

# Legacy effect of previous residue addition—influence of length of the moist period between residue additions on soil respiration, microbial biomass and nutrient availability

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**Abstract** In previous studies, we showed that microbial activity and nutrient availability after the second residue addition are influenced by the C/N ratio of the previously added residue, referred to as legacy effect. The aim of this experiment was to assess the influence of soil water content after the first residue addition on the legacy effect. A silt loam was amended with high C/N residue (mature wheat shoots, C/N 120, H) or low C/N residue (young kikuyu shoots, C/N 22, L) at 10 g kg<sup>-1</sup> twice, on day 0 and on day 29 with low C/N residue following high C/N residue or vice versa (HL or LH). After the first residue addition, the soil was maintained either constantly moist for 4 weeks or dried immediately after residue addition or after 1, 2 or 3 weeks. All dried treatments were rewetted on day 28 and the second residue added on day 29 after which all soils were maintained moist until end of the experiment (day 57). Respiration was measured over 57 days and microbial biomass C (MBC), N (MBN) and P (MBP) and available N and P were determined on days 0, 29 (before second residue addition), 43 and 57. Compared to constantly moist soil, only soil drying immediately after the first residue addition reduced cumulative respiration and increased MBC on day 29, and with low C/N residue, increased available N concentration. After the second residue addition, the previous moisture treatment influenced the legacy effect in HL, but not LH. Compared to the treatment that was maintained moist

after the first residue addition, cumulative respiration and MBC and available N concentration were higher in the treatment that was dried immediately after the first amendment. This can be explained by the greater amount of the previously added H residue left in the soil when the L residue was added compared to the treatment that had been moist throughout the experiment. It can be concluded that soil drying influences the legacy effect only when it occurs shortly after addition of the first residue, which is when decomposition rates are high.

**Keywords** C/N ratio · Immobilisation · Mineralisation · Nutrient availability · Rewetting

## Introduction

During summer in Mediterranean climates, top soils can remain dry for long periods of time which are occasionally interrupted by rainfall or irrigation events. In dry soils, microbial activity is low due to low water availability (Shi and Marschner 2014b). Some soil microbes may die due to loss of water from the cell, become dormant (Kakumanu et al. 2013) or compensate the strongly negative water potential in dry soil by accumulating osmoregulatory compounds which minimise water loss (Estop-Aragonés and Blodau 2012). The sudden decrease in soil water potential upon rewetting has been shown to induce a flush of respiration, which is followed by a rapid decrease in respiration rates (Mikha et al. 2005; Butterly et al. 2009). The respiration flush can be explained by increased substrate availability from cell lysis, release of intracellular osmoregulatory compounds accumulated during the dry period and exposure of previously protected organic matter due to aggregate breakdown (Mikha et al. 2005; Xiang et al. 2008; Navarro-García et al. 2012).

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Amendment of soil with plant residues can increase organic matter content, biological activity and nutrient availability and improve soil physical properties (Bending and Turner 1999; Sakala et al. 2000; Partey et al. 2014). Net mineralisation–immobilisation patterns in soil amended with organic materials are influenced by environmental conditions (Saccone et al. 2013), composition and abundance of the decomposer community (Kristiansen et al. 2004) and the chemical composition of the organic amendments (Thomas and Asakawa 1993). For example, the decomposition rate of organic materials is influenced by the nature of the organic C (Hadas et al. 2004), clay type (Vogel et al. 2015), clay content (Shi and Marschner 2014a) and soil moisture (Yu et al. 2014; Shi and Marschner 2015). Other factors influencing decomposition rate and nutrient release are the C/N ratio, lignin content and lignin/N ratio of the organic amendment. Microorganisms have a low C/N ratio (typically < 20); therefore, organic amendments with low C/nutrient ratio can satisfy the N demand of microbes and result in early net N mineralisation (Hadas et al. 2004). In contrast, the addition of plant residue with high C/N ratio can cause temporary net immobilisation of nutrients in the microbial biomass (Moritsuka et al. 2004). The decomposition rate of residues is negatively correlated with lignin content and lignin/C ratio because lignin is difficult to decompose (Vanlauwe et al. 1997). Residue properties such as C and N content, lignin/N ratio can also influence N<sub>2</sub>O emission (Chen et al. 2015). For example, N<sub>2</sub>O loss from anaerobic microsites may be smaller with low C/N than with high C/N residue because N<sub>2</sub>O release is negatively correlated with microbial activity (Begum et al. 2013).

In many ecosystems, plant residues from different species may be added to soils repeatedly, for example through leaf fall or root death. In Mediterranean climates, soils may be exposed to dry periods between residue additions. The effect of repeated addition of the same or different residue types on mineralisation and nutrient availability has been studied in moist soils (Duong et al. 2009; Marschner et al. 2015; Nguyen and Marschner 2015). It was shown that nutrient availability after the second addition of residue with the same or different C/N ratio depends not only on the C/N ratio of the second amendment, but also that of the first (Marschner et al. 2015; Nguyen and Marschner 2015). The legacy effect of the first residue addition on decomposition and nutrient availability after the second residue addition may be influenced by the extent to which the initially added residue has been decomposed because that determines how much is left in the soil when the second residue is added. Microbial activity is lower in dry than in moist soil; therefore, the extent of decomposition of the first amendment and thus the legacy effect could be influenced by the length of the moist and dry periods between the first and second residue addition.

The aim of this study was to determine the influence of the length of the moist period between the first and second residue

addition on soil respiration and nutrient availability. Residue treatments were high C/N followed by low C/N residue and low C/N followed by high C/N residue because the legacy effect was most pronounced in these treatments in our previous study (Marschner et al. 2015). We hypothesised that: (i) with decreasing length of the moist period after the first residue addition, cumulative respiration and microbial biomass would decrease; and (ii) the effect of the previous residue addition on the measured parameters (legacy effect) would decrease with length of moist period after the first residue addition.

