THE UNIVERSITY of ADELAIDE

Effects of drying-rewetting, previous and current soil water content on soil respiration, microbial biomass and nutrient availability in soils without or with plant residues differing in C/N ratio

Yanchen Zhang

Thesis submitted to The University of Adelaide in fulfilment of the requirements for the degree of Doctor Philosophy

Faculty of Sciences

School of Agriculture, Food and Wine

The University of Adelaide

2018

Table of contents

Acknowledgements

I would like to express my sincere gratitude to my principal supervisor, Prof Petra Marschner. It is great honour to be her student. Without her support, encouragement and guidance, I would not complete my fantastic journey in the PhD program. I appreciate the suggestions and comments from Petra about experimental design and scientific writing. There is no word I can say to thank her to spend so much time on revising my manuscripts. The knowledge and skills I have gained from Petra during the last three years are great treasures and will be beneficial for the rest of my career.

I also express my appreciation to my co-supervisor, Dr Clayton Butterly, independent advisor, Dr Ashlea Doolette and post-graduate coordinator Dr Jo Zhou for their suggestions and comments. I thank my mentor Dr John Radcliffe from CSIRO for his support and assistance during and after the Crawford Fund annual meeting.

I would like to thank Prof Scott Chang for providing me the opportunity to visit his lab in University of Alberta. I would like to express my gratitude to his critical comments to my research and his care about my life during my stay in Edmonton. I also appreciate all the help, suggestions and company from colleagues and students in University of Alberta.

The completion of my PhD would not have been possible without the assistance and support from professors, staff, friends and family in both Australia and China. I would like to thank Colin Rivers, Dr Hasbullah, Dr Shermeen Tahir, Dr Chaolei Yuan, Dr Trung Ta Nguyen, Erinne Stirling, Clinton Garrett, Anthelia Bond, Dr Ron Smernik, Dr Murray Unkovich, Mark Rollog, Dr Ran Xue, Dr Qiaoqi Sun, Hoang Thi Kim Khuyen, Bo Zheng, Juqi Li and many others for their help in my study and life. Your great help, encouragement and companies inspired me to go through out of the journey.

I am deeply indebted to my parents, for their love, continued support and understanding. I would also like to extend my sincerest thanks to my husband, Dr Yang Liu, for his sacrifice, understanding, patience and love.

Many thanks to The University of Adelaide for awarding me ASI and giving me a platform to study at this prestigious Australian university.

The trees and flowers at Waite were so lovely from time to time. Those peaceful early mornings and late afternoons I stayed in my office and the lab would be unforgettable memories.

Yanchen Zhang

5 September 2018

Adelaide

Abstract

Soil water content is a major factor influencing organic matter turnover and nutrient cycling through its effect on microbial activity, either directly or by modifying substrate availability. Organic soil amendments e.g. plant residue can improve soil fertility. The release of nutrients from plant residue is a complex process. Residue decomposition requires water for microbial growth and for diffusion of nutrients and by-products during decomposition. Recent studies showed that nutrient availability and microbial biomass after the addition of a second residue are influenced by the C/N ratio of the first residue amendment, which is referred to as a legacy effect. However, little is known about the effect of drying-rewetting (DRW) and variable soil water content on nutrient availability in soil amended with residues differing in C/N ratio and on the legacy effect of the previous residue addition. A better understanding of residue decomposition is important to help managing soil fertility using plant residues.

The aims of the study were to determine how soil respiration, microbial biomass and nutrient availability and the legacy effect are influenced by i) Drying and rewetting (DRW) cycle frequency between the first and second residue addition and residue addition upon rewetting, ii) current and previous water content after rewetting with three rewetting events iii) soil water content between the first and second residue addition and number of days between rewetting, and iv) previous and current soil water content in soil amended with residue differing in C/N ratio.

In the first experiment, soil was amended twice (days 0 and 32) with plant residues with either high (H) or low C/N ratio (L) giving treatments LH or HL. Soil was incubated for 64 days. Between the first and the second residue addition (day 0-32) the soil was maintained at 50% WHC or exposed to one, two or four DRW cycles. All treatments were kept at 50% WHC (optimal for soil respiration) during the second period (day 33-64). During the first period, N, P availability and microbial biomass C were higher in LH than HL in all moisture treatments.

Cumulative respiration was higher in LH than HL only in the constantly moist control. After the second residue addition compared with other water regimes, four DRW cycles stimulated decomposition of the low C/N ratio residue added on day 32, but the effect was transient as moisture treatment did not influence available N and MBN from day 48 onwards (8 days after the second residue amendment). The study showed that drying can temporarily increase N and P availability and reduce soil respiration, but after rewetting there was little difference to the constantly moist soil. Further, DRW between residue additions had little effect on the legacy effect of the first residue addition.

In the second experiment, soil was exposed to two wet-dry cycles with 5 days moist and 5 days dry each. Residues with high (H) or low C/N ratio (L) were added in eight residue treatments at different rates (10 or 20 g kg^{-1} soil) and timing (day 0 or day 10, before rewetting). Available N and P on day 11 were similar as on day 1 suggesting that if residues are added upon rewetting, nutrient release is not greater than if residues are added to moist soil. However, in the treatment where L had been added only on day 0, rewetting of dry soil induced N release from partially decomposed L residue left in the soil from the first period. When H was added to moist soil on day 0, MBN on days 1 and 5 was higher than in unamended soil. But when H was added on day 10, MBN increased only on day 11 indicating that with H microbial utilisation of residue N may be restricted if addition of residue was combined with rewetting. From day 11 to 20, MBN, available N and P were lower in LH than in HL, suggesting that the second residue had a strong effect and thus the legacy effect was weaker than if the soil was moist throughout the experiment.

Experiments 1 and 2 showed that DRW had little or no influence on the legacy effect of the first residue addition. In the third experiment, we investigated how current and previous water content after rewetting influences soil respiration, microbial biomass and nutrient availability in unamended soils. Soil was exposed to two wet-dry cycles (5 days moist, 3 days

dry) with soil being rewetted to 50%, 30% or 10% WHC on days 0 and 8. All treatments were rewet to 50% WHC on day 16 and maintained at this water content for 7 days (day 23). The flush of respiration after the first two rewetting events was more than two-fold higher with 50% than 10% WHC and the second flush was about five times lower than the first. After rewetting of all treatments to 50% WHC on day 16, the flush was three-fold greater in soil previously rewet to 10% WHC than soil rewet to 50% previously. In soil previously rewet to 10% WHC compared to that rewet to 50% WHC, MBN and available P on days 17 and 23 were about two-fold higher whereas available N did not differ between treatments. The greater respiration and microbial biomass after the third rewetting event in soil previously rewet to 10% WHC compared to that rewet to 50% WHC can be explained by the greater amount of available substrate remaining after the first two rewetting events. The study showed that rewetting of dry soil to low water content induces only a small flush of respiration and thus little decomposition of organic matter.

In the fourth experiment, soil was amended with high (H) or low C/N ratio (L) residue and then maintained at 10% or 50% WHC for 10 days after which the soil at 10% WHC was rapidly rewetted to 50% WHC. A second residue with a different C/N ratio than the first was added one, two or five days after rewetting. Rewetting of soil that was at 10% WHC in the first 10 days increased MBC and respiration after day 10 in soil amended with L. After day 10, MBN increased with number of days between rewetting and the second residue addition. After the second residue addition, respiration rate in the first three to four days and available N two days after residue addition were higher when residue was added five days after rewetting than if added after one day. But MBN was higher in treatments with residues added one day after rewetting compared to amendment after five days. It can be concluded that soil water content between the first and the second residue addition influenced soil respiration whereas the time between rewetting and the second residue addition affected N availability. However, neither

previous soil water content nor time between rewetting and the second residue addition influenced the legacy effect.

In the following two experiments, we studied how previous and current soil water content influence soil respiration, microbial biomass and nutrient availability in soils amended with residues differing in C/N ratio.

In the fifth experiment from day 1 to day 10, soil was incubated at 10%, 30% or 50% WHC and there were three residue treatments (unamended (C), amended with high (H) or low C/N ratio (L) residue). After sampling on day 10, soil water content was adjusted to 50% WHC and either H or L was added to soil. Therefore, the water content treatments were 10-50, 30-50 and 50-50. Cumulative respiration from day 1 to day 10, MBC and MBN on day 1 and available N and P on both day 1 and day 10 were lower at 10% than at 50% WHC. When L was added on day 10, cumulative respiration from day 11 to day 20, microbial biomass C and N on day 11 and available N on day 20 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 day 10. Differences in MBC, MBN, MBP and available N on day 11 between HL and LL and between LH and HH were greater with 10% WHC in the first period than with 50% WHC. The results showed that the water content between the first and second residue amendment affects the extent of the legacy effect after the second residue addition.

In the last experiment, soil was amended with low (L) or high C/N ratio (H) residues on days 0 and 10 and the soil was incubated at 10 or 50% WHC from day 1 to day 10, but unlike previous experiment the water content from day 10 to 20 was either 10 or 50% WHC, not only 50%. Therefore the water content treatments were 10-10, 10-50, 50-10 and 50-50. In LH at 50% WHC from day 11 to day 20, previous low water content (10% WHC) enhanced N

immobilisation. In HL with 10% WHC from day 11 to day 20, MBN, available N and P on day 20 were higher in soil that was at 50% WHC in the first 10 days compared to that at 10% WHC. The study showed that the influence of the previous water content on respiration and microbial biomass was stronger when the first amendment was low C/N residue because its decomposition was more affected by water content than that of high C/N residue.

It can be concluded that soil water content influences organic matter decomposition and therefore nutrient availability, particularly in soil amended with low C/N residue whereas drying and rewetting has only small and transient effects.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

I acknowledge that copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Yanchen Zhang

List of publications

Zhang Y, Marschner P (2016) Nutrient availability, soil respiration and microbial biomass after the second residue addition are influenced by the C/N ratio of the first residue added, but not by drying and rewetting between residue amendments. European Journal of Soil Biology 77:68-76

Zhang Y, Marschner P (2017) Residue addition combined with rewetting of dry soil–Effect of timing of residue addition on soil respiration, microbial biomass, nutrient availability and legacy effect. Geoderma 299, 83-90.

Zhang Y, Marschner P (2018) Respiration, microbial biomass and nutrient availability are influenced by previous and current soil water content in plant residue amended soil. Journal of Soil Science and Plant Nutrition, 2018, 18(1), 173-187.

Zhang Y, Marschner P (2017) 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. Journal of Soil Science and Plant Nutrition, 2017, 17(3), 594-608.

In preparation for submission

Zhang Y, Marschner P (2018) Previous and current water content influence soil respiration, microbial biomass and nutrient availability after rewetting of dry soil

Zhang Y, Marschner P (2018) Influence of previous and current water content on microbial biomass and nutrient availability is modulated by the order in which low and high C/N ratio residue are added

CHAPTER 1

Introduction and literature review

1.1 **Plant residues as soil amendment**

Soil is a dynamic natural system that interfaces with earth, air, water and life, providing critical ecosystem services for the sustenance of humanity (Needelman 2013). The increasing awareness of the progressive degradation of soils in farming systems has led to research on improving soil quality. Soil quality has been defined as the capacity of a soil to take up, store and recycle water, minerals and energy so that crop production is maximized and environmental degradation is minimized (Trasar-Cepeda et al. 2008). Decline in soil organic matter under intensive farming is recognized as a main cause of soil fertility decrease. Yu et al. (2014) reported that plant residue removal decreased soil organic carbon (C) content in arid climate, leading to aggregate instability. Amendment with organic materials such as crop residues can be a reliable and effective way to ameliorate soil structure and soil fertility (Cordell et al. 2009; Ng et al. 2014; Ros et al. 2003; Scotti et al. 2015).

Crop residues, in general, are parts of the plants left in the field after crops have been harvested or trashed (Kumar and Goh 1999). Incorporation of crop residues provides a source of readily available C and N in the soil, as well as other nutrients. It also provides energy for biological processes, and improves physical and chemical properties such as soil structural stability, water retention, cation exchange and buffer capacity (Baldock 2007; Diacono and Montemurro 2010). Recent studies showed that the application of organic amendments can reduce the adverse effects of soil compaction and improve transport properties of soil gases and water by affecting both biological processes (Beare et al. 2009) and pore characteristics (Mangalassery et al. 2013). Microbial biomass C is higher in soil amended with residues than if residues are removed (Govaerts et al. 2007; Lou et al. 2011; Salinas-Garcia et al. 2001). Incorporation of crop residues for 43 years increased soil organic C content by 21% to 29% in a wide range of soils (Pituello et al. 2016).

Apart from application of plant residue, residues are also added with root death and leaf fall during plant growth which may also lead to changes in the content of easily available C in soil. Therefore, further understanding of the influence of plant residue properties (e.g. C/N ratio) on decomposition process can help to improve land use management and soil productivity. Two main factors are considered to play important roles in plant residue decomposition; namely the nature of plant residues and their distribution to soil, referred to as internal factors, i.e. residue particle size, chemical composition, C/N ratios, and environmental factors, i.e. temperature, soil texture, pH, water content (Camiré et al. 1991; Swift et al. 1979).

1.2 Factors influencing plant residue decomposition

1.2.1 Internal factors

The amount of plant residue, its physical and chemical properties, such as particle size, C composition and C to nutrient ratios, are controlling factors for residue decomposition and nutrient transformation in terrestrial ecosystems (Scholes and Archer 1997; Swift et al. 1979). At the early stage of decomposition, soil animals and insects shred fresh plant residues into small particles and earthworms mix those particles into soils. Soil microbes have access to residue particles and their population increase quickly. Eventually, C and N availability may limit the growth of microbes, decreasing the rate of residue decomposition and organic matter mineralization (Reinertsen et al. 1984).

1.2.1.1 Chemical composition

One of the most important factors governing residue decomposition rate is its chemical composition (Heal et al. 1997). Plant residues are composed of simple and complex organic components with different susceptibility to decomposition, such as lignin, tannins, cellulose,

organic acids, amino acids and simple sugars (Kögel-Knabner 2002). The composition and relative abundance of these components vary widely depending on plant species, maturity and part (Kononova 1966; Martens 2000).

Organic compounds are decomposed by a range of soil microorganisms, each of which produces a particular group of enzymes (Wagner and Wolf 1999). Simple substrates e.g. sugars and amino acids are readily assimilated by microbes (Jones 1999). In favourable environments, simple sugars like glucose may be completely metabolized by soil microorganisms in one or two days. Free amino acids also readily utilized by soil microbes (van Hees et al. 2005). More resistant components (e.g. condensed tannins, waxes phenolics) tend to accumulate as residue decomposition progresses, because their breakdown requires special enzymes which are only released by some microbes (Preston et al. 2009). Lignin, a complex molecule comprising up to 20% by the mass of plant residues, is resistant to degradation by extracellular enzymes (Austin and Ballaré 2010).

The proportion of simple and complex compounds varies in plant residues. Residues with high concentrations of recalcitrant compounds, e.g. lignin, decompose more slowly than residues with higher soluble sugar (Johnson et al. 2007; Stewart et al. 2015). Broder and Wagner (1988) studied decomposition of corn, wheat straw and soybean residues in soil. Soybean residue which had highest proportion of soluble components had the highest decomposition rate in the initial 32 days of the study. The chemical composition of amended residues can also increase or suppress decomposition of native soil organic matter, the so-called priming effect (Kuzyakov et al. 2000). The priming effect is a result of the interactions between the transformation of the amended substances and the native soil cycles.

