL and HL in soil with 10% WHC previously, but in soil maintained at 50% WHC, it was about 30% higher with LL than HL. Available P changed little from d11 to d20 in CH, HH, LL and LH at all previous water contents. Available P also did not change during this time in the other treatments at 30 and 50% WHC (Figure 5d). But in soil kept at 10% previously, available P decreased 20-30% from d11 to d20 in CL and HL. Available P on d20 was about two-fold higher in LH than HH in soil previously kept at 10% WHC, but was only about 50% higher in soil with higher water contents. Available P was about 50% higher in LL than HL at all previous water contents.

## 4. Discussion

This experiment showed that after residue addition, low soil water content influences soil respiration, microbial biomass and nutrient availability not only directly, but also after adjustment to optimal water content. Further, it showed that the water content between the first and second residue amendment affects the e xtent of the legacy effect after the second residue addition. Differences in respiration, microbial biomass and nutrient availability were greatest between 10% WHC and the two higher water contents whereas the differences between 30 and 50% were small and inconsistent. Therefore the discussion will focus on10 and 50% WHC.

### *4.1. Between first and second residue addition (d1-d10)*

Cumulative respiration, MBC/N/P and available N and P were higher with L than with H or in unamended soil. This is in agreement with previous studies (Marschner *et al*., 2015; Nguyen *et al.*, 2016) and can be explained by the higher N, P and water extractable organic C concentration in L compared to H. Compared to the unamended soil, the measured parameters were only slightly higher with H indicating that nutrients in H were poorly available to soil microbes.

Compared to 50% WHC, cumulative respiration in the first period, MBC and MBN on d1 and available N and P on both d1 and d10 were lower at 10% WHC, with larger differences in soil with L than with H or unamended soil. This suggests that with H and in unamended soil, low nutrient availability limited microbes even at optimal water content. The lower cumulative respiration, MBC, MBN, available N and P in soil with L at 10% compared to 50% WHC can be explained by lower water availability to microbes as a result of stronger binding of water to soil particles and

in small pores as well as discontinuous water films which limit diffusion of substrates to microbes (Geisseler *et al*., 2011).

In soil amended with L, differences in MBC were smaller on d10 than d1 because MBC decreased from d1 to d10 at 50% WHC whereas there was little change over time at 10% WHC. Available N and P increased from d1 to d10 about two-fold at 10% WHC, but decreased during this time at 50% WHC. The decrease at 50% WHC can be explained by depletion of easily available organic N from L. At 10% WHC, decomposition was much slower and thus easily available organic N depleted more slowly. The increase in available N at 10% from d1 to d10 was greater than the decrease in MBN which suggests that available N was not only derived from microbial turnover. Apparently, even at this low water content some N was mineralised.

At the end of the first period, MBC was little affected by water content, but MBN and MBP were lower at 10% WHC than at 50%. This may be due to low N and P mineralisation, but could also be an indirect result of the low microbial activity. Inactive microbes have low N and P demand because they require fewer proteins and other cell components than active microbes (Vrede *et al*., 2002). Due the lower microbial activity, it can be assumed that more of the residue added on d1 was left on d10 at 10% compared to 50% WHC.

### *4.2. After second residue addition (d11-d20)*

As in the first 10 days, cumulative respiration in the second period was greater when L was added on d10 than with H addition which can be explained by the higher nutrient concentration of L compared to H. Cumulative respiration from d11 to d20 was generally higher than in the first period which is likely due to the greater amount of residue in the soil after the second addition which would include residue added on d10 and residue left in soil from first addition). In treatments where the same residue type was added on d1 and d10 (LL and HH), the difference in cumulative respiration between first and second period was greater at 10% than at 50% WHC. In the soil kept at 10% WHC in the first 10 days, there are several explanations for the increase in cumulative respiration in the second period. Firstly, the higher soil water content in the second period which would increase microbial activity. Secondly, a greater proportion of the previously added residue was left at 10% than at 50% WHC at the end of the first period, thus the amount of residue in the soil in the second period was greater