## Materials and methods

### Soil and residues

The silt loam used in this study was collected from 0 to 15 cm on the Waite Campus, The University of Adelaide (34°58' S, 138° 37' E). The area is in a semi-arid region and has a Mediterranean climate with hot and dry summers and cool, wet winters. The soil is a red-brown Earth in Australian soil classification (Isbell 2002) and a Calcic Rhodoxeralf according to US Soil Taxonomy (Chittleborough and Oades 1979). The soil was managed for over 80 years in the Waite Long-Term Rotation trial as permanent pasture and its properties are sand 27 %, silt 51 % and clay 22 %, pH (1:5 soil: water) 7.3, electrical conductivity (EC 1:5) 742  $\mu\text{S cm}^{-1}$ , total N 134 mg kg<sup>-1</sup> and P 461 mg kg<sup>-1</sup>, organic C 15 g kg<sup>-1</sup>, available N 15 mg kg<sup>-1</sup> and P 34 mg kg<sup>-1</sup>, microbial biomass C 122 mg kg<sup>-1</sup>, N 7 mg kg<sup>-1</sup> and P 1 mg kg<sup>-1</sup>, maximum water holding capacity (WHC) 327 g kg<sup>-1</sup>, and bulk density 1.3 g cm<sup>-3</sup>. After collection from several randomly selected sites on the plot, samples were pooled and dried at 40 °C in a fan-forced oven. This temperature is not unnatural because daytime temperatures in summer often exceed 40 °C in the top soil and soils are air-dried for several weeks. After air-drying, visible plant debris was removed and the soil sieved to <2 mm.

Two residues with distinct properties were used (Table 1): high C/N mature wheat shoots (*Triticum aestivum* L.) and low C/N young kikuyu shoots (*Pennisetum clandestinum* L.). These two plant species were chosen because wheat is an important cereal and its straw and stubble are often incorporated into the soil after harvest and kikuyu is a common pasture species and used as green manure. The residues were dried at 40 °C in a fan-forced oven, ground and sieved to particle size 0.25 to 1 mm.

### Experimental design

Before the start of the experiment, the air-dried soil was pre-incubated for 10 days at 22 °C and 50 % of WHC to reactivate the microbes and to stabilise their activity after rewetting. This

**Table 1** Total organic C, N, P, C/N and C/P ratio, available N and P, water-extractable C, and pH of high C/N (mature wheat shoots) and low C/N (young kikuyu shoots) residues ( $n = 3$ )

Properties	High C/N	Low C/N
Total organic C (g kg <sup>-1</sup> )	370.4	321.8
Total N (g kg <sup>-1</sup> )	3.3a	16.7b
Total P (g kg <sup>-1</sup> )	0.2a	2.9b
C/N ratio	120b	21.8a
C/P ratio	1675b	97a
Available N (mg kg <sup>-1</sup> )	112.9a	464.9b
Available P (mg kg <sup>-1</sup> )	40.2a	117.5b
Water extractable C (g kg <sup>-1</sup> )	32.3a	41.8b
pH (1:10)	6.7	6.5

Within rows, means followed by different letters are significantly different ( $P \leq 0.05$ )

water content was chosen because soil respiration was maximal at 50 % of WHC in our recent study with the same soil (Marschner et al. 2015).

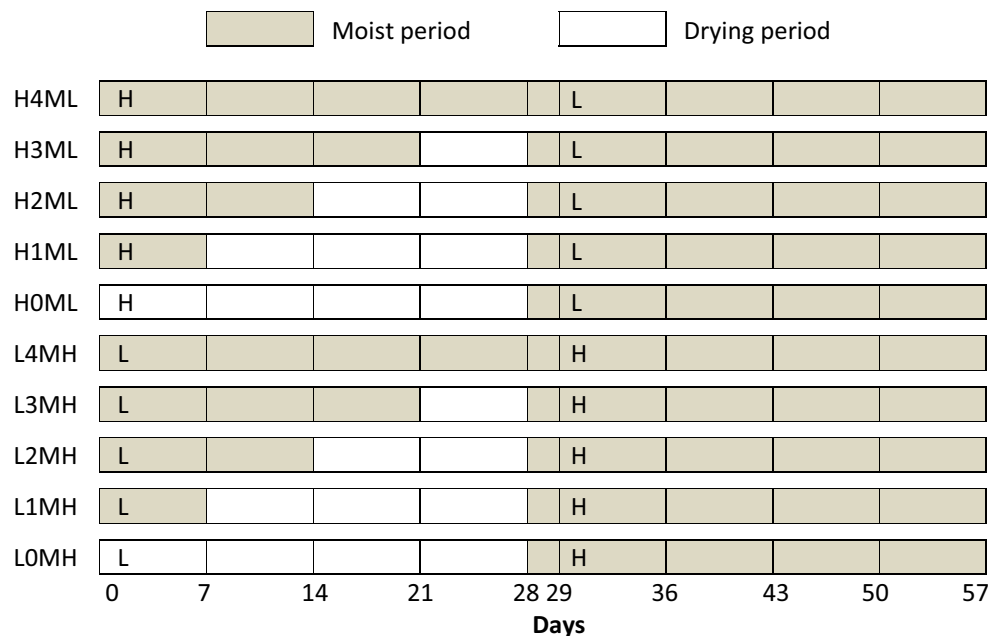
The pre-incubated soil was amended with high C/N residue (C/N 120, H) or low C/N residue (C/N 22, L) on day 0 and followed by the second amendment on day 29 with low or high C/N residue, respectively (Fig. 1). Thus, there were two residue treatments: HL and LH. At both first and second amendment, residues were added at 10 g kg<sup>-1</sup> soil and thoroughly mixed into the soil. In the 4 weeks after the first addition, soil moisture treatments with different length of the dry period were imposed. The soil was maintained constantly moist for 4 weeks (H4ML, L4MH), 3 weeks moist and 1 week dry (H3ML, L3MH), 2 weeks moist and 2 weeks dry (H2ML, L2MH), 1 week moist and 3 weeks dry (H1ML, L1MH) or dry for 4 weeks (H0ML, L0MH).