1.2.1.2. Particle size

Particle size influences the surface of residues exposed to decomposition relative to its volume (Tarafdar et al. 2001). Small particles decompose faster than larger ones because the higher surface area to volume ratio and greater dispersion in soil will increase the susceptibility to microbial attack (Ambus and Jensen 1997; Henriksen and Breland 2002). Ground or finely chopped residues are more susceptible to microbial attack than intact residues due to lack of intact lignified barrier tissue (Summerell and Burgess 1989). Tejada and Gonzalez (2007) found an increase in mineralization of cotton gin residues and wheat straw when the residues were ground to very fine particles when compared to larger particles. Another study also indicated that finely crushed maize straw residues (< 1 cm) increased native organic matter mineralization compared to larger particle size (1-10 cm and > 10 cm) (Tejada and Benítez 2014).

However, the effect of plant residue particle size on nutrient cycling is also an interaction with soil texture, plant residue chemical composition and soil faunal activity. For example, less N is released from fine leguminous residue particles than from coarser particles. This is was explained by physical protection by clay minerals and other mineral particles of the fine particles (Stickler and Frederick 1959). Jensen (1994) also demonstrated that fine residue particles may be physically protected against decomposition by adsorption to soil minerals.

1.2.1.3. Carbon to nutrient ratios

The C/N, C/P and N/P ratios of plant residues play a key role in the decomposition rate and nutrient release. Generally, organic matter decomposition is tightly controlled by C: N: P ratio (Hill et al. 2006), and C/N ratio and C/P ratio of residues are positively correlated

(Kwabiah et al. 2003; Nwoke et al. 2004). Kwabiah et al. (2001) found that the release of N and P were controlled by total P content in leaves. Leaves with low total P decomposed more slowly than those with high total P.

It is well-recognized that decomposition and N mineralisation rate are negatively correlated with residue C/N ratio (Frankenberger and Abdelmagid 1985; Martens 2000; Taylor et al. 1989). Residues with high C/N ratio or C/P ratio are decomposed slowly and can result in immediate net N immobilization in the microbial biomass (Moritsuka et al. 2004). In contrast, residues with low C/N ratio or C/P ratio are rapidly decomposed and induce net N mineralization as they satisfy the nutrient demand of microbes (Hadas et al. 2004; Janssen 1996; Trinsoutrot et al. 2000). Heterotrophic soil bacteria have a lower C/N ratio than their habitat. Generally, the amendment of plant residue into soil with C/N > 20 or C/P ratio > 200 can cause temporary immobilization (Enwezor 1976).

The C/P ratio can also influence the rate of nutrient release during decomposition and give an indication of the effect of decomposing plant residues on available P (Nwoke et al. 2004). During organic matter decomposition, P is assimilated by microorganisms and immobilized in the form of lipids, nucleoproteins and other organic compounds (Singh and Jones 1976). The immobilised P can become available after the death of microbial cells. Residues high in P decompose faster and release more P (Tian et al. 1992). However, soil P availability during residue decomposition is also influenced by P sorption capacity and the availability of native soil P (Singh and Jones 1976; Umrit and Friesen 1994).

Decomposition studies are usually carried out by adding residues from a single species (Aber and Melillo 1980; Preston et al. 2009; Trofymow et al. 2002; Zhang and Zak 1995). However, in natural systems, plant residues of different species often decompose together (Hooper and Vitousek 1997). The observed decomposition value differs from the expected

value in many studies of residue mixtures (Chapman and Koch 2007). The expected value is calculated by the average of individual litter species decomposition rates when they decompose alone. A review on plant litter mixtures showed differences in decomposition between measured and expected values in 67% of the reviewed studies. In those studies showing differences between expected values and observed values, 65% were synergistic (expected values < observed values) while 35% were antagonistic (expected values > observed values) (Gartner and Cardon 2004). Synergistic interactions can be explained by rapid adaption of decomposer community to the substrate mix (Maisto et al. 2011) and the availability of nutrients to microorganisms in litter mixtures (Mao and Zeng 2012). Antagonism has been attributed to inhibition of microbial growth or enzyme activity (Coq et al. 2011; De Marco et al. 2011; Dijkstra et al. 2011). However, there are also some studies with no interaction occurred in residue mixtures (Cobo et al. 2002; Xiang and Bauhus 2007). This could be explained by the spatial separation of microbial communities decomposing the individual components in mixtures. A recent study showed that nutrient availability and microbial biomass after the second residue addition are influenced by the C/N ratio of the previously added residue, which is referred as legacy effect (Marschner et al. 2015). For example, N availability after addition of low C/N residue is lower if it follows high C/N residue than if added to previously unamended soil. This legacy effect occurs because in the former case, soil microbes decompose not only the freshly added low C/N reside, but also the high C/N residue left in the soil from the previous amendment.

1.2.2 Environmental factors

The rate of residue decomposition depends on its internal factors and on those factors which affect the soil environment (Parr and Papendick 1978). The chemical and physical properties of a soil determine the nature of the environment in which microorganisms are found

(Alexander 1961). A range of external factors influence plant residue decomposition, e.g. temperature, soil texture, and soil water availability. Among them, soil water content is considered to have a stronger influence on the decomposition process than the other factors because of it regulates microbial activity (Brady and Weil 2002; Oades 1984).

1.2.2.1 Temperature

Increasing temperature can increase organic matter solubility (Chantigny et al. 2010) and affect microbial and enzyme activity (Allison et al. 2010; Curtin et al. 2012). Response of plant residue decomposition rates to temperature can range from weak (Fissore et al. 2009) to strong and persistent (Conant et al. 2008) because temperature sensitivity increases with recalcitrance of the organic material (Davidson and Janssens 2006). Enzyme kinetic theory suggests that more recalcitrant compounds will be decomposed at higher temperatures (Conant et al. 2008). Based on the kinetic theory of Arrhenius, temperature sensitivity increases with increasing activation energy.

Organic C fractions of plant residues have different temperature sensitivities because they differ in physical and chemical protection (Davidson and Janssens 2006; Hartley and Ineson 2008). Decomposition of stable soil organic matter has a higher temperature sensitivity than that of labile organic matter (Boddy et al. 2008; Feng and Simpson 2008). Studies show that decomposition of residues with high lignin content is more sensitive to increasing temperature than more biochemically labile residues (Bosatta and Ågren 1999; Fierer et al. 2005). Fierer et al. (2005) demonstrated that temperature sensitivity of litter decomposition was dependent on substrate quality with lower quality or residues with greater lignin content having a greater temperature sensitivity. Decomposition rates of the stable soil organic matter are not temperature sensitive within a temperature range of 5-35 ℃ (Giardina and Ryan 2000); while the decomposition of the labile soil organic matter is very temperature sensitive (Eliasson

et al. 2005; Luo et al. 2001; Trumbore et al. 1996). Temperature is an important factor regulating soil microbial activity and shaping soil microbial communities. In a study by Pietikäinen et al. (2005) temperature dependence of different groups of soil microorganisms varied, with bacterial growth rate maximizing at 30 ℃ and fungal growth at 25 ℃. This indicates that functional microbial communities in soil may respond differently temperature variation and thus influence decomposition.

1.2.2.2 Soil texture

Soil texture has direct and indirect effects on residue decomposition by altering soil aeration, surface area, pore size distribution, soil water availability and nutrient availability to microbes (Scott et al. 1996). Soil texture affects N and P transformations by influencing total organic matter accumulation and soil microbial activity. Texture influences soil physical environment by modifying pore size distribution and pore continuity, thus in turn modifying soil water availability, gas exchange and the movement of soil microorganisms (Elliott et al. 1980; Hassink et al. 1993). For example, the number of large pores is usually higher in sandy soils than in loamy and clay soils (Hassink 1992). Large pores facilitate aeration because O_2 diffusion is 10,000 fold greater in air than in water (Kirkham 2014). Sufficient oxygen supply is necessary for rapid decomposition because most decomposers in soil are aerobic. The smaller pores in clay soils on the other hand are often water-filled. Decomposition may therefore be reduced due to insufficient O_2 supply (Kirkham 2014).

Sandy soils are often water and nutrient deficient due to 1) low water retention in macropores ; 2) low cation exchange capacity which causes low capacity to retain nutrients and low pH buffer capacity, 3) weak bonding affinity with soil organic matter which results in high decomposition rate (Brady and Weil 2002; Gentile et al. 2013; Reuter 1994).

Clay concentration plays an important role because clay can limit organic matter decomposition by binding and occlusion in aggregates thereby decreasing its availability to microbes (Oades 1988; Roychand and Marschner 2013). Due to the predominance of small particles, clay soils can have restricted gas flow (Franzluebbers et al. 1996). Very small pores can protect plant residues from decomposers as the small diameter may restrict microbial access to organic material (Don and Schulze 2008; Kaiser and Zech 2000). Particles in the clay fraction provide large surface areas and numerous reactive sites for soil organic matter to be adsorbed by ligand exchange and polyvalent cation bridges (von Lützow et al. 2007). Soil organic matter may also be physically or chemically excluded from microbial attack or enzymatic reactions by aggregation (Sollins et al. 1996). Therefore, clay promotes organic matter stabilization (Merckx et al. 1985).

1.2.2.3 Water availability

Soil water availability is a major factor determining soil organic matter turnover and therefore nutrient cycling (Thomsen et al. 1999). Residue decomposition requires water for microbial growth and for diffusion of nutrients and by-products during decomposition. Water availability influences microbial activity by modifying substrate availability and affecting water potential. Water requirements for growth and survival of different microorganisms vary considerably and therefore, water availability has a selective effect on the total soil microflora (Parr and Papendick 1978).

Water potential is related to the energy level, indicating how readily available water is for movement and plant uptake (Warrick and Or 2007). Water potential is often divided into four components based on the origin of forces that retain the water, including matric, osmotic gravitational and pressure potentials (Parr and Papendick 1978). Matric potential exists because the attraction of water to soil solids provides a matric force. Osmotic potential is caused by

solutes such as inorganic salts and organic compounds which cluster water molecules around aggregates thereby reducing movement of soil water. The gravitational potential is induced by gravity (Brady and Weil 2002). Pressure potential is explained by the external pressure applied to the water. Water potential is a better measure of water availability to plants and microbes than water content because water potential is related to how water moves while water content only gives the amount of water in a soil. Water potential is expressed as energy per unit mass of water (joules/kg) or energy per unit volume of water (Bars or atmospheres). In most soils, organic matter mineralization increases with increasing water content between -5 to about -0.05 MPa, and then decreases due to oxygen deficiency. Bacterial respiration begins to decrease at -0.3 MPa and is almost negligible below -2 MPa (Wilson and Griffin 1975). Consequently, water availability alters mineralization rate.

Storage and flow of water in soils are related to the size of soil pores and controlled by the smallest pore size (Beven and Germann 1982). Micropores are less than 0.08 mm diameter, while macropores are more than 0.08 mm diameter (Brady and Weil 2002). Water drains freely from macropores by gravity but is held more tightly in micropores. Available pore space in soil is important for organic matter decomposition in soils. Decreasing soil water content concomitantly increases the portion of oxygen-filled soil pores (Moyano et al. 2013; Schimel et al. 2007), which reduces the mobility of dissolved organic matter and nutrients and disconnects microbes from substrates (Schimel et al. 2007). Soil microorganisms may produce osmolytes to reduce internal water potential and avoid death when water availability is low (Borken and Matzner 2009). When soil water contents approach water-logged conditions or exceed field capacity, the percentage of soil pore space filled with water (percent of water-filled pores, % WFP) is a better indicator of aerobic vs. anaerobic microbial activity than either water content or water potential (Linn and Doran 1984; Miller and Johnson 1964). Calculation of WFP only requires gravimetric soil water content and soil bulk density.

Soil pores vary with soil texture. Fine textured soils have large number of different sized pores and larger surface area to retain water than sandy soils (Leeper and Uren 1993). Soil aggregates also influence soil pores since they are the result of rearrangement of soil and organic particles. Soil aggregation can lead to formation of inter-aggregate pore spaces, which improves water penetration and aeration (Rawls et al. 2003) and increases nutrient transport to soil microbes.

1.3 Effect of drying-rewetting on decomposition

Surface soils may experience periods of drying followed by rapidly rewetting, particularly in Mediterranean ecosystems (Fierer and Schimel 2002). Soil undergoes complex physical, chemical and biological changes during drying and rewetting, including changes to soil structure, soil organic matter and microorganisms (Berg 2000; Hueso et al. 2012; Sørensen 1974). Many studies have investigated the effect of drying and rewetting on soil processes, including soil respiration, nutrient cycling, microbial biomass and plant nutrient uptake (Butterly et al. 2009; Halverson et al. 2000; Shi and Marschner 2014).

Drying of soil gradually confines soil water to thinner films around soil particles, suppresses microbial activity and biomass and reduces substrate diffusion to microbes (Stark and Firestone 1995; Yao et al. 2011). A large proportion of the microbial biomass can die, mainly those that are active (Bottner 1985). Studies showed that drying can induce changes in microbial community structure and fungi/bacteria ratio may decrease or increase (Denef et al. 2001; Fierer and Schimel 2002; Geisseler et al. 2011; Gordon et al. 2008). For example, Jensen et al. (2003) found that the process of drying in soil can shift community structure towards a greater proportion of fungi, since they are less affected by drought stress than bacteria. Some microbes are able to survive desiccation stress by regulating the concentration of internal osmotic solutes (Csonka and Hanson 1991). Osmoregulatory compounds including amino

acids in bacteria and polyols in fungi can survive after the drying phase (Killham and Firestone 1984; Mikha et al. 2005).

Rewetting of dry soils causes a burst of respiration, which can last up to six days, also known as the Birch effect (Birch 1958; Fierer and Schimel 2002; Li et al. 2010). Such pulses of mineralised C are often associated with mineralisation of other nutrients including N and P (Olsen and Court 1982; Sparling and Ross 1988). Both C and N mineralization rates are generally elevated for 1-4 days after rewetting of a dry soil (Birch 1958; Franzluebbers et al. 2000). Rewetting results in a rapid increase in water potential which can cause microbial cell lysis or death or release intracellular solutes (Bottner 1985; Halverson et al. 2000; Linn and Doran 1984). These labile C and N substrates can then be mineralized by the surviving microbes leading to a pulse of mineralised C and N (Prechtel et al. 2000). Drying and rewetting (DRW) cycles also cause the release of available P, which is thought to be primarily P released from the soil microbial biomass (Grierson et al. 1998; Turner et al. 2003). Sorption and microbial immobilisation can result in rapid removal of P that is released into the soil solution on rewetting in most soils (Butterly et al. 2009; Butterly et al. 2011).

Rapid rewetting also causes soil aggregates slaking because rapid entry of water into aggregates compresses the air within them and induces aggregate breakdown. Aggregate breakdown exposes physically protected organic matter. Therefore, previously unavailable organic matter is accessible to microbes (Adu and Oades 1978). Degens and Sparling (1995) suggested organic matter mineralization in soils which frequently experience DRW cycles was not affected by DRW cycles, because part of organic matter may be recalcitrant. The response to DRW may be related to the time since labile organic matter was added to the soil (Cosentino et al. 2006).

The influence of repeated DRW on microbial biomass and activity and microbial community composition has been studied (Halverson et al. 2000; Yao et al. 2011). The size of the respiration flush upon rewetting often decreases with increasing number of DRW cycles (Baumann and Marschner 2013; Wu and Brookes 2005). This has been explained by lower substrate availability and microbial death after several DRW cycles. However, less is known about the effect of wetting intensity, which is the amount of added water during the rewetting phase (Borken and Matzner 2009; Xu and Luo 2012). Theoretically, the size of the mineralization pulse is expected to increase with the amount of applied water as aggregate slaking and microbial solute release should be intensified for the starved microbes. In dry soil, the strongly negative water potential may lead to the death of some microorganisms, while cells of surviving microbes may burst with the decrease in water potential after rewetting (Yao et al. 2011). However, Schmitt et al. (2010) reported there was little effect of rewetting size (8, 20 and 50 mm water per day) on soil respiration in a forest soil due to the heterogeneous water infiltration along preferential flow paths and hydrophobicity.

