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# Soil respiration, microbial biomass and nutrient availability in soil amended with high and low C/N residue – Influence of interval between residue additions



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#### ABSTRACT

It is well known that soil microbial biomass and activity and nutrient availability following plant residue addition are influenced by residue composition. In a previous study, Marschner et al. (2015) showed that in soil amended twice with residues of different C/N, the C/N of the previous residue addition influenced nutrient release after the second amendment which can be referred to as legacy effect. In that study, the interval between residue additions was 20 days. In this study, high C/N residue (mature wheat shoots, C/ N 120, H) or low C/N residue (young kikuyu shoots, C/N 22, L) were added at 10 g kg<sup>-1</sup> on day 0 to a silt loam followed by a second amendment (at 10 g kg<sup>-1</sup>) on days 10, 20 or 30 with low C/N residue following high C/N residue or vice versa (HL or LH). Respiration was measured between residue additions and over 30 days after the second addition of residues. Microbial biomass C, N and P and available N and P were measured every 10 days. Cumulative respiration over 30 days after the second residue addition was higher in HL than LH and higher when the second amendment occurred 10 days after the first compared to 30 days. There were no differences in microbial biomass C, N and P and available N and P between HL and LH when the second residue was added 10 days after the first, indicating a strong legacy effect. When the second residue was added 20 or 30 days after the first, available N concentrations were lower in LH than HL, but the MBC concentration was higher in LH than HL thus a legacy effect was present for MBC, but not for available N. It can be concluded that the legacy effect on N availability is short-lived (10 days), but the C/N ratio of a previously added low C/N residue can stimulate microbial growth after addition of high C/N residue even when added 30 days before.

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#### 1. Introduction

Plant residue application can increase biological activity and nutrient availability and improve soil physical properties (Bending and Turner, 1999). Decomposition rate and temporal nutrient release pattern are affected by the biochemical and physical properties of plant residues (Tian et al., 1992; Bending and Turner, 1999) and the abundance and community structure of the decomposers (Kristiansen et al., 2004). They are also influenced by environmental factors such as soil moisture and temperature (Hobbie, 1996) as well as annual temperature and rainfall (Trofymow et al., 2002; Saccone et al., 2013).

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http://dx.doi.org/10.1016/j.soilbio.2015.12.020 0038-0717/© 2016 Elsevier Ltd. All rights reserved. Biochemical properties of residues such as C/N ratio, lignin and cellulose contents and soluble organic C concentration are important for predicting nutrient release from organic material (Preston and Trofymow, 2000; Hadas et al., 2004; Moritsuka et al., 2004). Microorganisms have a low C/N ratio (typically <20) compared to most plant materials; organic amendments with low C/N ratio can satisfy the nutrient demand of microbes and result in early net nutrient mineralisation (Hadas et al., 2004). In contrast, the incorporation of organic materials with high C/N ratio induces temporary net immobilization of nutrients in the microbial biomass (Moritsuka et al., 2004).

Decomposition and nutrient release of individual and mixed residues have been studied extensively (Tian et al., 1992; Sakala et al., 2000; Gartner and Cardon, 2004). In many ecosystems plant residues from different species may be added repeatedly, for example through leaf fall or root death. The effect of repeated addition of the same residue type on mineralisation and nutrient



availability was studied by Duong et al. (2009). Recently, Marschner et al. (2015) showed in an experiment where a second residue with the same or a different C/N ratio was added 20 days after the first amendment, that nutrient availability after the second addition depends not only on the C/N ratio of the second amendment, but also that of the first. This suggests the existence of a legacy effect. In our earlier study (Marschner et al., 2015), we found that the previously added low C/N residue stimulated soil respiration, microbial growth and nutrient availability after addition of high C/N residue compared to high C/N residue following high C/N residue.

The present study further investigates the legacy effect. The aim of this study was to determine the influence of the interval between additions of residues with different C/N ratio on soil respiration, microbial biomass and nutrient availability. We hypothesized that the effect of the C/N ratio of the first residue addition on cumulative respiration, microbial biomass and N and P availability after the second residue amendment (the legacy effect) will (i) become smaller with increasing interval between residue additions, and (ii) become smaller over time after the second residue addition.