Then all drying treatments were rewetted on day 28 and the second residue added on day 29 (1 day after rewetting). After the second residue addition, the soil was maintained at 50 % of WHC until end of the experiment (day 57).

After each residue addition, 30 g dry soil equivalent was filled into PVC cores with 1.85-cm radius, 5-cm height and a nylon net base (7.5 μm, Australian Filter Specialist) and adjusted to a bulk density of 1.3 g cm<sup>-3</sup> by packing the soil in the cores to the appropriate height. For the second residue addition, the soil was removed from the cores, residues added, mixed and then returned to the cores. The cores were placed individually into 1-L jars with gas-tight lids equipped with septa to allow quantification of headspace CO<sub>2</sub> concentration as described below. The jars were incubated in the dark at 22–23 °C. During the moist periods, soil moisture was maintained at 50 % of WHC by checking the water content every few days by weight and adding reverse osmosis (RO) water if necessary. Drying was achieved by placing small pouches containing self-indicating silica gel into the glass jars from days 21, 14, 7 and 0 for H3ML and L3MH, H2ML and L2MH, H1ML and L1MH, and H0ML and L0MH, respectively. The silica gel was exchanged daily. The soil dried to constant weight (6 % of WHC) in 7 days.

Cores were destructively sampled on days 0 (5 h after residue addition), 29 (1 day after rewetting, before second residue addition), 43 (2 weeks after second residue addition) and 57 (4 weeks after second residue addition) for analysis of available N and P, microbial biomass C, N and P, pH and EC (Fig. 1). Microbial biomass C, N and P were not determined on day 0 because release of C, N and P by chloroform

**Fig. 1** Schematic diagram of treatments for soil amended with high (H) or low (L) C/N residues on days 0 and 29 with different length of dry period between the first and second residue addition



from freshly added residues could lead to overestimation of microbial biomass (Domenach et al. 1994).

### Analyses and calculations

Soil texture was determined by the hydrometer method (Gee and Or 2002). The pH and electrical conductivity (EC) were 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 C 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<sub>2</sub>SO<sub>4</sub> and a 6:1 mixture of HNO<sub>3</sub> and HClO<sub>4</sub>, respectively. Total N was measured by a modified Kjeldahl method (Bremner and Sulvaney 1982). Total P in the digest was measured by the phosphovanado-molybdate method according to Hanson (1950). Water-extractable organic C was determined by shaking 1 g residue with 30 ml RO water for 1 h. The extract was centrifuged at 1509g for 10 min and filtered through a Whatman#42 filter paper. The water-extractable organic C concentration of the extract was determined after K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and H<sub>2</sub>SO<sub>4</sub> oxidation by titration with acidified (NH<sub>4</sub>)<sub>2</sub>Fe(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O (Anderson and Ingram 1993). Solutions with different sucrose concentrations (0.01–1 mg C ml<sup>-1</sup>) were used as standards.

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 measured colorimetrically according to the nitroprusside/dichloro-S-triazine modification of the Bertelot indophenol reaction after Forster (1995). Nitrate-N was determined by reduction of nitrate by vanadium (III) combined with detection by the acidic Griess reaction as described by Miranda et al. (2001). Available P was extracted by the anion exchange resin (#55164; BDH Chemicals, Poole, England) method (Kouno et al. 1995), and the P concentration was determined by formation of a blue molybdophosphate complex in an acid matrix which was reduced with ascorbic acid and measured colorimetrically (Murphy and Riley 1962). This method was also used to determine microbial biomass P using hexanol as fumigant (Kouno et al. 1995). Microbial biomass P was calculated as the difference in P concentration between fumigated and non-fumigated. A correction factor for P sorption was not used because an earlier study with this soil found that recovery of a P spike was 98 % (Butterly et al. 2010).

Soil microbial biomass C and N were determined by chloroform fumigation–extraction (Vance et al. 1987). Soil samples were fumigated with chloroform for 48 h followed by shaking at 1:5 ratio with 0.5 M K<sub>2</sub>SO<sub>4</sub>. The C concentration

in the extract of fumigated and non-fumigated soils was determined by adding 0.0667 M K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and concentrated H<sub>2</sub>SO<sub>4</sub>. The remaining K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> was titrated with 0.033 M acidified (NH<sub>4</sub>)<sub>2</sub>Fe(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O (Anderson and Ingram 1993). The difference in C concentration between fumigated and non-fumigated soil was divided by 0.38 to calculate MBC (Vance et al. 1987). Microbial biomass N (MBN) was determined after Moore et al. (2000). Ammonium-N in the extracts was determined as described above for available N. MBN was calculated as the difference in ammonium-N concentration between fumigated and non-fumigated samples divided by 0.57 which is the proportionality factor to convert ammonium to microbial biomass N (Moore et al. 2000).

Soil respiration was determined by measuring the CO<sub>2</sub> concentration in the headspace of the jars using a Servomex 1450 infrared gas analyser (Servomex, UK) as described by Setia et al. (2011). After each measurement (T<sub>1</sub>), the jars were vented to refresh the headspace using a fan and then resealed followed by determination of the CO<sub>2</sub> concentration (T<sub>0</sub>). The CO<sub>2</sub> evolved during a given interval was calculated as the difference in CO<sub>2</sub> concentration between T<sub>1</sub> and T<sub>0</sub>. Due to the upper detection limit of the gas analyser (2 % CO<sub>2</sub>) and the decrease in respiration rate over time after residue addition, respiration was measured daily in the first 6 days after addition of residues and then every second day. Linear regression based on injection of known amounts of CO<sub>2</sub> in the jars was used to define the relationship between CO<sub>2</sub> concentration and detector reading. Cumulative respiration was calculated as the sum of respiration rates [in mg CO<sub>2</sub>-C (g soil and day)<sup>-1</sup>] for the first and the second period.

### Statistical analysis

There were four replicates per treatment and sampling time. Data were analysed by two-way ANOVA [residue treatments × water treatments (length of dry period)] 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.