Wetting intensity can also be expressed as soil moisture increment (*ΔSWC*) (Lado-Monserrat et al. 2014). At a given water content after rewetting, the increment of soil moisture is highly dependent on the pre-wetting soil moisture. The higher the pre-wetting soil moisture, the lower the *ΔSWC*. Borken and Matzner (2009) showed a higher soil water content before wetting decreases cumulative C and N mineralization rates after rewetting.

The size of respiration and nutrient flush after rewetting also depends on substrate availability (Berryman et al. 2013). Previous studies have shown that plant residue amendment enhances microbial responses to rewetting. McIntyre et al. (2009) found that respiration rate after rewetting was twice as high in amended soils compared with unamended soils. Miller et al. (2005) reported that a 10% increase of soil respiration after rewetting is due to a previous litter addition compared with unamended soil. Cosentino et al. (2006) also suggested that the

response to DRW may be related to the time when the labile organic matter was added to the soil by changing cohesion and hydrophobicity of aggregates. A recent study suggested litter addition modified *ΔSWC* sensitivity of the Birch effect, which highlighted the importance of how much C is available at rewetting (Lado-Monserrat et al. 2014).

1.4 Research gaps

The literature review showed that many factors influence decomposition of residues added to soils. Most studies have elucidated the influence of single residue amendment on microbial biomass and nutrient release. In the field, plant residues of different species or different plant parts often intermingle and decompose together (Hooper and Vitousek 1997; Song et al. 2010). Previous studies in our group showed that nutrient availability and microbial biomass after the second residue addition are influenced by the properties of the previously added residue, which is referred as legacy effect (Marschner et al. 2015). However, several knowledge gaps remain which will be addressed in this study.

Firstly, little is known about the effect of drying-rewetting on nutrient availability in soil amended with residues differing in C/N ratio and on the legacy effect of the previous residue addition. In the field, residue addition may also coincide with rewetting, e.g. when harvest residues are incorporated into soil just after or during a rainfall event. Nutrient release upon rewetting in residue amended soil may depend on amount and C/N ratio of the residue and occurrence of dry-wet cycles relative to residue amendment.

Secondly, there remain significant gaps in our understanding as how variable soil water content influences microbial biomass and nutrient availability in soils. With intermittent rainfall, soil water content can vary among drying-rewetting events. For example, soil may be rewet to water contents below optimal. Further, in residue amended soils, 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 effect of soil water content and length of time at lower water content between the first and second residue addition on soil respiration, microbial biomass and nutrient availability is still unclear.

Thirdly, soil water content may also vary after the second residue addition. Whether the influence of variable soil water content on soil respiration, microbial biomass and nutrient availability is modulated by the order in which the residues are applied needs further investigation.

1.5 Aims

The aims of the PhD project were

i) To determine the effect of drying-rewetting cycle frequency between the first and second residue addition on the legacy effect and the effect of residue addition upon rewetting on microbial activity, biomass and nutrient availability and on the legacy effect (Chapters 2 and 3).

ii) To investigate how current and previous water content after rewetting influence soil respiration, microbial biomass and nutrient availability with three rewetting events (Chapter 4).

iii) To determine the effect of soil water content between the first and second residue addition and number of days between rewetting of soil with previous lower water content on soil respiration, microbial biomass and nutrient availability (Chapter 5).

iv) To assess the effect of previous and current soil water content on soil respiration, microbial biomass and nutrient availability in soil amended with residue differing in C/N ratio (Chapters 6 and 7).

References

Aber JD and Melillo JM (1980) Litter decomposition: measuring relative contributions of organic matter and nitrogen to forest soils. *Canadian Journal of Botany* **58**, 416-421.

Adu J and Oades J (1978) Physical factors influencing decomposition of organic materials in soil aggregates. *Soil Biology and Biochemistry* **10**, 109-115.

Alexander M (1961) Introduction to Soil Microbiology. John Wiley and Sons, Inc., New York.

Allison SD, Wallenstein MD and Bradford MA (2010) Soil-carbon response to warming dependent on microbial physiology. *Nature Geoscience* **3**, 336

Ambus P and Jensen ES (1997) Nitrogen mineralization and denitrification as influenced by crop residue particle size. *Plant and Soil* **197**, 261-270.

Austin AT and Ballaré CL (2010) Dual role of lignin in plant litter decomposition in terrestrial ecosystems. *Proceedings of the National Academy of Sciences* **107**, 4618-4622.

Baldock, JA (2007) Composition and cycling of organic carbon in soil. In: Marschner, P., Rengel, Z. (Eds.), Nutrient Cycling in Terrestrial Ecosystems. Springer Verlag, Berlin Heidelberg, pp. 1-35.

Baumann K, and Marschner P (2013) Effects of salinity on microbial tolerance to drying and rewetting. *Biogeochemistry* **112**, 71-80.

Beare M, Gregorich E and St-Georges P (2009) Compaction effects on CO_2 and N_2O production during drying and rewetting of soil. *Soil Biology and Biochemistry* **41**, 611-621.

Berg B (2000) Litter decomposition and organic matter turnover in northern forest soils. *Forest ecology and Management* **133**, 13-22

Berryman E, Marshall JD, Rahn T, Litvak M and Butnor J (2013) Decreased carbon limitation of litter respiration in a mortality-affected piñon-juniper woodland. *Biogeosciences* **10**, 1625-1634.

Beven K, Germann P (1982) Macropores and water flow in soils. *Water Resources Research* **18**, 1311-1325.

Birch H (1958) The effect of soil drying on humus decomposition and nitrogen availability. *Plant and soil* **10**, 9-31.

Boddy E, Roberts P, Hill PW, Farrar J and Jones DL (2008) Turnover of low molecular weight dissolved organic C (DOC) and microbial C exhibit different temperature sensitivities in Arctic tundra soils. *Soil Biology and Biochemistry* **40**, 1557-1566.

Borken W and Matzner E (2009) Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. *Global Change Biology* **15**, 808-824.

Bosatta E and Ågren GI (1999) Soil organic matter quality interpreted thermodynamically. *Soil Biology and Biochemistry* **31**, 1889-1891.

Bottner P (1985) Response of microbial biomass to alternate moist and dry conditions in a soil incubated with ^{14}C- and ^{15}N-labelled plant material. *Soil Biology and Biochemistry* **17**, 329-337.

Brady NC and Weil RR (2002) The nature and properties of soils. 13th ed. Prentice Hall, Upper Saddle River, NJ.

Broder M and Wagner G (1988) Microbial colonization and decomposition of corn, wheat, and soybean residue. *Soil Science Society of America Journal* **52**, 112-117.

Butterly C, Bünemann E, McNeill A, Baldock J and Marschner P (2009) Carbon pulses but not phosphorus pulses are related to decreases in microbial biomass during repeated drying and rewetting of soils. *Soil Biology and Biochemistry* **41**, 1406-1416.

Butterly CR, McNeill AM, Baldock JA and Marschner P (2011) Rapid changes in carbon and phosphorus after rewetting of dry soil. *Biology and Fertility of Soils* **47**, 41-50.

Camiré C, Côté B and Brulotte S (1991) Decomposition of roots of black alder and hybrid poplar in short-rotation plantings: nitrogen and lignin control. *Plant and Soil* **138**, 123-132.

Chantigny MH, Curtin D, Beare MH and Greenfield LG (2010) Influence of temperature on water-extractable organic matter and ammonium production in mineral soils. *Soil Science Society of America Journal* **74**, 517-524.

Chapman SK and Koch GW (2007) What type of diversity yields synergy during mixed litter decomposition in a natural forest ecosystem? *Plant and Soil* **299**, 153-162.

Cobo J, Barrios E, Kass DCL and Thomas RJ (2002) Decomposition and nutrient release by green manures in a tropical hillside agroecosystem. *Plant and Soil* **240**, 331-342.

Conant RT, Steinweg JM, Haddix ML, Paul EA, Plante AF and Six J (2008) Experimental warming shows that decomposition temperature sensitivity increases with soil organic matter recalcitrance. *Ecology* **89**, 2384-2391.

Coq S, Weigel J, Butenschoen O, Bonal D and Hättenschwiler S (2011) Litter composition rather than plant presence affects decomposition of tropical litter mixtures. *Plant and Soil* **343**, 273-286.

Cordell D, Drangert JO and White S (2009) The story of phosphorus: global food security and food for thought. *Global Environmental Change* **19**, 292-305.

Cosentino D, Chenu C and Le Bissonnais Y (2006) Aggregate stability and microbial community dynamics under drying–wetting cycles in a silt loam soil. *Soil Biology and Biochemistry* **38**, 2053-2062.

Csonka LN and Hanson AD (1991) Prokaryotic osmoregulation: genetics and physiology. *Annual Reviews in Microbiology* **45**, 569-606.

Curtin D, Beare MH and Hernandez-Ramirez G (2012) Temperature and moisture effects on microbial biomass and soil organic matter mineralization. *Soil Science Society of America Journal* **76**, 2055-2067.

Davidson EA and Janssens IA (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature* **440**, 165-173.

De Marco A, Meola A, Maisto G, Giordano M and De Santo AV (2011) Non-additive effects of litter mixtures on decomposition of leaf litters in a Mediterranean maquis. *Plant and Soil* **344**, 305-317.

Degens B and Sparling G (1995) Repeated wet-dry cycles do not accelerate the mineralization of organic C involved in the macro-aggregation of a sandy loam soil. *Plant and Soil* **175**, 197-203.

Denef K, Six J, Bossuyt H, Frey SD, Elliott ET, Merckx R and Paustian K (2001) Influence of dry–wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. *Soil Biology and Biochemistry* **33**, 1599-1611.

Diacono M and Montemurro F (2010) Long-term effects of organic amendments on soil fertility. A review. *Agronomy for Sustainable Development* **30**, 401-422.

Dijkstra JA, Westerman EL and Harris LG (2011) The effects of climate change on species composition, succession and phenology: a case study. *Global Change Biology* **17**, 2360-2369.

Don A and Schulze ED (2008) Controls on fluxes and export of dissolved organic carbon in grasslands with contrasting soil types. *Biogeochemistry* **91**, 117-131.

Eliasson PE, McMurtrie RE, Pepper DA, Strömgren M, Linder S and Ågren GI (2005) The response of heterotrophic CO2 flux to soil warming. *Global Change Biology* **11**, 167-181.

Elliott ET, Anderson R, Coleman DC, and Cole C (1980) Habitable pore space and microbial trophic interactions. *Oikos* **35**, 327-335.

Enwezor W (1976) The mineralization of nitrogen and phosphorus in organic materials of varying C: N and C: P ratios. *Plant and Soil* **44**, 237-240.

Feng X and Simpson MJ (2008) Temperature responses of individual soil organic matter components. *Journal of Geophysical Research* **113**, C03036.

Fierer N, Craine JM, McLauchlan K and Schimel JP (2005) Litter quality and the temperature sensitivity of decomposition. *Ecology* **86**, 320-326.

Fierer N and Schimel JP (2002) Effects of drying–rewetting frequency on soil carbon and nitrogen transformations. *Soil Biology and Biochemistry* **34**, 777-787.

Fissore C, Giardina CP, Swanston CW, King GM and Kolka RK (2009) Variable temperature sensitivity of soil organic carbon in North American forests. *Global Change Biology* **15**, 2295-2310.

Frankenberger W and Abdelmagid H (1985) Kinetic parameters of nitrogen mineralization rates of leguminous crops incorporated into soil. *Plant and soil* **87**, 257-271.

Franzluebbers A, Haney R, Honeycutt C, Schomberg H and Hons F (2000) Flush of carbon dioxide following rewetting of dried soil relates to active organic pools. *Soil Science Society of America Journal* **64**, 613-623.

Franzluebbers A, Haney R, Hons F and Zuberer D (1996) Active fractions of organic matter in soils with different texture. *Soil Biology and Biochemistry* **28**, 1367-1372.

Gartner TB and Cardon ZG (2004) Decomposition dynamics in mixed-species leaf litter. *Oikos* **104**, 230-246.

Geisseler D, Horwath WR and Scow KM (2011) Soil moisture and plant residue addition interact in their effect on extracellular enzyme activity. *Pedobiologia* **54**, 71-78.

Gentile R, Vanlauwe B and Six J (2013) Integrated Soil Fertility Management: Aggregate carbon and nitrogen stabilization in differently textured tropical soils. *Soil Biology and Biochemistry* **67**, 124-132.

Giardina CP and Ryan MG (2000) Evidence that decomposition rates of organic carbon in mineral soil do not vary with temperature. *Nature* **404**, 858-861.

Gordon H, Haygarth PM and Bardgett RD (2008) Drying and rewetting effects on soil microbial community composition and nutrient leaching. *Soil Biology and Biochemistry* **40**, 302-311.

Govaerts B, Mezzalama M, Unno Y, Sayre KD, Luna-Guido M,Vanherck, K. et al. (2007) Influence of tillage, residue management, and crop rotation on soil microbial biomass and catabolic diversity. *Applied Soil Ecology* **37**, 18-30.

Grierson P, Comerford N and Jokela E (1998) Phosphorus mineralization kinetics and response of microbial phosphorus to drying and rewetting in a Florida spodosol. *Soil Biology and Biochemistry* **30**, 1323-1331.

Hadas A, Kautsky L, Goek M and Kara EE (2004) Rates of decomposition of plant residues and available nitrogen in soil, related to residue composition through simulation of carbon and nitrogen turnover. *Soil Biology and Biochemistry* **36**, 255-266.

Halverson LJ, Jones TM and Firestone MK (2000) Release of intracellular solutes by four soil bacteria exposed to dilution stress. *Soil Science Society of America Journal* **64**, 1630-1637.

Hartley IP and Ineson P (2008) Substrate quality and the temperature sensitivity of soil organic matter decomposition. *Soil Biology and Biochemistry* **40**, 1567-1574.

Hassink J (1992) Effects of soil texture and structure on carbon and nitrogen mineralization in grassland soils. *Biology and Fertility of Soils* **14**, 126-134.

Hassink J, Bouwman L, Zwart K and Brussaard L (1993) Relationships between habitable pore space, soil biota and mineralization rates in grassland soils. *Soil Biology and Biochemistry* **25**, 47-55.

Heal OW, Anderson JM and Swift MJ (1997) Plant litter quality and decomposition: a historical overview. Driven by Nature: Plant Litter Quality and Decomposition (eds G. Cadisch & K.E. Giller), pp. 3–30. CAB International, Wallingford, UK.

Henriksen TM and Breland TA (2002) Carbon mineralization, fungal and bacterial growth, and enzyme activities as affected by contact between crop residues and soil. *Biology and Fertility of Soils* **35**, 41-48.

Hill B, Elonen C, Jicha T, Cotter A, Trebitz A and Danz N (2006) Sediment microbial enzyme activity as an indicator of nutrient limitation in Great Lakes coastal wetlands. *Freshwater Biology* **51**, 1670-1683.

Hooper DU and Vitousek PM (1997) The effects of plant composition and diversity on ecosystem processes. *Science* **277**, 1302-1305.

Hueso S, García C and Hernández T (2012) Severe drought conditions modify the microbial community structure, size and activity in amended and unamended soils. *Soil Biology and Biochemistry* **50**, 167-173.