#### 2. Materials and methods

#### 2.1. Soil and residues

The silt loam used in this study was collected from 0 to 15 cm at 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 cool, wet winters, and hot and dry summers. The soil is a Redbrown earth in Australian soil classification (Isbell, 2002) and a Xeralf according to US Soil Taxonomy (Chittleborough and Oades, 1979). The soil was managed for over 80 years in the Waite Longterm Rotation trial as permanent pasture and has the following properties: sand 27%, silt 51% and clay 22%, pH (1:5 soil:water) 7.3, electrical conductivity (EC 1:5 soil and water) 742  $\mu$ S cm<sup>-1</sup>, total N 134 mg kg<sup>-1</sup> and total P 461 mg kg<sup>-1</sup>, total organic carbon (TOC) 15 g kg<sup>-1</sup>, available N 15 mg kg<sup>-1</sup>, available P 34 mg kg<sup>-1</sup>, maximum water holding capacity (WHC) 327 g kg<sup>-1</sup> and bulk density  $1.3 \text{ g cm}^{-3}$ . After collection from several randomly selected sites on the plot, the soil was 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-dry 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): mature wheat shoots (*Triticum aestivum* L.) with high C/N and young kikuyu shoots (*Pennisetum clandestinum* L.) with low C/N. These two plant species were chosen because wheat is an important cereal and its straw and stubble often incorporated into the soil after harvest. Kikuyu is a common pasture species and used as

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

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

green manure. The residues were dried at 40  $^{\circ}$ C in a fan-forced oven, ground and sieved to particle size 0.25–1 mm.

#### 2.2. Experimental design

Before the start of the experiment, the air-dried soil was preincubated for 10 days at 23 °C and 50% of WHC to reactivate the microbes and to stabilise their activity after rewetting. This water content was chosen based on our recent study where we used the same soil (Marschner et al., 2015).

There were six treatments (Fig. 1): soil amended with high C/N residue (C/N 120, H) on day 0, followed by low C/N residue (C/N 22, L) addition on days 10 (HL10), 20 (HL20) or 30 (HL30) and soil amended with low C/N residue on day 0 with addition of high C/N residue on days 10 (LH10), 20 (LH20) or 30 (LH30). The residues were added at 10 g kg<sup>-1</sup> soil at both times. These intervals between the first and the second residue addition were based on an earlier study with the same soil and residues (Marschner et al., 2015) where the second residue was added 20 days after the first and a legacy effect was found. The present study was conducted to investigate how a shorter or longer interval between residue additions compared to Marschner et al. (2015) influences the legacy effect.

After the initial 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 (0.75 µm, Australian Filter Specialist) and packed to a bulk density of 1.3 g cm<sup>-3</sup> by adjusting the height of the soil in the cores. Then the cores were placed individually into 1 L jars with gas-tight lids equipped with septa to allow guantification of headspace CO<sub>2</sub> concentration as described below. The jars were incubated in the dark at 22-23 °C. 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. Cores were destructively sampled on days 0 (5 h after residue addition), 10, 20, 30, 40, 50 and 60 (end of experiment) for analysis of available N and P, microbial biomass C, N and P, pH and EC (Fig. 1). However, microbial biomass C, N and P were not determined on day 0 because release of C, N and P by chloroform from freshly added residues could lead to overestimation of microbial biomass (Domenach et al., 1994). The final harvest of all treatments was 30 days after the second addition, therefore treatments with the second amendment on days 10 (HL10, LH10), 20 (HL20, LH20), and 30 (HL30, LH30) ended on days 40, 50 and 60, respectively (Fig. 1). In a given 10-day period, only the cores to be sampled at the end of the period were placed in the



Fig. 1. Schematic diagram of treatments for soil amended with high (H) and low (L) C/ N residues on days 0 and 10 or 20, or 30.

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