## Results

### Residue properties

High C/N residue (mature wheat shoots) had lower total N and P, available N and P, water extractable organic C concentrations, but higher C/N and C/P ratios than low C/N residue (young kikuyu shoots). The two residues had similar total organic C and pH (Table 1).

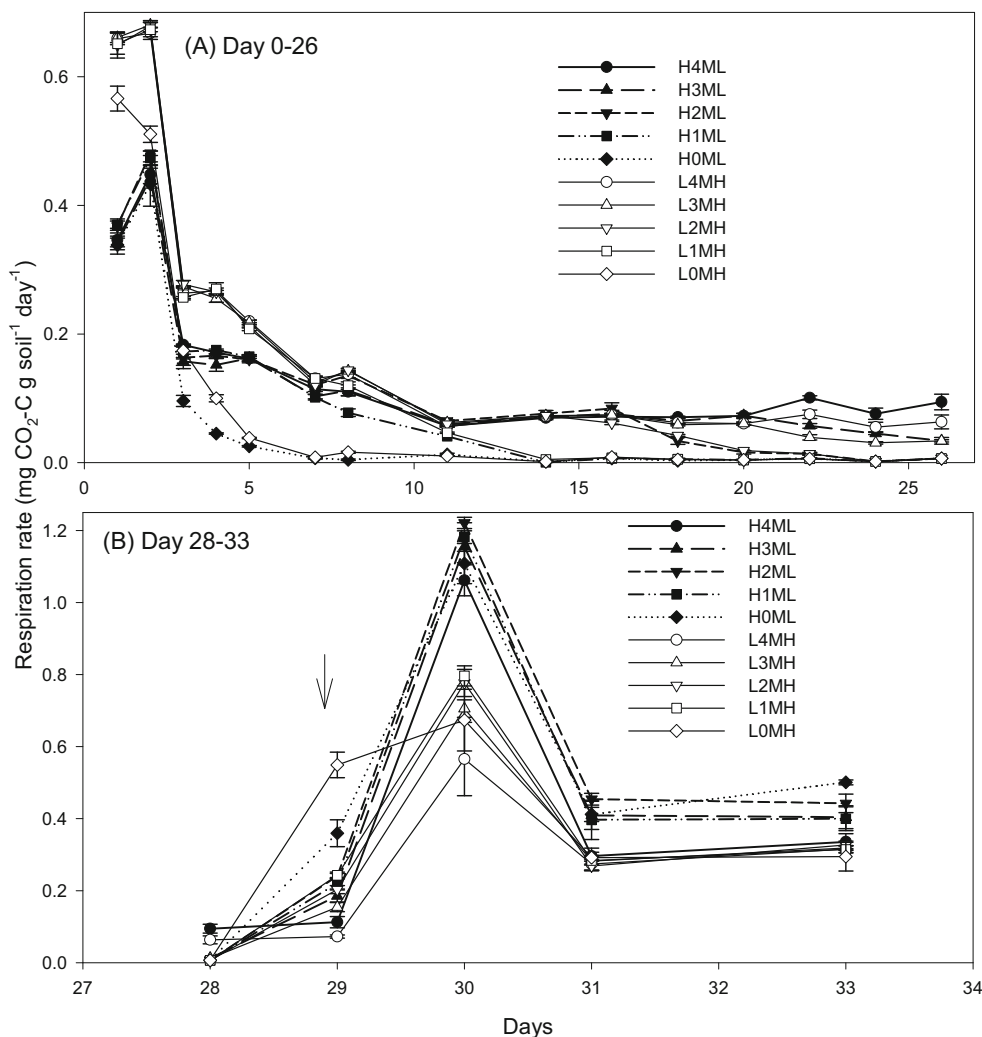
### Respiration

After the first residue addition, respiration rates were highest in the first 5 days and stable from day 10 onwards (Fig. 2a). In treatments where the soil was moist in the first week (1, 2, 3 and 4 M), respiration rates in the first 5 days were higher with low compared to high C/N residue. In treatment 0 M where the soil was dried immediately after residue addition, respiration rates rapidly declined in the first 3 days and were lower than in treatments with moist soil until day 10. In 0 M, respiration rates were higher with low compared to high C/N residue only in the first 2 days. In 1 M, in which soils were dried after the first week, drying reduced respiration rate in the second week, but the reduction by drying was much smaller than when soils were dried immediately after residue addition. Drying 2 or 3 weeks after the first residue addition (in 2 and 3 M) had no effect on respiration rate. Rewetting of treatments 1, 2, 3 and 4 M on day 28 induced a two to sixfold increase in

respiration rate (Fig. 2b). This increase was greatest in 0 M where the soils were dry longest. Only in 4 M, respiration rates on day 29 were higher with low compared to high C/N residue. Residue addition on day 29 increased respiration rates in all moisture treatments. Respiration rates on day 30 were about twofold higher when low C/N followed high C/N residue on day 29 (HL) than when high C/N followed low C/N residue (LH). Respiration rates rapidly declined from day 30 to 31. Respiration rates remained about 30 % higher in HL than LH until day 33 except in the constantly moist treatments (4 M). Compared to the first addition, respiration rates on the first day after residue addition were twice as high in HL than when high C/N residue was added on day 0, but were similar in LH as after low C/N residue addition on day 0.