Janssen B (1996) Nitrogen mineralization in relation to C: N ratio and decomposability of organic materials. In: Progress in Nitrogen Cycling Studies. Springer, pp 69-75.

Jensen E (1994) Mineralization-immobilization of nitrogen in soil amended with low C: N ratio plant residues with different particle sizes. *Soil Biology and Biochemistry* **26**, 519-521.

Jensen KD, Beier C, Michelsen A and Emmett BA (2003) Effects of experimental drought on microbial processes in two temperate heathlands at contrasting water conditions. *Applied Soil Ecology* **24**, 165-176.

Johnson JM-F, Barbour NW and Weyers SL (2007) Chemical composition of crop biomass impacts its decomposition. *Soil Science Society of America Journal* **71**, 155-162.

Jones D (1999) Amino acid biodegradation and its potential effects on organic nitrogen capture by plants. *Soil Biology and Biochemistry* **31**, 613-622.

Kaiser K and Zech W (2000) Dissolved organic matter sorption by mineral constituents of subsoil clay fractions. *Journal of Plant Nutrition and Soil Science* **163**, 531-535.

Killham K and Firestone M (1984) Salt stress control of intracellular solutes in streptomycetes indigenous to saline soils. *Applied and Environmental Microbiology* **47**, 301-306.

Kirkham MB (2005) Principles of soil and plant water relations. Amsterdam, the Netherlands: Elsevier Academic Press.

Kögel-Knabner I (2002) The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. *Soil Biology and Biochemistry* **34**, 139-162.

Kononova MM (1966) Soil organic matter. Pergamon Press, Oxford, New York.

Kumar K and Goh K (1999) Crop residues and management practices: effects on soil quality, soil nitrogen dynamics, crop yield, and nitrogen recovery. In: Advances in agronomy, vol 68. Elsevier, pp 197-319

Kuzyakov Y, Friedel J and Stahr K (2000) Review of mechanisms and quantification of priming effects. *Soil Biology and Biochemistry* **32**, 1485-1498.

Kwabiah A, Palm C, Stoskopf N and Voroney R (2003) Response of soil microbial biomass dynamics to quality of plant materials with emphasis on P availability. *Soil Biology and Biochemistry* **35**, 207-216.

Kwabiah A, Stoskopf N, Voroney R and Palm C (2001) Nitrogen and Phosphorus Release from Decomposing Leaves under Sub-Humid Tropical Conditions[1]. *Biotropica* 33, 229-240.

Lado-Monserrat L, Lull C, Bautista I, Lidón A and Herrera R (2014) Soil moisture increment as a controlling variable of the "Birch effect". Interactions with the pre-wetting soil moisture and litter addition. *Plant and soil* **379**, 21-34.

Leeper GW and Uren NC (1993) Soil science: an introduction. Melbourne University Press, Carlton.

Li X, Miller AE, Meixner T, Schimel JP, Melack JM and Sickman JO (2010) Adding an empirical factor to better represent the rewetting pulse mechanism in a soil biogeochemical model. *Geoderma* **159**, 440-451.

Linn D and Doran J (1984) Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. *Soil Science Society of America Journal* **48**, 1267-1272.

Lou Y, Xu M, Wang W, Sun X and Zhao K (2011) Return rate of straw residue affects soil organic C sequestration by chemical fertilization. *Soil and Tillage Research* **113**, 70-73.

Luo Y, Wan S, Hui D and Wallace LL (2001) Acclimatization of soil respiration to warming in a tall grass prairie. Nature **413**, 622-625.

Maisto G, De Marco A, Meola A, Sessa L and De Santo AV (2011) Nutrient dynamics in litter mixtures of four Mediterranean maquis species decomposing in situ. *Soil Biology and Biochemistry* **43**, 520-530.

Mangalassery S, Sjögersten S, Sparkes D, Sturrock C and Mooney S (2013) The effect of soil aggregate size on pore structure and its consequence on emission of greenhouse gases. *Soil and Tillage Research* **132**, 39-46.

Mao R and Zeng DH (2012) Non-additive effects vary with the number of component residues and their mixing proportions during residue mixture decomposition: a microcosm study. *Geoderma* **170**, 112-117.

Marschner P, Hatam Z and Cavagnaro T (2015) Soil respiration, microbial biomass and nutrient availability after the second amendment are influenced by legacy effects of prior residue addition. *Soil Biology and Biochemistry* **88**, 169-177.

Martens DA (2000) Plant residue biochemistry regulates soil carbon cycling and carbon sequestration. *Soil Biology and Biochemistry* **32**, 361-369.

McIntyre RE, Adams MA, Ford DJ and Grierson PF (2009) Rewetting and litter addition influence mineralisation and microbial communities in soils from a semi-arid intermittent stream. *Soil Biology and Biochemistry* **41**, 92-101.

Merckx R, Den Hartog A and Van Veen J (1985) Turnover of root-derived material and related microbial biomass formation in soils of different texture. *Soil Biology and Biochemistry* **17**, 565-569.

Mikha MM, Rice CW and Milliken GA (2005) Carbon and nitrogen mineralization as affected by drying and wetting cycles. *Soil Biology and Biochemistry* **37**, 339-347.

Miller AE, Schimel JP, Meixner T, Sickman JO and Melack JM (2005) Episodic rewetting enhances carbon and nitrogen release from chaparral soils. *Soil Biology and Biochemistry* **37**, 2195-2204.

Miller RD and Johnson D (1964) The effect of soil moisture tension on carbon dioxide evolution, nitrification, and nitrogen mineralization. *Soil Science Society of America Journal* **28**, 644-647.

Moritsuka N, Yanai J, Mori K and Kosaki T (2004) Biotic and abiotic processes of nitrogen immobilization in the soil-residue interface. *Soil Biology and Biochemistry* **36**, 1141-1148.

Moyano FE, Manzoni S and Chenu C (2013) Responses of soil heterotrophic respiration to moisture availability: An exploration of processes and models. *Soil Biology and Biochemistry* **59**, 72-85.

Needelman B (2013) What are soils. Nature Education Knowledge.

Ng EL, Patti A, Rose M, Schefe C, Wilkinson K, Smernik R and Cavagnaro T (2014) Does the chemical nature of soil carbon drive the structure and functioning of soil microbial communities? *Soil Biology and Biochemistry* **70**, 54-61.

Nwoke O, Vanlauwe B, Diels J, Sanginga N and Osonubi O (2004) The distribution of phosphorus fractions and desorption characteristics of some soils in the moist savanna zone of West Africa. *Nutrient Cycling in Agroecosystems* **69**, 127-141.

Oades J (1988) The retention of organic matter in soils. *Biogeochemistry* **5**, 35-70.

Oades JM (1984) Soil organic matter and structural stability: mechanisms and implications for management. *Plant and soil* **76**, 319-337.

Olsen R and Court M (1982) Effect of wetting and drying of soils on phosphate adsorption and resin extraction of soil phosphate. *Journal of Soil Science* **33**, 709-717.

Parr J and Papendick R (1978) Factors affecting the decomposition of crop residues by microorganisms. p. 100-129. In W.R.Oschwald (ed.) Crop residue management systems. ASA Special Publication 31. ASA, CSSA and SSSA, Madison, WI.

Pietikäinen J, Pettersson M and Bååth E (2005) Comparison of temperature effects on soil respiration and bacterial and fungal growth rates. *FEMS Microbiology Ecology* **52**, 49-58.

Pituello C, Polese R, Morari F and Berti A (2016) Outcomes from a long-term study on crop residue effects on plant yield and nitrogen use efficiency in contrasting soils. *European journal of agronomy* **77**, 179-187.

Prechtel A, Alewell C, Michalzik B and Matzner E (2000) Different effect of drying on the fluxes of dissolved organic carbon and nitrogen from a Norway spruce forest floor. *Journal of Plant Nutrition and Soil Science* **163**, 517-521.

Preston CM, Nault JR and Trofymow J (2009) Chemical changes during 6 years of decomposition of 11 litters in some Canadian forest sites. Part 2. ^{13}C abundance, solid-state ^{13}C NMR spectroscopy and the meaning of "lignin". *Ecosystems* **12**, 1078-1102.

Rawls W, Pachepsky YA, Ritchie J, Sobecki T and Bloodworth H (2003) Effect of soil organic carbon on soil water retention. *Geoderma* **116**, 61-76.

Reinertsen SA, Elliott L, Cochran V and Campbell G (1984) Role of available carbon and nitrogen in determining the rate of wheat straw decomposition. *Soil Biology and Biochemistry* **16**, 459-464.

Reuter G (1994) Improvement of sandy soils by clay-substrate application. *Applied Clay Science* **9**, 107-120.

Ros M, Hernandez MT and García C (2003) Soil microbial activity after restoration of a semiarid soil by organic amendments. *Soil Biology and Biochemistry* **35**, 463-469.

Roychand P and Marschner P (2013) Respiration in a sand amended with clay–effect of residue type and rate. *European Journal of Soil Biology* **58**, 19-23.

Salinas-Garcia J, Baez-Gonzalez A, Tiscareno-Lopez M and Rosales-Robles E (2001) Residue removal and tillage interaction effects on soil properties under rain-fed corn production in Central Mexico. *Soil and Tillage Research* **59**, 67-79.

Schimel J, Balser TC and Wallenstein M (2007) Microbial stress-response physiology and its implications for ecosystem function. *Ecology* **88**, 1386-1394.

Schmitt A, Glaser B, Borken W and Matzner E (2010) Organic matter quality of a forest soil subjected to repeated drying and different re-wetting intensities. *European Journal of Soil Science* **61**, 243-254.

Scholes R and Archer S (1997) Tree-grass interactions in savannas. *Annual Review of Ecology and Systematics* **28**, 517-544.

Scott NA, Cole CV, Elliott ET and Huffman SA (1996) Soil textural control on decomposition and soil organic matter dynamics. *Soil Science Society of America Journal* **60**, 1102-1109.

Scotti R, Bonanomi G, Scelza R, Zoina A and Rao M (2015) Organic amendments as sustainable tool to recovery fertility in intensive agricultural systems. *Journal of Soil Science and Plant Nutrition* **15**, 333-352.

Shi A and Marschner P (2014) Drying and rewetting frequency influences cumulative respiration and its distribution over time in two soils with contrasting management. *Soil Biology and Biochemistry* **72**, 172-179.

Singh B and Jones J (1976) Phosphorous Sorption and Desorption Characteristics of Soil as Affected by Organic Residues. *Soil Science Society of America Journal* **40**, 389-394.

Sollins P, Homann P and Caldwell BA (1996) Stabilization and destabilization of soil organic matter: mechanisms and controls. *Geoderma* **74**, 65-105.

Song F, Fan X and Song R (2010) Review of mixed forest litter decomposition researches. *Acta Ecologica Sinica* **30**, 221-225.

Sørensen LH (1974) Rate of decomposition of organic matter in soil as influenced by repeated air drying-rewetting and repeated additions of organic material. *Soil Biology and Biochemistry* **6**, 287-292.

Sparling G and Ross D (1988) Microbial contributions to the increased nitrogen mineralization after air-drying of soils. *Plant and Soil* **105**, 163-167.

Stark JM and Firestone MK (1995) Mechanisms for soil moisture effects on activity of nitrifying bacteria. *Applied and Environmental Microbiology* **61**, 218-221.

Stewart CE, Moturi P, Follett RF and Halvorson AD (2015) Lignin biochemistry and soil N determine crop residue decomposition and soil priming. *Biogeochemistry* **124**, 335-351.

Stickler F and Frederick L (1959) Residue particle size as a factor in nitrate release from legume tops and roots 1. *Agronomy Journal* **51**, 271-274.

Summerell B and Burgess L (1989) Decomposition and chemical composition of cereal straw. *Soil Biology and Biochemistry* **21**, 551-559.

Swift MJ, Heal OW and Anderson JM (1979) Decomposition in terrestrial ecosystems. University of California Press, Berkeley, CA.

Tarafdar JC, Meena SC and Kathju S (2001) Influence of straw size on activity and biomass of soil microorganisms during decomposition. *European Journal of Soil Biology* **37**, 157-160.

Taylor BR, Parkinson D and Parsons WF (1989) Nitrogen and lignin content as predictors of litter decay rates: a microcosm test. *Ecology* **70**, 97-104.

Tejada M and Benítez C (2014) Effects of crushed maize straw residues on soil biological properties and soil restoration. *Land Degradation and Development* **25**, 501-509.

Tejada M and Gonzalez J (2007) Application of different organic wastes on soil properties and wheat yield. *Agronomy Journal* **99**, 1597-1606.

Thomsen IK, Schjønning P, Jensen B, Kristensen K and Christensen BT (1999) Turnover of organic matter in differently textured soils: II. Microbial activity as influenced by soil water regimes. *Geoderma* **89**, 199-218.

Tian G, Kang B and Brussaard L (1992) Biological effects of plant residues with contrasting chemical compositions under humid tropical conditions—decomposition and nutrient release. *Soil Biology and Biochemistry* **24**, 1051-1060.

Trasar-Cepeda C, Leirós M and Gil-Sotres F (2008) Hydrolytic enzyme activities in agricultural and forest soils. Some implications for their use as indicators of soil quality. *Soil Biology and Biochemistry* **40**, 2146-2155.

Trinsoutrot I, Recous S, Bentz B, Lineres M, Cheneby D and Nicolardot B (2000) Biochemical quality of crop residues and carbon and nitrogen mineralization kinetics under nonlimiting nitrogen conditions. *Soil Science Society of America Jounal* **64**, 918-926.

Trofymow JA, Moore TR, Titus B et al. (2002) Rates of litter decomposition over 6 years in Canadian forests: influence of litter quality and climate. *Canadian Journal of Forest Research* **32**, 789-804.

Trumbore SE, Chadwick OA and Amundson R (1996) Rapid exchange between soil carbon and atmospheric carbon dioxide driven by temperature change. *Science* 272, 393-396.

Turner BL, Driessen JP, Haygarth PM and Mckelvie ID (2003) Potential contribution of lysed bacterial cells to phosphorus solubilisation in two rewetted Australian pasture soils. *Soil Biology and Biochemistry* **35**, 187-189.

Umrit G and Friesen DK (1994) The effect of C:P ratio of plant residues added to soils of contrasting phosphate sorption capacities on P uptake by Panicum maximum (Jacq.). *Plant and Soil* **158**, 275-285.

van Hees PA, Jones DL, Finlay R, Godbold DL and Lundström US (2005) The carbon we do not see—the impact of low molecular weight compounds on carbon dynamics and respiration in forest soils: a review. *Soil Biology and Biochemistry* **37**, 1-13.

von Lützow M, Kögel-Knabner I, Ekschmitt K, Flessa H, Guggenberger G, Matzner E and Marschner B (2007) SOM fractionation methods: relevance to functional pools and to stabilization mechanisms. *Soil Biology and Biochemistry* **39**, 2183-2207.

Wagner GH and Wolf DC (1999) Carbon transformations and soil organic matter formation. In: Sylvia DM, Fuhrmann JJ, Hartel PG and Zuberer DA (Eds.), Principles and Applications of Soil Microbiology, Prentice Hall, New Jersey, pp. 218–258.

Warrick AW and Or D (2007) 2. Soil water concepts. In: Developments in Agricultural Engineering, vol 13. Elsevier, pp 27-59.

Wilson JM and Griffin D (1975) Water potential and the respiration of microorganisms in the soil. *Soil Biology and Biochemistry* **7**, 199-204.

Wu J and Brookes P (2005) The proportional mineralisation of microbial biomass and organic matter caused by air-drying and rewetting of a grassland soil. *Soil Biology and Biochemistry* **37**, 507-515.