at 10% WHC. Thirdly, rewetting of the soil on d10 may have resulted in a flush of microbial activity (Wu & Brookes, 2005). This is supported by the greater increase in MBC from d10 to d11 in soil previously kept at 10% WHC than that maintained at 50% WHC. However, since the soil was at 10% WHC, not air-dry, the flush is likely to be smaller than commonly observedm upon rewetting of dry soil (Chowdhury *et al*., 2011). And lastly, microbes in soil at 10% are likely to be starved due to limited diffusion which may induce very high activity when soils are rewet and residues are added. The more rapid decrease in MBC from d11 to d20 at 10% WHC could be explained by depletion of available substrates which would be fastest when initial uptake was high. It is also possible that MBC on d11 was overestimated because of release of organic C from freshly added residues, but this would be the case at all previous water contents.

The previous water content had no clear effect on MBN and available N on d11. However in treatments with L (CL, HL, LL, LH), MBN and available N increased from d11 to d20 and were higher on d20 in soil previously kept at 10% WHC than that maintained at 50% WHC. This suggests that N mineralisation rate increased slowly after rewetting on d10, but then remained high whereas it did not change or even decreased in soil maintained at 50% WHC. Available

N on d20 was higher in LL and CL than HL because only L was in the soil in CL and LL whereas in HL, microbes decomposing H left from the first addition immobilised N which is evident in the high MBN concentration on d20 in HL. In CH and HH, available N and MBN were low throughout the second period and not influenced by previous water content indicating that the low N concentration in H limited N mineralisation and uptake. This is in contrast to cumulative respiration in the second period and MBC on d11 in CH and HH which were higher in soil previously kept at 10% WHC than that maintained at 50% WHC. Thus, while organic C mineralisation was stimulated by previous low water content, N likely limited microbial activity and growth in CH and HH.

In the treatments that were unamended in the first period (CL and CH), the previous water content generally had a greater effect on the measured parameters in CL than CH. In unamended soil, mineralisation of native organic matter in the first period was greater at 50% than at 10% WHC as shown in the higher cumulative respiration and MBC on day 10. Thus in CL, not only the added L residue was decomposed, but also any remaining available native soil organic matter of which there was more left at 10% than at 50% WHC which resulted in higher respiration, microbial biomass and available nutrients in soil previously kept at 10% WHC. In CH, N likely limited decomposition of both added H and available native soil organic matter. As in our previous studies (Marschner *et al*., 2015; Nguyen *et al.*, 2016), nutrient availability and microbial biomass in the second period were influenced by the C/N ratio of the initially added residue (legacy effect). Available N and MBN were lower in HL than LL and lower in HH than LH which can be explained by microbes decomposing H and L together in the treatments HL and LH. Nitrogen released by microbes decomposing L can be taken up by those decomposing H. Nutrient transfer from low to high C/N residue was shown in previous studies (Schimel *et al*., 2007; Schwendener *et al*., 2005). This transfer from L to H would increase MBN and available N in LH compared to HH, but decrease them in HL compared to LL. Based on this concept, it is likely that the extent of the legacy effect depends on amount of previously added residue left in the soil when the second residue is added. The more of the first amendment is left when thesec ond residue is added, the stronger the legacy effect as it was the case in Zheng & Marschner (2017). Thus, our hypothesis was that the legacy effect is stronger when the water content in the first period is low than when it is high. This hypothesis can be confirmed because differences in MBC, MBN, MBP and available N on d11 between HL and LL and between LH and HH were greater when the water content in the first period was 10% WHC compared to 50% WHC. This was most pronounced in available N and MBN where the differences between HL and LL and between LH and HH were three-fold greater with 10% WHC in first period than with 50% WHC. The previous water content had less effect on the extent of the legacy effect on d20; because by then, decomposition had occurred at optimal water content for 10 days which is likely to over-ride the effect of the previous water content.

## 5. Conclusions

Low soil water content after the first residue addition reduced residue decomposition, particularly the low C/N residue. This increased the influence of the first residue on soil respiration and N availability after the second residue addition and adjustment to optimal water content. The implications of these findings are that residues added to dry soil which is later rewet have a longer lasting effect on nutrient availability than expected from studies in constantly moist soil. This should be taken into account when calculating fertiliser requirements. To better understand nutrient

fluxes in soil, $^{15}N$ labelled residue could be used in future experiments.

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