In the first 29 days, before the second residue addition cumulative respiration was about 30 % higher with low C/N residue than with high C/N residue (Fig. 3a). Cumulative respiration did not differ between treatments with 2 or more

**Fig. 2** Respiration rate (mg CO<sub>2</sub>-C g soil<sup>-1</sup> day<sup>-1</sup>) from days 1 to 33 in soil with different length of dry period between the first and second residue addition with high C/N followed by low C/N residue (H4ML, H3ML, H2ML, H1ML, H0ML) or low C/N followed by high C/N residue (L4MH, L3MH, L2MH, L1MH, L0MH) on day 29 (*n* = 4, values are means ± SE). Arrow indicates the day of the second residues addition. For treatment abbreviations, see Fig. 1

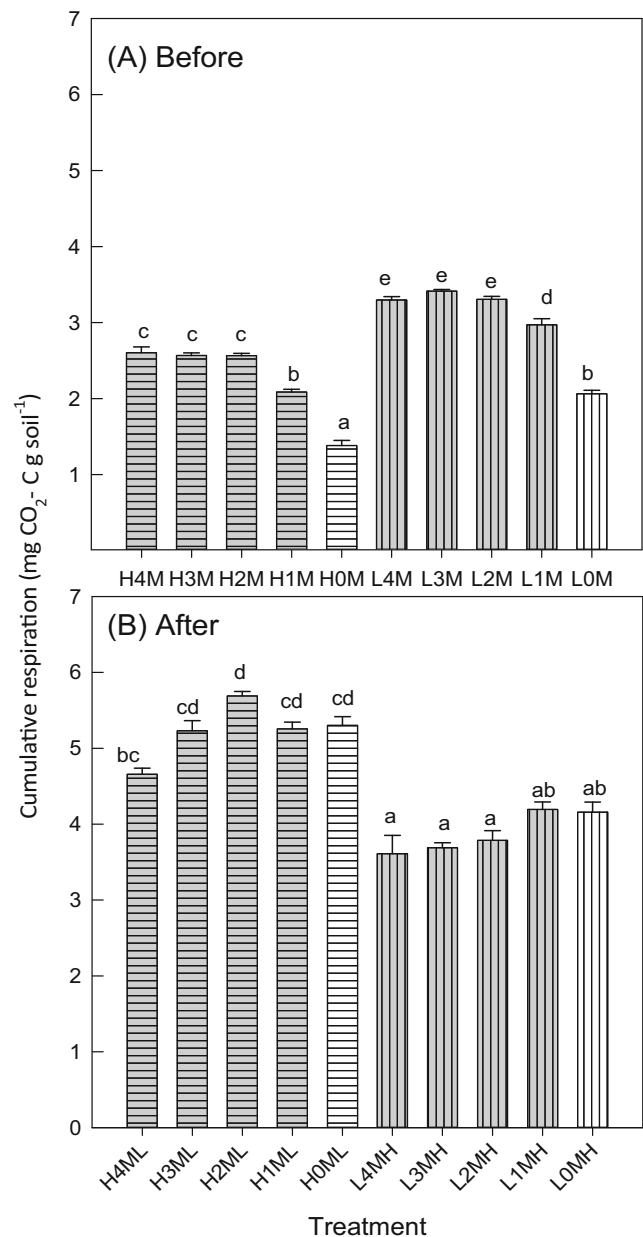


weeks moist (2, 3 and 4 M), but was 20 % lower with only 1 week moist (1 M) and 40 % lower when the soil was dried immediately after residue addition (0 M) (Fig. 3a). Cumulative respiration in HL after the second residue addition until the end of the experiment was about 30 % higher than LH and nearly twice as high than after first addition of high C/N residue (Fig. 3). In LH, the moisture treatment after the first residue addition had no significant effect on cumulative respiration after the second addition (when all soils were maintained moist) (Fig. 3b). In HL, cumulative respiration after the second residue addition was about 15 % lower in 4 M compared to treatments with a dry period, but this difference was significant only compared to the treatment with 2 weeks moist (H2ML). In both residue treatments, cumulative respiration from day 0 to 57 was 10 % lower in 0 M than in the treatments with at least 1 week moist (Table S1). Among the latter treatments, cumulative respiration was lowest in 4 M and highest in 2 M, with greater differences in HL than LH.

### Microbial biomass

Microbial biomass C (MBC) did not differ between HL and LH on days 29 and 43 (Fig. 4). On day 29 (1 day after rewetting but before the second residue addition), MBC was up to twofold higher in 0 M compared to the treatments with at least 1 week moist (1, 2, 3 and 4 M). Moisture treatments did not differ in MBC on day 43. On day 57, MBC was about 40 % higher in LH than HL in treatments with at least 1 week moist (Fig. 4c). In HL, MBC was about 40 % higher in the treatment which had remained dry for 4 weeks in the first period (0 M) than in the treatments with at least 1 week moist. However, MBC did not differ among moisture treatments with at least 1 week moist in LH.

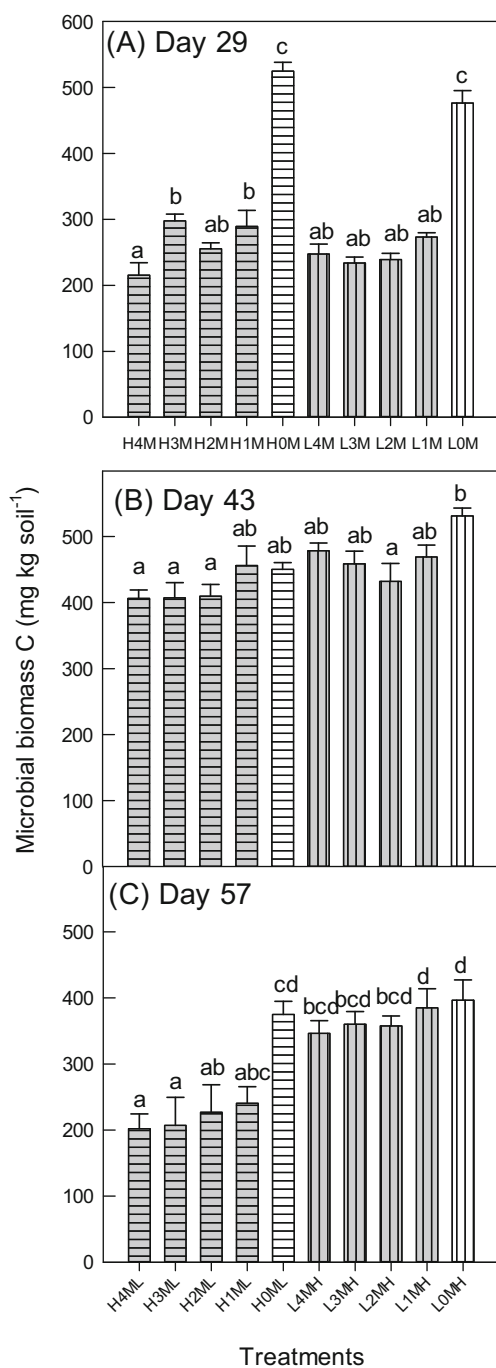
Microbial biomass N (MBN) on day 29 (before the second residue addition) was 10 % higher in treatments with low (LH) compared to high C/N residue (HL) (Fig. 5a). In a given residue treatment, MBN on day 29 was not influenced by moisture treatment in the first period. On day 43, MBN was 10 % higher in HL than LH in treatments with no or 1 week moist (0 and 1 M), but did not differ in treatments with 2 or more weeks moist (2, 3 and 4 M) (Fig. 5b). Moisture treatment in the first period influenced MBN on day 43 only in HL where it was 30 % higher in treatments with no or 1 week moist (0 and 1 M) than in treatments with 2 or more weeks moist (2, 3 and 4 M). Microbial biomass N on day 57 did not differ between HL and LH except in the treatment which was dried immediately after the first residue addition (0 M) where it was 10 % higher in HL than LH (Fig. 5c). In HL, but not LH, MBN was about 30 % lower in treatments with 3 or 4 moist