Xiang W and Bauhus J (2007) Does the addition of litter from N-fixing Acacia mearnsii accelerate leaf decomposition of Eucalyptus globulus? *Australian Journal of Botany* **55**, 576-583.

Xu X and Luo X (2012) Effect of wetting intensity on soil GHG fluxes and microbial biomass under a temperate forest floor during dry season. *Geoderma* **170**, 118-126.

Yao S-H, Zhang B and Hu F (2011) Soil biophysical controls over rice straw decomposition and sequestration in soil: the effects of drying intensity and frequency of drying and wetting cycles. *Soil Biology and Biochemistry* **43**, 590-599.

Yu B, Stott P, Di X and Yu H (2014) Assessment of land cover changes and their effect on soil organic carbon and soil total nitrogen in Daqing Prefecture, China. *Land Degradation and Development* **25**, 520-531.

Zhang Q and Zak JC (1995) Effects of gap size on litter decomposition and microbial activity in a subtropical forest. *Ecology* **76**, 2196-2204.

CHAPTER 2

Nutrient availability, soil respiration and microbial biomass after the second residue addition are influenced by the C/N ratio of the first residue added, but not by drying and rewetting between residue amendments

Statement of Authorship

Title of Paper	Nutrient availability, soil respiration and microbial biomass after the second residue addition are influenced by the C/N ratio of the first residue added, but not by drying and rewetting between residue amendments
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Zhang Y, Marschner P (2016) Nutrient availability, soil respiration and microbial biomass after the second residue addition are influenced by the C/N ratio of the first residue added, but not by drying and rewetting between residue amendments. European Journal of Soil Biology 77:68-76

Principal Author

Name of Principal Author (Candidate)	Yanchen Zhang
Contribution to the Paper	Performed the experiment, analyses of soils, data analysis and interpretation, and manuscript writing.
Overall percentage (%)	70%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 24/08/2018

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Petra Marschner
Contribution to the Paper	Supervised development of the work, data interpretation, manuscript evaluation and correction and acted as the corresponding author.
Signature	Date 24/08/2018

European Journal of Soil Biology 77 (2016) 68–76

Contents lists available at ScienceDirect

European Journal of Soil Biology

journal homepage: http://www.elsevier.com/locate/ejsobi

Original article

Nutrient availability, soil respiration and microbial biomass after the second residue addition are influenced by the C/N ratio of the first residue added, but not by drying and rewetting between residue amendments

Yanchen Zhang, Petra Marschner*

School of Agriculture, Food and Wine, The University of Adelaide, South Australia, 5005, Australia

ARTICLE INFO

Article history:
Received 30 July 2016
Received in revised form
14 October 2016
Accepted 27 October 2016

Handling Editor: C.C. Tebbe

Keywords:
Dry-rewetting cycles
Legacy effect
Microbial biomass
Nutrient availability
Residue addition
Respiration

ABSTRACT

It has been shown that the C/N ratio of the first residue amendment influences soil respiration and nutrient availability after the addition of a second residue, which is referred to as legacy effect. However, little is known about the effect of dry-rewet (DRW) cycles on nutrient availability in soil amended with residues differing in C/N ratio and on the legacy effect. A loamy soil was amended twice (days 0 and 32) with plant residues with either high (H) or low (L) C/N ratio to give the treatments low than high (LH) or high then low C/N residue (HL). Between the first and the second residue addition the soil was maintained at 50% WHC or exposed to one, two or four DRW cycles. After the second residue addition all treatments were kept at 50% WHC until day 64. During the first period, N and P availability and microbial biomass C were higher in LH than HL in all moisture treatments. Cumulative respiration (mg CO_2-C g $soil^{-1}$) was higher in LH than HL only in the constantly moist treatment. Available N and P concentration were higher in dry soil than moist soils in 1 DRW and 2 DRW. After the second residue addition, moisture and residue treatments did not differ in available N and P concentration, which confirmed the legacy effect, but indicated that drying only temporarily increased N and P availability and that the previous moisture treatment did not influence the legacy effect.

1. Introduction

Food production will have to be doubled by 2050 to achieve global food security [1] and climate change is predicted to alter global precipitation patterns [2] which may make achieving food security more difficult. Shifts in precipitation patterns could have particularly strong effects in the Mediterranean climate zone, where hot dry summers are interrupted by occasional rainfalls and soils are exposed to dry-rewetting (DRW) cycles.

During drying, soil water is held more tightly between soil particles, which limits substrate diffusion and microbial activity [3,4]. Rewetting of dry soils is known to result in a respiration flush [5]. The respiration flush has been attributed to increased substrate availability [6]. Shi and Marschner [7] showed that cumulative respiration of soil exposed to DRW depends on the length of the dry period relative to that of the moist period. The respiration flush upon rewetting often decreases with increasing number of DRW cycles which may be explained by lower substrate availability and microbial death after several DRW cycles. It has also been shown that the respiration flush after a long dry period is smaller than after a short one, possibly because a larger proportion of soil microbes die during a long dry period. Most DRW studies were carried out in unamended soils, but in agricultural and natural ecosystems, organic materials such as plant residues (e.g. harvest residues, or litter) may be added to soil and are a nutrient source for plants and soil microbes. A recent study showed that the effect of DRW on soil respiration and microbial biomass is more pronounced in residue amended than unamended soil but that the pattern in response to dry and moist periods was not influenced by amendment [8]. Sørensen [9] reported that the longer organic material was incubated in soil, the lower its mineralisation during DRW cycles.

Nutrient release from plant residues is a complex process influenced by internal factors such as residue C composition, nutrient ratios (e.g. C/N), and external factors, e.g. temperature and

* Corresponding author.
E-mail address: petra.marschner@adelaide.edu.au (P. Marschner).

http://dx.doi.org/10.1016/j.ejsobi.2016.10.006
1164-5563/

soil water content [10]. It is well-known that decomposition and N mineralisation rate are negatively correlated with the residue C/N ratio [11]. Addition of plant residue with C/N < 20 results in net N mineralisation as it can satisfy the nutrient demand of microbes whereas residues with higher C/N ratio lead to at least temporary net N immobilisation [12]. Recently, we showed that N and P availability after residue addition is also influenced by the C/N ratio of the previously added residue, which is referred to as legacy effect [13].

However, little is known about the effect of DRW on nutrient availability in soil amended with residues differing in C/N ratio and on the legacy effect of the previous residue addition. This information will be useful for predicting nutrient release from plant residues in Mediterranean climate. The aim of this experiment was to determine the effect of DRW cycle frequency between the first and second residue addition on the legacy effect. The soil was maintained moist after the second residue addition to separate DRW from residue legacy effect. Our first hypothesis was that nutrient release and soil respiration after the first residue addition will decrease with increasing number DRW cycles, particularly with low C/N residue. The second hypothesis was that changes in nutrient availability and soil respiration induced by DRW after the first residue addition will influence nutrient availability after the second residue addition, thus the legacy effect.

2. Materials and methods

2.1. Soil and plant residues

A loamy soil was collected in early spring 2015 from 0 to 10 cm depth in Urrbrae, South Australia (Longitude 138°38′3.2″ E, Latitude 34°58′0.2″S) that had been under pasture for more than 80 years. This site has a Mediterranean climate with cool, wet winters and hot, dry summers interrupted by short, heavy rainfall events. The soil is a Red-brown Earth according to Australian soil classification and classified as Rhodoxeralf according to US Soil Taxonomy. Soil was collected along a randomly selected transect in three 2 × 2 m plots which were at least 10 m apart. In each sampling plot, after removal of plants and surface litter, five samples of topsoil (0–10 cm) were collected. Then the soil was sieved to <2 mm followed by air-drying in a fan-forced oven at 40°C. Soil from all sampling points was pooled and thoroughly mixed before subsamples were taken for the experiment. The soil has the following properties: 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 organic 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 residue, and mature wheat straw (*Triticum aestivum* L.) as high C/N residue (Table 1). These two plant species were used because they are commonly grown 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. Total N, total P, available N and P were 5–10 times higher in low C/N ratio residue (young faba bean) than in high C/N ratio residue (wheat straw) (Table 1). Therefore low C/N residue had significantly lower C/N ratio and C/P ratios than high C/N residue. Water extractable C concentration was nearly two-fold higher in low C/N ratio residue than in high C/N ratio residue. The residues had a similar pH.

Table 1
Total organic C, N, P, C/N ratio and C/P ratio, available N and P, water-extractable C, and pH of low C/N (young faba bean) and high C/N (wheat straw) residues (n = 4). Different letters indicate significant differences between residues (P < 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

2.2. Experimental design

Before the start of the experiment, the air-dried soil was incubated for 10 days at 20–25 °C in the dark at 50% of maximum WHC to activate the soil microbes and to stabilise soil respiration after rewetting of air-dry soil. This water content was chosen because previous studies with this soil have shown that microbial activity is maximal at 50% WHC [13].

Soil was amended twice with plant residues with either high or low C/N ratio at a rate of 10 g kg^{-1} for each addition. At the start of the experiment, high C/N residue was added to half of the soil and low C/N residue to the other half. The second residue with a different C/N ratio than the first was added after 32 days giving the residue treatments high C/N followed by low C/N (HL) and low C/N followed by low C/N (LH) (Fig. 1). These treatments were chosen because they gave the strongest legacy effect in previous study [13]. At each amendment, residues were thoroughly mixed with the soil. Then 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 23–25 °C.

The following moisture treatments were imposed between the first and second residue addition where moist refers to 50% of WHC, dry to about 2% WHC: constantly moist (32 days at 50% WHC), 1 DRW cycle (16 days dry then 16 days moist), 2 DRW cycles (2 times 8 days dry then 8 days moist), 4 DRW cycles (4 times 4 days dry then 4 days moist) (Fig. 1). After the second residue amendment, the soil was maintained at 50% WHC for 32 days in all treatments. 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.

At the start of the dry period, the soil was dried using pouches with silica gel as described in Shi and Marschner [7]. Preliminary studies showed that the soil (30 g in a sealed 1 L glass jar) dries within 3 days with 24 g silica (three bags with 8 g per bag) which were replaced by dry silica pouches daily during the dry period. The removed pouches were dried overnight in an oven. After three days the water content was 2% WHC and thereafter soil weight remained constant. Rapid rewetting was carried out as described in Shi and Marschner [7] by adding water in a circular motion. Four cores per treatment were destructively sampled on days 8 (dry in 1 DRW and 2 DRW), 16 (dry in 1 DRW), 24 (dry in 2 DRW), 32, 40, 48, 56 and 64, giving a total of 256 cores.

2.3. Measurements

Soil texture was determined according to the rapid textural analysis. Soil maximum water holding capacity was measured in a sintered glass funnel connected to a 100 mm water column ($\psi_m = -10$ kPa).Soil was placed in rings in the sintered glass funnel, thoroughly wetted and allowed to drain for 48 h. Dry weight of the

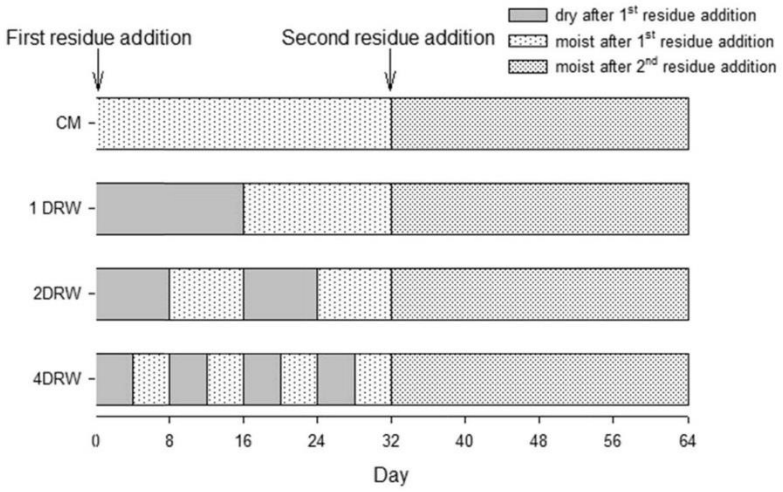

Fig. 1. Schematic diagram of experimental design.

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 h shaking at 25 °C. Total organic C in soil and plant residues was measured according to Walkley and Black [14]. Total N was extracted using the Kjeldahl method [15]. 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 phosphovanadomolydate. Water extractable organic C was determined by shaking 1 g residue with 30 ml RO water for 1 h. The extract was then centrifuged at 3000 rpm for 10 min and filtered through a Whatman#42 filter paper. 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) [16]. Jars were vented with a fan to refresh the headspace 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 t1 and t0 [16]. 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 h end-over-end shaking with 2 M KCl at a 1:5 soil to extractant ratio. Available P was extracted by the anion exchange resin method, the P concentration was determined colorimetrically. Microbial biomass C and N were determined by chloroform fumigation-extraction with 0.5 M K_2SO_4 at 1:4 soil to extractant ratio [17]. Organic C concentration in the extract was measured by titration with 0.033 M acidified $(NH_4)_2Fe(SO_4)_2 \cdot 6H2O$ after dichromate oxidation [18]. The chloroform-labile C concentration is the difference between fumigated and non-fumigated soil, which was multiplied by 2.64 to calculate MBC [19]. Microbial biomass N was calculated as the difference in NH_4^+ concentration between fumigated and non-fumigated samples divided by 0.57 which is the proportionality factor to convert ammonium to MBN. Microbial biomass P was determined with the anion exchange method [20] using hexanol as fumigant. Microbial biomass P is the difference in P concentration between fumigated and un-fumigated soil [20]. No correction factor was used for P because recovery of a P spike in this soil was 98% (Butterly, pers. comm.).

2.4. Statistical analysis

There were four replicate cores for each treatment and sampling time. Data was tested for homogeneity and equal variance. Two-way repeated measures analysis of variance (ANOVA) with fixed factors moisture treatment (CM, 1 DRW, 2 DRW, 4 DRW) and residue treatment (HL or LH) was carried out in SPSS (IBM NY, USA). If the ANOVA showed significant effects of the fixed factors and time ($p < 0.05$), Tukey's multiple comparison test at 95% confidence interval was used. One-way ANOVA was used to compare the properties of two plant residues.

3. Results

3.1. Respiration

In LH, respiration rates declined in the first 10 days in CM and then remained stable (Fig. 2a). In HL, respiration rates in CM increased from day 2 to day 4, but then declined gradually until day 18 after which they remained stable until day 32 (Fig. 2b). Respiration rates were lower in HL than LH until about day 18 after which they were similar In the two residue treatments. In all DRW treatments, respiration rate decreased as the soil dried and was not detectable in dry soil. Rewetting of dry soil resulted in a strong increase in respiration rates on the first day followed by a rapid decline in the following 2–5 days. The flush upon rewetting was greatest in 1 DRW. In 2 DRW and 4 DRW, flush size was greater in the first rewetting event than in the following events. The initial respiration flush was lower in HL than LH, but higher respiration rates were maintained longer (2–3 days) in HL before they declined. The second residue addition on day 32 induced a flush of respiration which was greater in HL than LH. In LH this flush was greater in 1 DRW than in the other treatments whereas in HL it was higher in all DRW treatments compared to CM. Respiration rates then declined and remained stable from day 40.