**Fig. 3** Cumulative respiration over 29 days before the second residue addition (a) and 28 days after the second residue addition (b) in soil with different length of dry period between the first and second residue addition with high C/N followed by low C/N residue (H4ML, H3ML, H2ML, H1ML, H0ML) or low C/N followed by high C/N residue (L4MH, L3MH, L2MH, L1MH, L0MH) ( $n=4$ , values are means  $\pm$  SE). Bars with different letters are significantly different ( $P \leq 0.05$ ). For treatment abbreviations, see Fig. 1

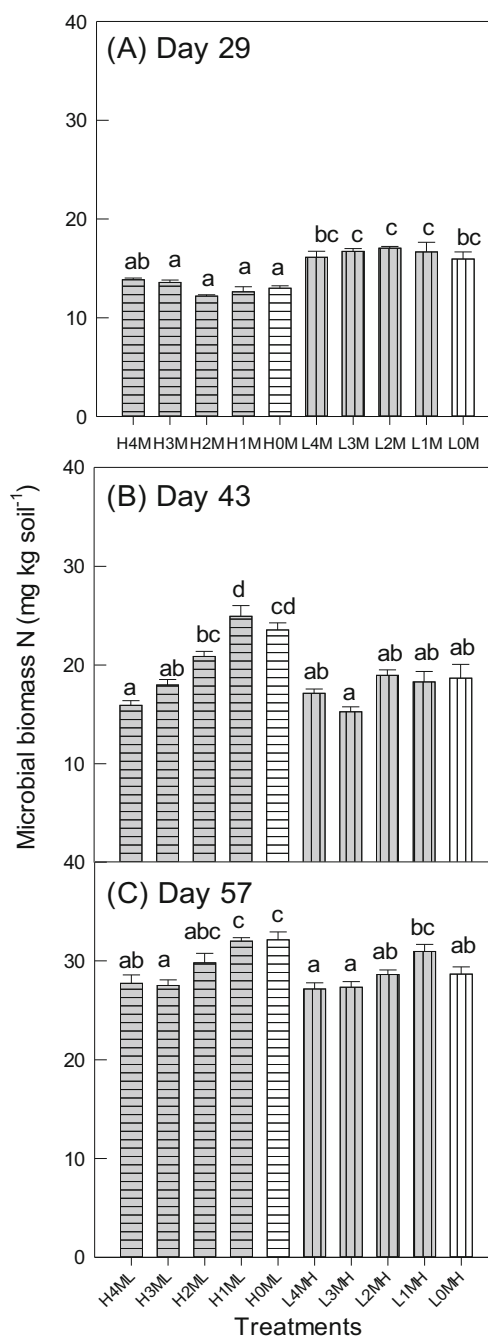
weeks in the first period (3 and 4 M) than in those no or 1 moist week (0 and 1 M).

Microbial biomass P (MBP) on day 29 was about 20 % higher in soil with low C/N (LH) than with high C/N residue (HL) (Fig. S1A). Moisture treatment in the first period influenced MBP only in LH where it was 10 % higher in soil that had been constantly moist or dry for 1 week (4 and 3 M) than



**Fig. 4** Microbial biomass C on days 29 (1 day after rewetting, before second residue addition **a**), 43 (2 weeks after second residue addition **b**) and 57 (4 weeks after second residue addition **c**) in soil with different length of dry period between the first and second residue addition with high C/N followed by low C/N residue (H4ML, H3ML, H2ML, H1ML, H0ML) or low C/N followed by high C/N residue (L4MH, L3MH, L2MH, L1MH, L0MH) ( $n = 4$ , values are means  $\pm$  SE). At a given sampling day, bars with different letters are significantly different ( $P \leq 0.05$ ). For treatment abbreviations, see Fig. 1

in soils have been dry for 4 weeks. Residue treatments did not differ in MBP on day 43 except in the treatments that had been dry for 4 weeks (0 M) in the first period where MBP



**Fig. 5** Microbial biomass N on days 29 (1 day after rewetting, before second residue addition **a**), 43 (2 weeks after second residue addition **b**) and 57 (4 weeks after second residue addition **c**) in soil with different length of dry period between the first and second residue addition with high C/N followed by low C/N residue (H4ML, H3ML, H2ML, H1ML, H0ML) or low C/N followed by high C/N residue (L4MH, L3MH, L2MH, L1MH, L0MH) ( $n = 4$ , values are means  $\pm$  SE). At a given sampling day, bars with different letters are significantly different ( $P \leq 0.05$ ). For treatment abbreviations, see Fig. 1

was 10 % higher in H0ML than in L0MH (Fig. S1B). MBP on day 57 was not influenced by residue or moisture treatment (Fig. S1C).