At the end of the first period, cumulative respiration was higher in CM than the DRW treatments in LH, but the reverse was true in

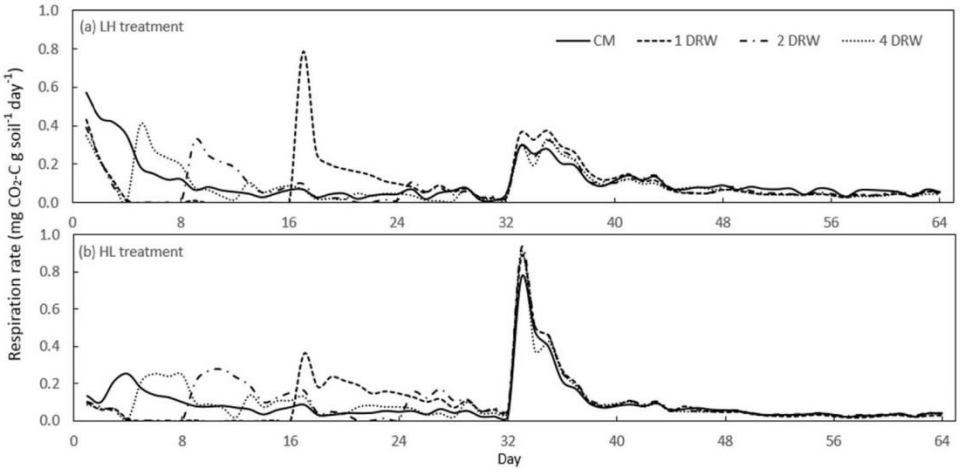

Fig. 2. Respiration rate after the first (days 0–32) and after the second residue addition (days 40–64) in soil amended with low followed by high C/N residue (a, LH) or with high followed by low C/N residue (b, HL) with different moisture treatments between first and second residue addition: constantly moist (CM), one, two or four dry rewet cycles (1 DRW, 2 DRW, 4DRW) (n = 4, means ± SE).

HL (Fig. 3 and Fig. S1). In LH, cumulative respiration in the first period was higher in CM than the DRW treatments, particularly 2 DRW (Fig. 3a). In HL, cumulative respiration in the first period did not differ among moisture treatments (Fig. 3b). Cumulative respiration increased strongly after the second residue addition, particularly in HL. Cumulative respiration in the second period was generally greater in HL than LH. In LH, the previous moisture treatment had no significant effect on cumulative respiration in the second period. In HL, cumulative respiration after the second residue addition was lower in CM than the DRW treatments, particularly 4 DRW.

3.2. Available N and P

In the first period, the available N concentration was 4–10 fold higher in LH than HL (Fig. 4a and b, Table S1). It was lower in CM than in the DRW treatments. In LH, the available N concentration in CM was higher on days 8 and 16 than on days 24 and 32. But in HL, it was highest on day 32. In the DRW treatments of LH, the available N concentration was highest on day 8. But in HL, there was no consistent effect of time on available N concentration. With HL in 1 DRW the available N concentration was higher in dry soil (days 8 and 16) than after rewetting while in 2 DRW, the available N concentration was highest on day 8 when the soil was dry and then declined until day 24. From day 24–32 (moist period), the available N concentration slightly decreased in LH, but nearly doubled in HL.

Eight days after the second residue addition (day 40), the available N concentration was similar as on day 32 (before second residue addition) in all moisture treatments in LH, but more than 8-fold higher in HL. The available N concentration in the second period was highest on day 40. It decreased more than two-fold from day 40 to day 48, then increased by about 30% until day 56 after which it remained stable. On day 40 the available N concentration in LH was higher in CM than in the DRW treatments. But in HL, it was highest in 4 DRW. Among the DRW treatments in HL, the available N concentration on day 40 increased with number of DRW

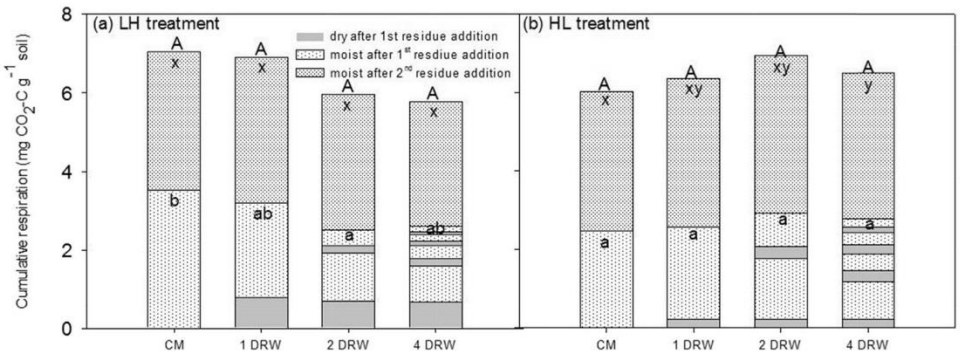

Fig. 3. Cumulative respiration after the first (days 0–32) and after the second residue addition (days 40–64) in soil amended with low followed by high C/N residue (a, LH) or with high followed by low C/N residue (b, HL) with different moisture treatments between first and second residue addition: constantly moist (CM), one, two or four dry rewet cycles (1 DRW, 2 DRW, 4DRW) (n = 4, means ± SE). Different letters indicate significantly different cumulative respiration between days 0 and 32 (a, b, c.) and between days 33 and 64 (x, y, z). Capital letters (A, B, C) indicate significant differences in total cumulative respiration (day 0–64) ($P \leq 0.05$).

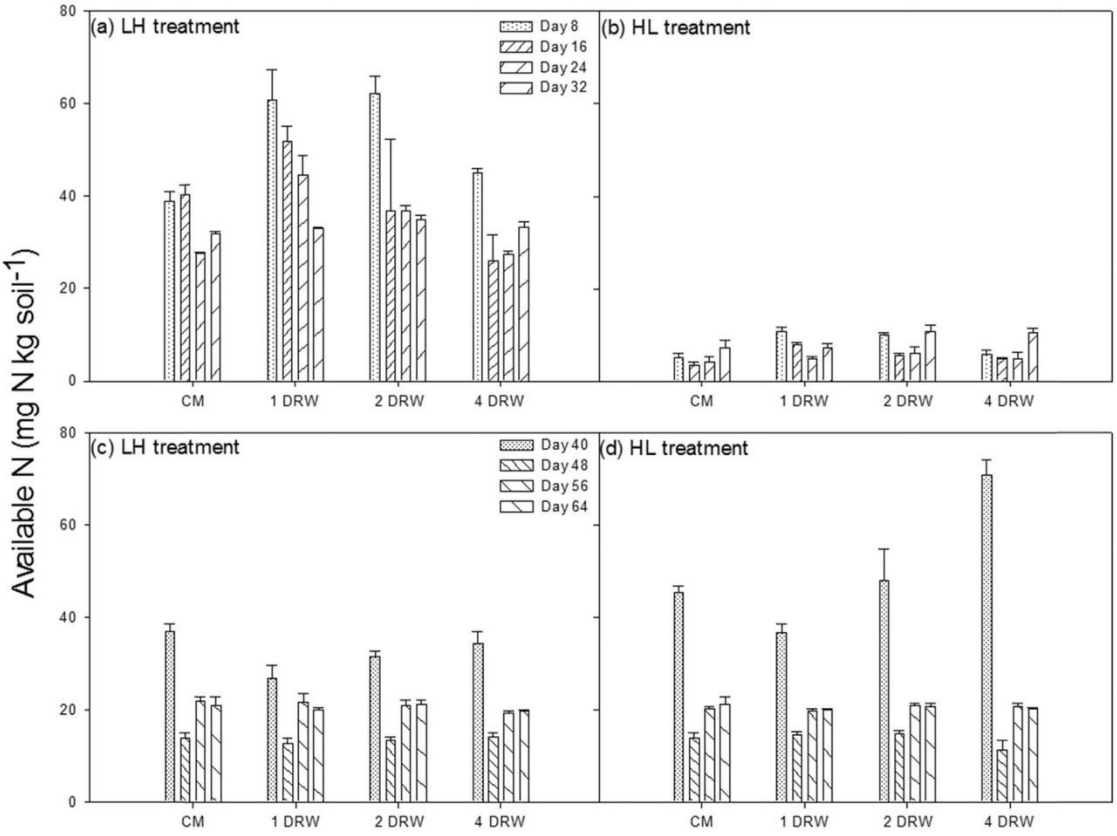

Fig. 4. Available N concentration after the first (a, b, days 8, 16, 24, 32) and after the second residue addition (c, d, days 40, 48, 56, 64) in soil amended with low followed by high C/N residue (a, c, LH) or with high followed by low C/N residue (b, d, HL) with different moisture treatments between first and second residue addition: constantly moist (CM), one, two or four dry rewet cycles (1 DRW, 2 DRW, 4DRW) (n = 4, means ± SE). For letters indicating significant differences see Table S1.

cycles. On the other sampling days after the second residue addition, moisture and residue treatments did not differ in available N concentration.

The available P concentration in the first period was 2–3 fold higher in LH than HL (Fig. 5a and b, Table S2). When the soil had been dry for at least 8 days (days 8 and 16 in 1 DRW and days 8 and 24 in 2 DRW), the available P concentration was higher than in CM. There was no difference in available P concentration among moisture treatments on day 32. After the second residue addition, the available P concentration was lower on days 40 and 48 than on the other two sampling days. The available P concentration was not influenced by residue treatment or the previous moisture treatment.

3.3. Microbial biomass

Microbial biomass C in the first period was 10%–50% lower in HL than LH (Fig. 6a and b, Table S3). In CM, it was highest on day 16 and lowest on day 32. In the DRW treatments, it was also highest on day 16 but lowest on day 8 in 1 DRW and 2 DRW (dry period) whereas it was lowest on day 32 in 4 DRW. Although the soil remained dry in 1 DRW from day 8 to day 16, MBC increased about three-fold in LH and 500-fold in HL. On day 24, MBC was highest in CM, but there were no differences among moisture treatments on day 32.

The second residue addition induced a large increase in MBC. Compared to day 32, MBC on day 40 was 2–3 fold higher in LH and 4–5 fold higher in HL. In all treatments, MBC decreased from day 40 to day 48 followed by an increase to day 56. In CM, MBC was higher in HL than in LH on days 48, 56 and 64, but there were no consistent differences between residue treatments in the DRW treatments.

Microbial biomass N in the first period did not differ between HL and LH except for day 16 in CM and in 4 DRW where it was higher in LH than HL (Fig. 7a and b, Table S4). Microbial biomass N was lower in dry soil (days 8 and 16 1 DRW and days 8 and 24 in 2 DRW) than in CM. Only in HL, MBN on day 8 increased with increasing number of DRW cycles. In 4 DRW, MBN was lower on day 32 than on the previous sampling times. In general, there was no difference in MBN on day 32 between residue or moisture treatments.

Eight days after the second residue addition (day 40) MBN was higher than on day 32, with a greater increase in HL (three-fold) than LH (two-fold) and a greater increase in the DRW treatments than in CM. Microbial biomass N in the second period did not differ between residue treatments except on day 40 where it was higher in HL than LH (Fig. 7 c, d, Table S4). Later, there were no differences among residue and moisture treatments. In all treatments, MBN was lowest on day 64.

Microbial biomass P in the first period was about two-fold higher in LH than HL (Fig. S2, Table S5). Only on day 8, MBP was

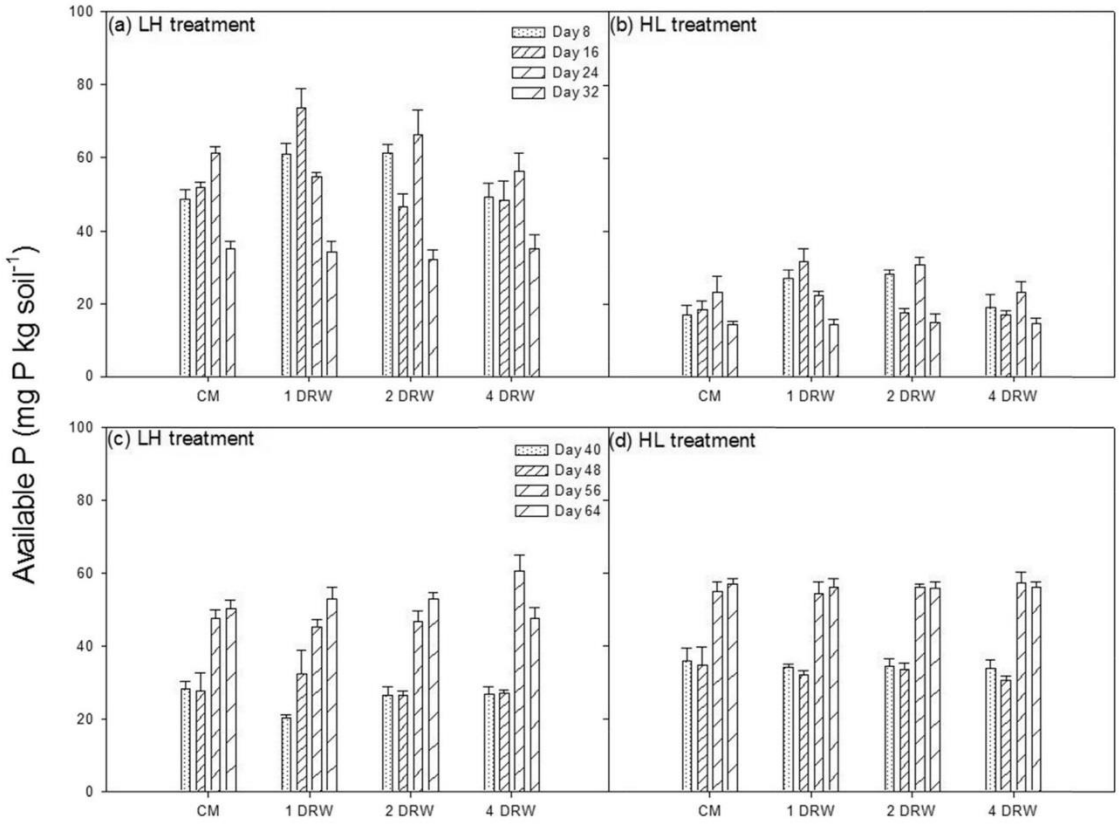

Fig. 5. Available P concentration after the first (a, b, days 8, 16, 24, 32) and after the second residue addition (c, d, days 40, 48, 56, 64) in soil amended with low followed by high C/N residue (a, c, LH) or with high followed by low C/N residue (b, d, HL) with different moisture treatments between first and second residue addition: constantly moist (CM), one, two or four dry rewet cycles (1 DRW, 2 DRW, 4DRW) (n = 4, means ± SE). For letters indicating significant differences see Table S2.

higher in LH than in HL. Moisture treatment did not have a consistent effect on MBP. Microbial biomass P was lowest on day 32. Eight days after the second residue addition (day 40), MBP was about two-fold higher than on day 32. There was no difference among moisture or residue treatments on MBP in the second period. Microbial biomass P was lower on days 40 and 48 than on day 64.

4. Discussion

This study showed that drying can temporarily increase N and P availability and reduce soil respiration, but after rewetting there was little difference to the constantly moist soil. Nutrient availability, microbial biomass and soil respiration after the second residue addition were influenced by the legacy effect, but the previous moisture treatment did not influence the legacy effect. Therefore, both the first (nutrient release and soil respiration after the first residue addition will decrease with increasing number DRW cycles, particularly with low C/N residue) and the second hypothesis (changes in nutrient availability and soil respiration induced by DRW after the first residue addition will influence nutrient availability after the second residue addition, thus the legacy effect) have to be declined.

4.1. Period after the first residue addition (days 0–32)

In agreement with our previous study, nutrient availability and MBC were higher in LH compared to HL in the first period which can be explained by the lower C/N ratio of the L compared to H residue [13]. Although available N and P concentrations were higher in LH than HL, MBC was only slightly higher in LH and cumulative respiration was slightly higher in LH than HL only in the constantly moist treatment and 1DRW treatment. This suggests that DRW reduced the ability of microbes to take up mineralised nutrients.

The higher respiration flush in the first compared to the following rewetting events is also in agreement with previous studies [21,22]. The lower flush in later rewetting events has been explained by death of a proportion of the microbial biomass and reduced substrate availability upon rewetting [6,23]. In this experiment, it is likely due to two factors: (i) a lower microbial biomass after several DRW cycles and (ii) depletion of easily available compounds from the residues.

The decrease in respiration rate when the soils dried and the low respiration in dry soil compared to moist soil was also found in previous DRW studies [6,7]. Lower respiration rates in dry soil can be explained by low water availability to microbes and limited diffusion of substrate [24]. However, in 1 DRW MBC increased from

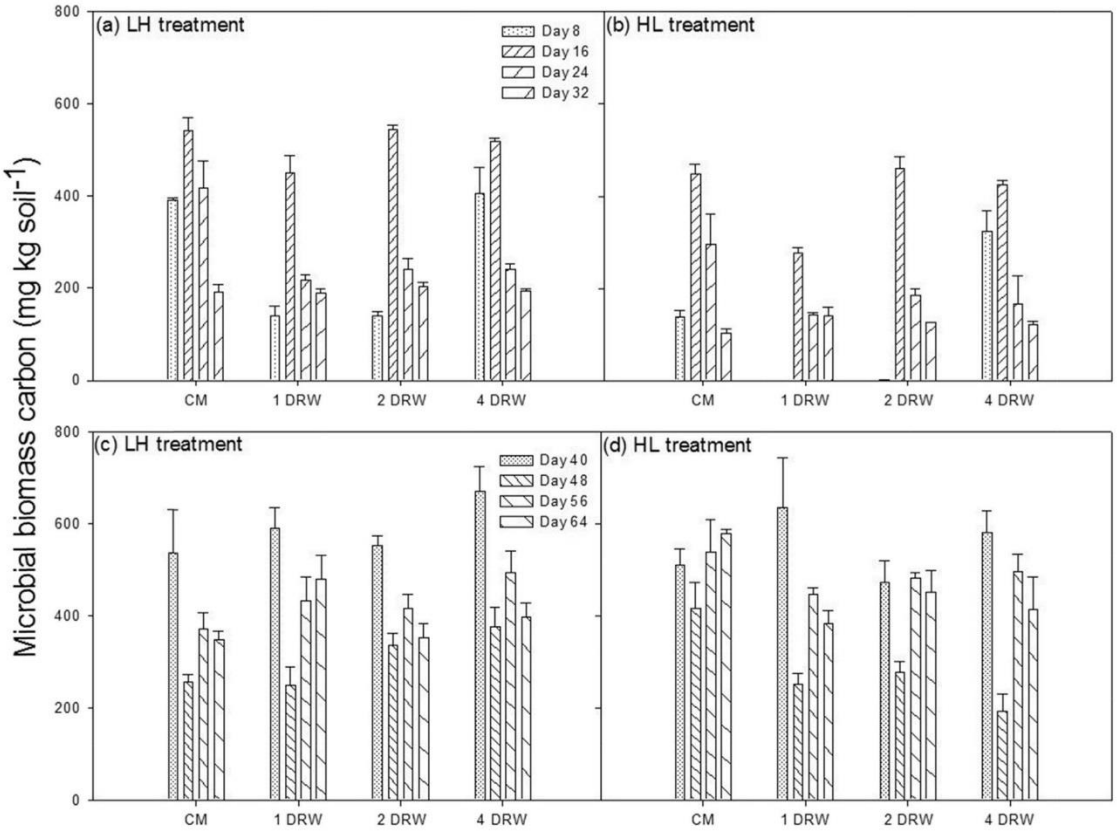

Fig. 6. Microbial biomass carbon concentration after the first (a, b, days 8, 16, 24, 32) and after the second residue addition (c, d, days 40, 48, 56, 64) in soil amended with low followed by high C/N residue (a, c, LH) or with high followed by low C/N residue (b, d, HL) with different moisture treatments between first and second residue addition: constantly moist (CM), one, two or four dry rewet cycles (1 DRW, 2 DRW, 4DRW) (n = 4, means ± SE). For letters indicating significant differences see Table S3.

day 8–16 although the soil remained dry. This suggests that a proportion of the microbial biomass was able to access nutrients, possibly through direct contact with the added residues in pores that remained water-filled or biofilms. In 2 DRW MBC also increased from the first to the second dry period (day 8 compared to day 24), but the second dry period followed a 8-day moist period at the end of which MBC was about twice as high. Therefore, the higher MBC on day 24 compared to day 8 in 2DRW does not indicate growth in dry soil but instead survival of about 50% of the biomass from the previous moist period.

In treatments that had been dry from day 0 to day 8 (1 DRW, 2 DRW), the available N concentration on day 8 was higher than in CM. There are at least two possible reasons for the higher N availability in dry soil. Firstly, some N mineralisation occurred as the soils dried and because microbial growth and thus N uptake was reduced, this N remained available. In CM on the other hand, the increase in MBN from day 16–24 coincided with a decrease in available N which indicates that mineralised N was immobilised. Secondly, inorganic N may have been released from the residues upon rewetting as plant residues contain inorganic N and P [25,26] which could be released when cells burst upon rewetting. This rewetting flush would occur when the extractant (KCl) is added to the dry soil. Higher P availability in dry soil compared to moist soil has been shown in previous studies and was attributed to rapid rewetting during extraction [27,28]. The available N concentration on day 32 was higher in 2 DRW and 4 DRW than in CM. However, the differences were quite small (10–40% higher) and the relative difference between HL and LH was similar in all moisture treatments.

4.2. Period after the second residue addition (days 32–64)

There was a clear legacy effect because cumulative respiration, MBC, MBN and available N and P were similar in LH and HL after the second residue addition. The differences between soil amended with high compared to low C/N residue observed in the first period did not occur after the second residue addition which is in agreement with our previous studies on the legacy effect [13,29]. The lack of differences between HL and LH can be explained by the presence of the previously added residue in the soil when the second residue is added. Cumulative respiration at the end of the first period was 3–3.5 mg CO_2-C/g soil. The residue addition rate was 10 g kg^{-1} (approximately 4 g C kg^{-1}, see Table 1). Organic amendments can induce an increase or decrease decomposition of native soil organic matter which is referred to as priming [30]. Priming effects can vary substantially and the magnitude of priming in our experiment may also differ between HL and LH. In our study, we can not distinguish the source of CO_2-C (soil organic

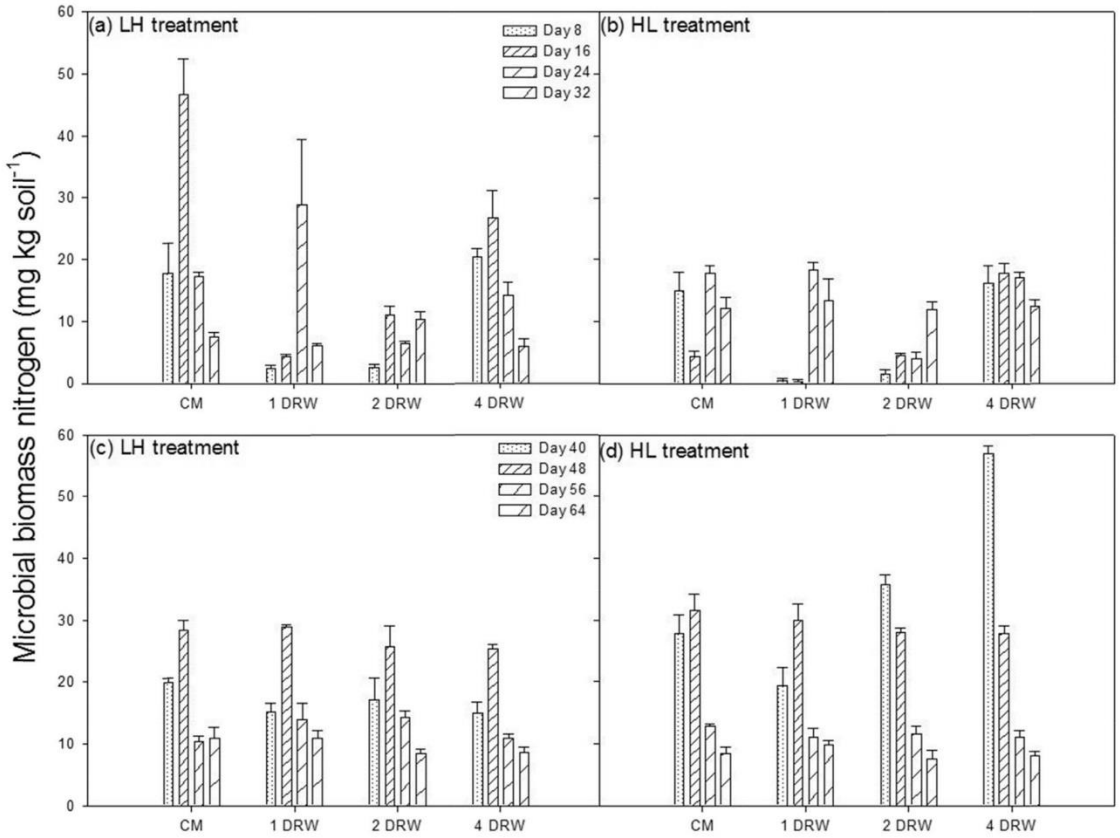

Fig. 7. Microbial biomass nitrogen concentration after the first (a, b, days 8, 16, 24, 32) and after the second residue addition (c, d, days 40, 48, 56, 64) in soil amended with low followed by high C/N residue (a, c, LH) or with high followed by low C/N residue (b, d, HL) with different moisture treatments between first and second residue addition: constantly moist (CM), one, two or four dry rewet cycles (1 DRW, 2 DRW, 4DRW) (n = 4, means ± SE). Values in the same column followed by different letters are significantly different ($P \leq 0.05$). For letters indicating significant differences see Table S4).

matter or added residues) and can therefore not determine the magnitude of priming. For simplicity it can be assumed that there is no priming effect. Then it can be calculated that about 10%–25% of the residue added on day 0 was still in the soil when the second residue was added. If positive priming occurred, this may be an overestimation of residue decomposition. But even if the proportion of first residue in the soil is smaller, it is likely that in LH, the remaining L residue was decomposed together with the freshly added H residue and in HL, the remaining H residue was decomposed together with the freshly added L residue. In both cases, N or P released by microbes decomposing L residue could have been taken up by microbes decomposing H residue.

In general, the moisture treatment before the second residue addition did not influence the legacy effect. In HL, the concentrations of available N and MBN on day 40 (8 days after the second residue addition) were 56 and 14% higher in 4 DRW than in CM which suggests that 4 DRW stimulated decomposition of the freshly added L residue. However, this effect was transient because the moisture treatment did not influence available N and MBN concentrations from day 48 onwards. Thus, although DRW influenced respiration, microbial biomass and N and P availability in the first period, the effect disappeared after all treatments were maintained moist for 8 days with the second residue added. It is possible that residue addition reduced the effect of DRW on microbes because of supply of easily decomposable compounds which is likely to improve microbial recovery after rewetting.

5. Conclusion

The experiment showed that DRW influences nutrient availability, soil respiration and microbial biomass in residue amended soil. In dry soil, N and P availability were two to three times higher than in moist soil. But after the second residue addition and maintenance of moist soil, the measured parameters were influenced primarily by the legacy effect of the first residue addition whereas the prior moisture regime had little effect. The legacy effect was evident because after the first residue addition, N and P availability were two to three-fold greater with L than with H, but after the second residue amendment, nutrient availability did not differ between LH and HL. Thus, changes in precipitation pattern in Mediterranean climate in the future may influence nutrient availability, but are unlikely to influence decomposition in a following constantly moist period. However, the DRW cycles in this experiment were short compared to the field where, in Mediterranean climate, soils may remain dry for weeks or months. Such long dry periods may have a stronger effect on processes after rewetting than in this experiment.

Acknowledgement

Yanchen Zhang received a scholarship from The University of Adelaide.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejsobi.2016.10.006.

References

[1] M.A. Parry, M.J. Hawkesford, Food security: increasing yield and improving resource use efficiency, Proc. Nutr. Soc. 69 (2010) 592–600.
[2] F. Mpelasoka, K. Hennessy, R. Jones, B. Bates, Comparison of suitable drought indices for climate change impacts assessment over Australia towards resource management, Int. J. Climatol. 28 (2008) 1283–1292.
[3] A. Shi, P. Marschner, The number of moist days determines respiration in drying and rewetting cycles, Biol. Fertil. Soils 51 (2015) 33–41.
[4] S.-R. Xiang, A. Doyle, P.A. Holden, J.P. Schimel, Drying and rewetting effects on C and N mineralization and microbial activity in surface and subsurface California grassland soils, Soil Biol. Biochem. 40 (2008) 2281–2289.
[5] H. Birch, The effect of soil drying on humus decomposition and nitrogen availability, Plant soil 10 (1958) 9–31.
[6] N. Fierer, J.P. Schimel, A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil, Soil Sci. Soc. Am. J. 67 (2003) 798–805.
[7] A. Shi, P. Marschner, Drying and rewetting frequency influences cumulative respiration and its distribution over time in two soils with contrasting management, Soil Biol. Biochem. 72 (2014) 172–179.
[8] D. Cosentino, C. Chenu, Y. Le Bissonnais, Aggregate stability and microbial community dynamics under drying–wetting cycles in a silt loam soil, Soil Biol. Biochem. 38 (2006) 2053–2062.
[9] L.H. Sørensen, Rate of decomposition of organic matter in soil as influenced by repeated air drying-rewetting and repeated additions of organic material, Soil Biol. Biochem. 6 (1974) 287–292.
[10] D. Stott, L. Elliott, R. Papendick, G. Campbell, Low temperature or low water potential effects on the microbial decomposition of wheat residue, Soil Biol. Biochem. 18 (1986) 577–582.
[11] D.A. Martens, Plant residue biochemistry regulates soil carbon cycling and carbon sequestration, Soil Biol. Biochem. 32 (2000) 361–369.
[12] N. Moritsuka, J. Yanai, K. Mori, T. Kosaki, Biotic and abiotic processes of nitrogen immobilization in the soil-residue interface, Soil Biol. Biochem. 36 (2004) 1141–1148.
[13] P. Marschner, Z. Hatam, T. Cavagnaro, Soil respiration, microbial biomass and nutrient availability after the second amendment are influenced by legacy effects of prior residue addition, Soil Biol. Biochem. 88 (2015) 169–177.
[14] A. Walkley, I.A. Black, An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method, Soil Sci. 37 (1934) 29–38.
[15] H. McKenzie, H.S. Wallace, The Kjeldahl determination of nitrogen: a critical study of digestion conditions-temperature, catalyst, and oxidizing agent, Aust. J. Chem. 7 (1954) 55–70.
[16] R. Setia, P. Marschner, J. Baldock, D. Chittleborough, P. Smith, J. Smith, Salinity effects on carbon mineralization in soils of varying texture, Soil Biol. Biochem. 43 (2011) 1908–1916.
[17] J. Moore, S. Klose, M. Tabatabai, Soil microbial biomass carbon and nitrogen as affected by cropping systems, Biol. Fertil. Soils 31 (2000) 200–210.
[18] J. Anderson, J. Ingram, Colorimetric Determination of Ammonium, Tropical Soil Biology and Fertility, a Handbook of Methods, second ed., CAB International, Wallingford, UK, 1993, pp. 73–74.
[19] E. Vance, P. Brookes, D. Jenkinson, An extraction method for measuring soil microbial biomass C, Soil Biol. Biochem. 19 (1987) 703–707.
[20] K. Kouno, Y. Tuchiya, T. Ando, Measurement of soil microbial biomass phosphorus by an anion exchange membrane method, Soil Biol. Biochem. 27 (1995) 1353–1357.
[21] K. Baumann, P. Marschner, Effects of salinity on microbial tolerance to drying and rewetting, Biogeochemistry 112 (2013) 71–80.
[22] M.M. Mikha, C.W. Rice, G.A. Milliken, Carbon and nitrogen mineralization as affected by drying and wetting cycles, Soil Biol. Biochem. 37 (2005) 339–347.
[23] W. Borken, E. Matzner, Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils, Glob. Change Biol. 15 (2009) 808–824.
[24] J.M. Stark, M.K. Firestone, Mechanisms for soil moisture effects on activity of nitrifying bacteria, Appl. Environ. Microbiol. 61 (1995) 218–221.
[25] R.C. Granstedt, R.C. Huffaker, Identification of the leaf vacuole as a major nitrate storage pool, Plant Physiol. 70 (1982) 410–413.
[26] M.J. McLaughlin, A. Alston, J. Martin, Phosphorus cycling in wheat pasture rotations. II. The role of the microbial biomass in phosphorus cycling, Soil Res. 26 (1988) 333–342.
[27] R. Bramley, N. Barrow, The reaction between phosphate and dry soil. II. The effect of time, temperature and moisture status during incubation on the amount of plant available P, J. Soil Sci. 43 (1992) 759–766.
[28] B.L. Turner, P.M. Haygarth, Changes in bicarbonate-extractable inorganic and organic phosphorus by drying pasture soils, Soil Sci. Soc. Am. J. 67 (2003) 344–350.
[29] T.T. Nguyen, T.R. Cavagnaro, H.T.T. Ngo, P. Marschner, Soil respiration, microbial biomass and nutrient availability in soil amended with high and low C/N residue—Influence of interval between residue additions, Soil Biol. Biochem. 95 (2016) 189–197.
[30] Y. Kuzyakov, J. Friedel, K. Stahr, Review of mechanisms and quantification of priming effects, Soil Biol. Biochem. 32 (2000) 1485–1498.