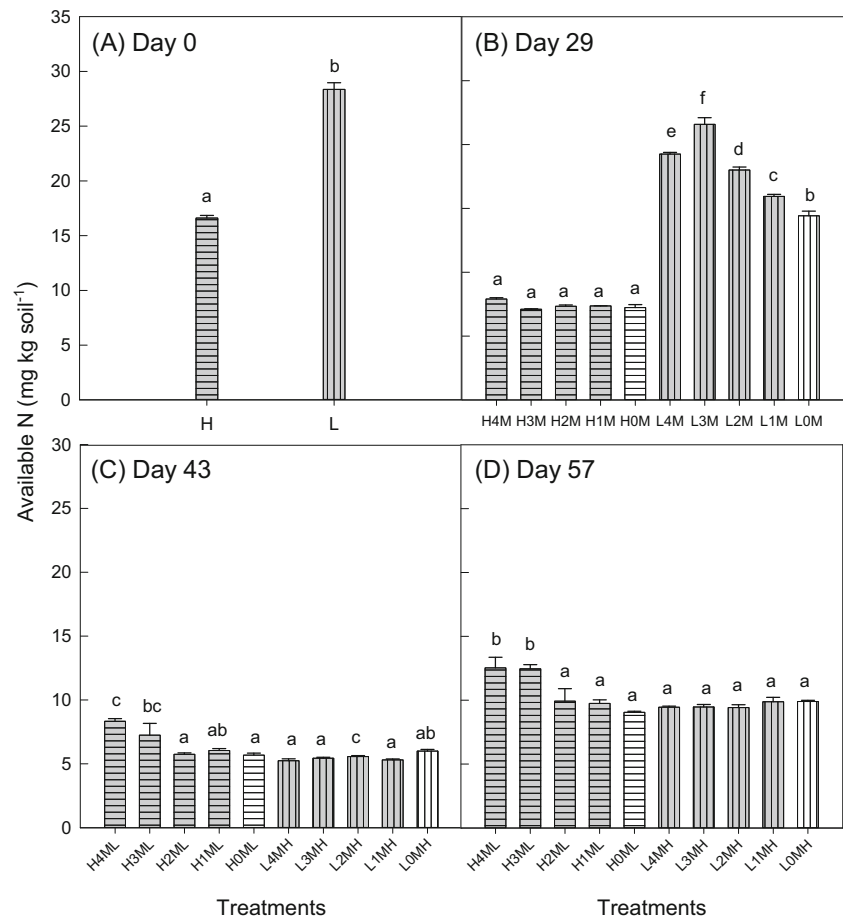
## Available N and P

On day 0, the available N concentration was nearly twice as high with low compared to high C/N residue (Fig. 6a). Available N concentrations were lower on the following sampling times. On day 29, after rewetting and before the second residue addition, available N concentrations were at least two-fold higher with low compared to high C/N residue (Fig. 6b). With high C/N residue (HL), the water content in the first period had no effect on available N concentrations, but with low C/N residue (LH), the available N concentration was lowest in the treatment where the soil was dried immediately after residue addition (0 M). The available N concentration increased as the number of moist weeks increased from 1 to 3 (1 to 3 M), but was lower in the continuously moist treatment (4 M) compared to that with 3 moist weeks (3 M). The available N concentration on day 43 (2 weeks after the second residue addition, Fig. 6c) in the LH treatments was less than half of that with low C/N residues on day 29. Available N concentrations were higher on day 57 (Fig. 6d) than on day 43. The effect of residue and previous moisture treatment was similar on days 43 and 57. The previous moisture treatment did not influence available N concentrations in LH treatments.

In HL, the available N concentration was 15 % higher in the continuously moist treatment and the treatment with 3 moist weeks (4 and 3 M) than in treatments with 2 or less moist weeks (2, 1 and 0 M).

The available P concentration on day 0 was about 40 % higher with low compared to high C/N residue (Fig. S2A). On day 29, the difference in available P concentration between the two residue treatments was smaller than on day 0; it was about 20 % higher with low compared to high C/N residue. In both residues, the available P concentration was about 10 % lower in the treatment where the soil was dried immediately after the first residue addition (0 M) than in the treatment with the highest concentration (2 moist weeks, 2 M). On day 43, there were no significant differences in available P concentration between HL and LH except in the treatment where the soil was dried immediately after the first residue addition (0 M), where it was 10 % higher in H0ML than in L0MH. The moisture treatment in the first period influenced the available P concentration only in LH, it was about 10 % lower in 0 M than in 3 M. Available P concentrations on day 57 were lower than on the previous sampling dates, and there were no significant differences between residue or moisture treatments.

**Fig. 6** Available N concentration on days 0 (a), 29 (1 day after rewetting, before second residue addition b), 43 (2 weeks after second residue addition c) and 57 (4 weeks after second residue addition d) in soil with different length of dry period between the first and second residue addition with high C/N followed by low C/N residue (H4ML, H3ML, H2ML, H1ML, H0ML) or low C/N followed by high C/N residue (L4MH, L3MH, L2MH, L1MH, L0MH) ( $n = 4$ , values are means  $\pm$  SE). At a given sampling day, bars with different letters are significantly different ( $P \leq 0.05$ ). For treatment abbreviations, see Fig. 1





## Discussion

This experiment showed that the length of the moist period between the first and the second residue addition influences nutrient availability, but we cannot unequivocally accept the first hypothesis. The length of the moist period after the first residue addition influenced the measured parameters only when comparing treatments where the soils were dried immediately after residue addition (0 M) to those kept moist for 4 weeks (4 M). The second hypothesis has to be declined because the moisture treatment after the first residue addition had very little impact on the measured parameters after the second residue addition, except for MBC and available N concentrations on day 57.

### Period between first and second residue addition (days 0–29)

In agreement with previous studies (Yao et al. 2011; Yan and Marschner 2014; Shi and Marschner 2015), soil respiration rates decreased as the soil dried compared to moist soil and remained low during the dry period. This is due to lower water availability to microbes and reduced substrate diffusion in dry soils (Yao et al. 2011). Cumulative respiration after the first residue addition was reduced compared to 4 weeks moist only when the soil was dried immediately after residue addition or 1 week later (0 and 1 M), but not when the soils were dried 2 or 3 weeks after the first residue addition (2 and 3 M). This can be explained by the changes in respiration rate over time. Respiration rates were high in the first 5–7 days after residue addition, but then declined and remained low. Drying therefore reduced cumulative respiration only if it occurred during the time when respiration rates were high in moist soil, i.e. first 7–10 days. Later, drying also reduced respiration rates, but the impact on cumulative respiration was small because respiration rates were low and most of the CO<sub>2</sub> had already been released in the previous moist period. Due to the lower cumulative respiration in 0 and 1 M compared to the constantly moist treatment (4 M), more of the initially added residue was left in the former when the soil was rewet and the second residue was added.

The length of the moist period influenced available N concentrations on day 29 when low C/N residue was added, but not with high C/N residue. With high C/N residue, the available N concentration was low in all moisture treatments, indicating that there was little net N mineralisation. The higher N availability with low C/N residue in treatments with 3 or 4 moist weeks compared to those with shorter moist periods suggests that N mineralisation in the first 2 weeks after residue addition (0, 1 and 2 M) was reduced by drying, although cumulative respiration was only lower in 0 and 1 M than in 4 M. It should be noted that treatments with a dry period were rewet on day 28 and N availability was measured the

following day. The flush in microbial activity after rewetting of dry soil may also lead to a temporary increase in N mineralisation (Scheu and Parkinson 1994). We cannot rule out that this was the case in this experiment because we did not measure N availability before rewetting. However, this N mineralisation flush is unlikely to be a major contributing factor in this experiment because then available N concentrations would have been expected to be higher where more of the previously added low C/N residue was still left in the soil (i.e. 0 and 1 M) compared to 4 M. More likely is that the available N measured on day 29 had accumulated over the previous 4 weeks.

It is not clear what caused the high MBC on day 29 in 0 M compared to the other moisture treatments. It may be organic C released by chloroform from residues that had been dry for 4 weeks. Another possible explanation is that this MBC comes from microbes that colonised the residue immediately after addition to soil on day 0 and grew until the soil water content was too low. These microbes may have remained dormant until rewetting.

### Period after second residue addition (day 30–58)

Respiration rates and cumulative respiration after the second residue addition were lower in LH compared to HL, which can be explained by two factors. Firstly, by the greater decomposability of low compared to high C/N residue (Partey et al. 2014; Marschner et al. 2015; Nguyen and Marschner 2015). Secondly, by the greater amount of residue (total from first and second residue addition) in the soil in HL compared to LH because cumulative respiration in the first period was lower with high than with low C/N residue. Thus, it can be assumed that more of the previously added high C/N residue than of the low C/N residue was still in the soil when the second residue was added. The greater amount of residue can also explain why cumulative respiration in the second period was greater than in the first. The greater MBC on day 57 compared to day 29 in most LH treatments (except L0MH) suggests that microbes decomposing high C/N residue were able to utilise N released by microbes decomposing the previously added low C/N residue. Transfer of N between microbes decomposing high and low C/N residue has been shown in experiments with residue mixtures (Schimel and Haettenschwiler 2007; Schwendener et al. 2005). This increase from day 29 to day 57 also occurred in MBN with a stronger increase in HL than LH, which can be explained by the higher N concentration in the low C/N residue added in the second period in HL.

In agreement with our previous study (Marschner et al. 2015), a legacy effect was evident on days 43 and 57. In the second period available N concentration was similar in HL and LH whereas it was more than twice as high in low C/N compared to high C/N residue on day 29. The legacy effect can be explained by interactions between microbes

decomposing the previously added residue and those decomposing the second amendment similar to those suggested in residue or litter mixtures (Gartner and Cardon 2004) such as the N transfer mentioned above, but also includes other synergistic effects. In HL, the available N concentration was influenced by the moisture treatment in the first period because it was higher in 3 and 4 M than in the treatments with shorter moist period (0, 1 and 2 M). The higher available N concentration can be explained by the smaller amount of high C/N residue in the soil when the low C/N residue was added in 3 and 4 M. Thus, uptake of N released from decomposition of low C/N residue was small. In LH on the other hand, the moisture treatment before the second residue addition did not influence available N concentrations after the second residue addition. Therefore, soil drying shortly after the first residue influenced the legacy effect on N availability only in HL. In HL, MBC on day 57 was lower than in LH in most moisture treatments. The only exception is 0 M where the MBC did not differ between HL and LH which is probably due to the larger amount of residue in the soil in the second period in 0 M compared to the other moisture treatments. The larger amount of residue remaining at the end of the first period when the soil was dried immediately after residue addition allowed maintenance of a large biomass until the end of the experiment. The finding that the moisture regime only influenced MBC in HL suggests that high microbial biomass after 4 weeks is mainly maintained by high C/N residue, but only if low C/N residue is also left over from the first addition and decomposed at the same time.

## Conclusion

The length of the moist period had little effect on the measured parameters after the first and second residue addition. Respiration, microbial biomass and N availability were different than in the constantly moist treatment only when the soil was dried immediately after the first residue addition. This highlights the importance of the first few days after residue addition for decomposition. In future experiments, soils could be dried between 1 and 7 days after residue addition to investigate the impact of water content on this first phase of residue decomposition in greater detail. The study confirmed the legacy effect which should be taken into account when estimating nutrient availability after residue addition. Mechanisms underlying the legacy effect could be studied using  $^{13}\text{C}$ ,  $^{15}\text{N}$  or  $^{32}\text{P}$  labelled residues.

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