Supplementary data

Table S1. Available N concentration (NH_4^++NO_3^-) (mg N kg^{-1}) after the first (days 8, 16, 24, 32), and after the second residue addition (days 40, 48, 56, 64) in soil amended with low followed by high C/N residue (LH) or with high followed by low C/N residue (HL) with different moisture treatments between first and second residue addition: constantly moist (CM), one, two or four dry rewet cycles (1 DRW, 2 DRW, 4DRW) (n=4, means ± SE). Values in the same column followed by different letters are significantly different ($P \leq 0.05$).

Residue treatment	LH				HL			
Moisture treatment	CM	1 DRW	2 DRW	4 DRW	CM	1 DRW	2 DRW	4 DRW
Day	Available N (mg kg^{-1})							
8	39.0±2.0^{a}	60.7±6.7^{b}	62.1±4.0^{b}	45.0±1.0^{a}	5.2 ± 0.9^{a}	10.7±1.0^{b}	10.0±0.5^{b}	5.9±1.0^{a}
16	40.3±2.3^{bc}	51.9±3.1^{c}	36.8±15.6^{ab}	26.1±5.6^{a}	3.5±0.6^{a}	8.0±0.4^{c}	5.5±0.6^{b}	4.9±0.3^{b}
24	27.6±0.4^{a}	44.5±4.1^{c}	36.7±1.4^{b}	27.5±0.5^{a}	4.3±1.1^{a}	4.9±0.6^{a}	6.1±1.3^{a}	5.0±1.2^{a}
32	31.8±0.5^{a}	33.0±0.3^{ab}	35.0±0.8^{c}	33.3±1.2^{b}	7.2±1.6^{a}	7.2±1.0^{a}	10.8±1.4^{b}	10.6±1.0^{b}
40	36.9±1.7^{c}	26.7±2.9^{a}	31.5±1.2^{b}	34.3±2.5^{bc}	45.2±1.5^{b}	36.6±1.9^{a}	47.9±6.9^{b}	70.7±3.4^{c}
48	13.9±1.1^{a}	12.6±1.1^{a}	13.4±0.6^{a}	14.0±0.9^{a}	13.8±1.3^{b}	14.4±0.9^{b}	14.6±0.7^{b}	11.3±1.9^{a}
56	21.9±0.9^{b}	21.6±1.8^{b}	20.9±1.1^{ab}	19.3±0.4^{a}	20.2±0.5^{ab}	19.8±0.3^{a}	20.8±0.6^{b}	20.6±0.7^{ab}
64	20.9±1.7^{a}	19.8±0.6^{a}	21.2±0.9^{a}	19.6±0.3^{a}	21.1±1.6^{a}	20.0±0.3^{a}	20.5±0.9^{a}	20.1±0.2^{a}

Table S2. Available P concentration (mg P kg^{-1}) after the first (days 8, 16, 24, 32), and after the second residue addition (days 40, 48, 56, 64) in soil amended with low followed by high C/N residue (LH) or with high followed by low C/N residue (HL) with different moisture treatments between first and second residue addition: constantly moist (CM), one, two or four dry rewet cycles (1 DRW, 2 DRW, 4DRW) (n=4, means ± SE). Values in the same column followed by different letters are significantly different ($P \leq 0.05$).

Residue treatment	LH				HL			
Moisture treatment	CM	1 DRW	2 DRW	4 DRW	CM	1 DRW	2 DRW	4 DRW
Day	Available P (mg kg^{-1})							
8	48.7±2.7^{a}	61.1±2.9^{b}	61.3±2.3^{b}	49.2±3.9 ^{a}	16.9±2.6^{a}	26.9±2.4^{b}	28.1±1.1^{b}	19.1±3.4^{a}
16	51.9±1.6^{a}	73.5±5.4^{b}	46.6±3.6^{a}	48.4±5.3 ^{a}	18.3±2.5^{a}	31.8±3.4^{b}	17.7±1.0^{a}	17.1±0.9^{a}
24	61.3±1.9^{ab}	55.0±1.1^{a}	66.3±6.8^{b}	56.3±5.1^{a}	23.1±4.4^{a}	22.2±1.3^{a}	30.8±2.1^{b}	23.2±3.0^{a}
32	35.2±1.9^{a}	34.1±3.1^{a}	32.3±2.6^{a}	35.1±3.9^{a}	14.3±1.0^{a}	14.2±1.5^{a}	14.9±2.3^{b}	14.6±1.4^{a}
40	28.2±2.0^{b}	20.1±0.8^{a}	26.4±2.2^{b}	26.2±2.0^{b}	35.8±3.6^{a}	33.9±1.1 ^{a}	34.2±2.1^{a}	33.6±2.5^{a}
48	27.6±4.9^{a}	32.2±6.6^{a}	26.5±1.1^{a}	27.0±0.8^{a}	34.6±5.1^{a}	31.9±1.1^{a}	33.5±1.7^{a}	30.5±1.2^{a}
56	47.4±2.4^{a}	45.1±2.4^{a}	46.6±3.1 ^{a}	60.6±4.2 ^{b}	54.8±2.7^{a}	54.4±3.0 ^{a}	56.1±0.8^{a}	57.4±3.0^{a}
64	50.3±2.3^{a}	52.9±3.2^{a}	52.8±2.0^{a}	47.5±2.9^{a}	56.9±1.6^{a}	56.2±2.4^{a}	55.7±1.8^{a}	56.2±1.3^{a}

Table S3. Microbial biomass C (mg kg^{-1} soil) after the first (days 8, 16, 24, 32), and after the second residue addition (days 40, 48, 56, 64) in soil amended with low followed by high C/N residue (LH) or with high followed by low C/N residue (HL) with different moisture treatments between first and second residue addition: constantly moist (CM), one, two or four dry rewet cycles (1 DRW, 2 DRW, 4DRW) (n=4, means ± SE). Values in the same column followed by different letters are significantly different ($P \leq 0.05$).

Residue treatment	LH				HL			
Moisture treatment	CM	1 DRW	2 DRW	4 DRW	CM	1 DRW	2 DRW	4 DRW
Day	Microbial biomass C (mg kg^{-1} soil)							
8	391±6^{b}	141±19^{a}	140±10^{a}	407±55^{b}	139±14^{b}	0±0^{a}	1±2^{a}	324±44^{b}
16	543±27^{b}	451±36^{a}	544±10^{b}	519±7^{b}	448±23^{bc}	277±13^{a}	461±25^{c}	425±9^{b}
24	417±60^{b}	217±12^{a}	242±23^{a}	242±10^{a}	296±66^{b}	142±5^{a}	186±13^{a}	167±62^{a}
32	191±17^{a}	190±9^{a}	203±10^{a}	195±5^{a}	103±9^{a}	141±18^{c}	126±1^{bc}	122±7^{b}
40	535±96^{a}	590±44^{ab}	551±23^{a}	670±53^{b}	510±36^{ab}	633±110^{c}	472±46^{a}	580±47^{bc}
48	255±18^{a}	248±40^{a}	337±25^{b}	374±42^{b}	416±55^{c}	250±24^{b}	277±23^{b}	192±37^{a}
56	370±37^{a}	432±52^{ab}	415±32^{a}	493±46^{b}	538±69^{b}	447±13^{a}	482±12^{ab}	495±38^{ab}
64	348±20^{a}	478±53^{b}	353±30^{a}	397±30^{a}	578±9^{b}	384±29^{a}	451±47^{a}	414±69^{a}

Table S4. Microbial biomass N (mg kg^{-1} soil) after the first (days 8, 16, 24, 32), and after the second residue addition (days 40, 48, 56, 64) in soil amended with low followed by high C/N residue (LH) or with high followed by low C/N residue (HL) with different moisture treatments between first and second residue addition: constantly moist (CM), one, two or four dry rewet cycles (1 DRW, 2 DRW, 4DRW) (n=4, means ± SE). Values in the same column followed by different letters are significantly different ($P \leq 0.05$).

Residue treatment	LH				HL			
Moisture treatment	CM	1 DRW	2 DRW	4 DRW	CM	1 DRW	2 DRW	4 DRW
Day	Microbial biomass N (mg kg^{-1} soil)							
8	17.7±5.0^{b}	2.3±0.5^{a}	2.6±0.4^{a}	20.3±1.4^{b}	15.0±2.9^{b}	0.4±0.3^{a}	1.5±0.8^{a}	16.2±2.9^{b}
16	46.7±5.8^{c}	4.4±0.3^{a}	11.0±1.4^{a}	26.8±4.3^{b}	4.4±0.9^{b}	0.4±0.2^{a}	4.5±0.3^{b}	17.7±1.7^{c}
24	17.3±0.7^{ab}	28.8±10.8^{b}	6.5±0.2^{a}	14.3±2.0^{a}	17.8±1.2^{b}	18.3±1.3^{b}	4.0±1.1^{a}	17.0±0.9^{b}
32	7.6±0.7^{a}	6.2±0.3^{a}	10.3±1.2^{b}	6.0±1.2^{a}	12.2±1.6^{a}	13.4±3.6^{a}	12.0±1.2^{a}	12.5±1.0^{a}
40	19.9±0.8^{b}	15.2±1.3^{a}	17.1±3.6^{ab}	14.9±1.8^{a}	27.8±3.1^{ab}	19.3±3.1^{a}	35.8±1.6^{b}	56.9±7.3^{c}
48	28.3±1.7^{a}	28.9±0.3^{a}	25.7±3.4^{a}	25.3±0.8^{a}	31.5±2.7^{a}	29.9±2.6^{a}	28.0±0.8^{a}	27.8±1.3^{a}
56	10.4±0.9^{a}	13.9±2.5^{bc}	14.2±1.2^{c}	10.9±0.7^{ab}	12.8±0.5^{a}	11.0±1.4^{a}	11.5±1.3^{a}	11.1±1.0^{a}
64	10.8±1.8^{a}	10.8±1.3^{a}	8.5±0.7^{a}	8.6±0.9^{a}	8.5±1.0^{ab}	9.8±0.8^{b}	7.5±1.6^{a}	8.0±0.8^{ab}

Table S5. Microbial biomass P (mg kg^{-1} soil) after the first (days 8, 16, 24, 32), and after the second residue addition (days 40, 48, 56, 64) in soil amended with low followed by high C/N residue (LH) or with high followed by low C/N residue (HL) with different moisture treatments between first and second residue addition: constantly moist (CM), one, two or four dry rewet cycles (1 DRW, 2 DRW, 4DRW) (n=4, means ± SE). Values in the same column followed by different letters are significantly different ($P \leq 0.05$).

Residue treatment	LH				HL			
Moisture treatment	CM	1 DRW	2 DRW	4 DRW	CM	1 DRW	2 DRW	4 DRW
Day	. Microbial biomass P (mg kg^{-1} soil)							
8	34.5±2.5^{b}	42.4±4.7^{b}	19.0±5.5^{a}	39.0±1.3^{b}	17.8±4.5^{a}	16.7±3.7^{a}	12.3±1.6^{a}	26.0±3.0^{b}
16	33.0±4.3^{a}	25.0±3.0^{a}	26.0±5.9^{a}	29.8±2.2^{a}	22.1±3.1^{a}	24.6±2.8^{a}	23.6±0.6^{a}	23.2±3.8^{a}
24	27.9±3.7^{a}	34.1±3.6^{a}	27.6±3.3^{a}	33.3±3.6^{a}	26.8±5.3^{a}	24.9±2.8^{a}	20.4±3.1^{a}	26.9±2.9^{a}
32	13.4±2.6^{a}	12.8±1.3^{a}	14.0±2.8^{a}	11.7±5.9^{a}	12.5±1.6^{a}	15.4±8.7^{a}	14.2±1.8^{a}	11.4±2.2^{a}
40	24.8±3.1^{a}	26.4±1.6^{a}	20.9±1.8^{a}	26.1±3.8^{a}	26.5±5.1^{a}	28.1±2.4^{a}	27.1±1.2^{a}	27.5±4.5^{a}
48	19.7±5.5^{a}	21.3±3.4^{a}	26.0±3.6^{a}	21.5±1.0^{a}	25.4±3.0^{ab}	24.8±2.7^{ab}	20.8±0.7^{a}	27.8±3.6^{b}
56	41.2±7.5^{b}	40.2±2.0^{b}	36.0±4.0^{ab}	26.2±3.0^{a}	46.3±3.6^{a}	40.4±6.5^{a}	45.8±2.1^{a}	39.1±7.4^{a}
64	40.7±4.5^{a}	37.3±2.1^{a}	44.1±5.6^{a}	39.9±3.8^{a}	46.2±5.4^{a}	38.2±4.6^{a}	42.6±3.0^{a}	42.1±2.7^{a}

Figure S1. Cumulative respiration over 64 days in LH treatment (a) and HL treatment (b) with different moisture treatments imposed during the first 32 days: constantly moist (CM), one, two or four dry rewet cycles (1 DRW, 2 DRW, 4 DRW) (n=4, means ± SE).

Figure S2. Microbial biomass phosphorus concentration after the first (a, b, days 8, 16, 24, 32) and the second residue addition (c, d, days 40, 48, 56, 64) in soil amended with low followed by high C/N residue (a, c, LH) or with high followed by low C/N residue (b, d, HL) with different moisture treatments between first and second residue addition: constantly moist (CM), one, two or four dry rewet cycles (1 DRW, 2 DRW, 4DRW) (n=4, means ± SE). Values in the same column followed by different letters are significantly different ($P \leq 0.05$).

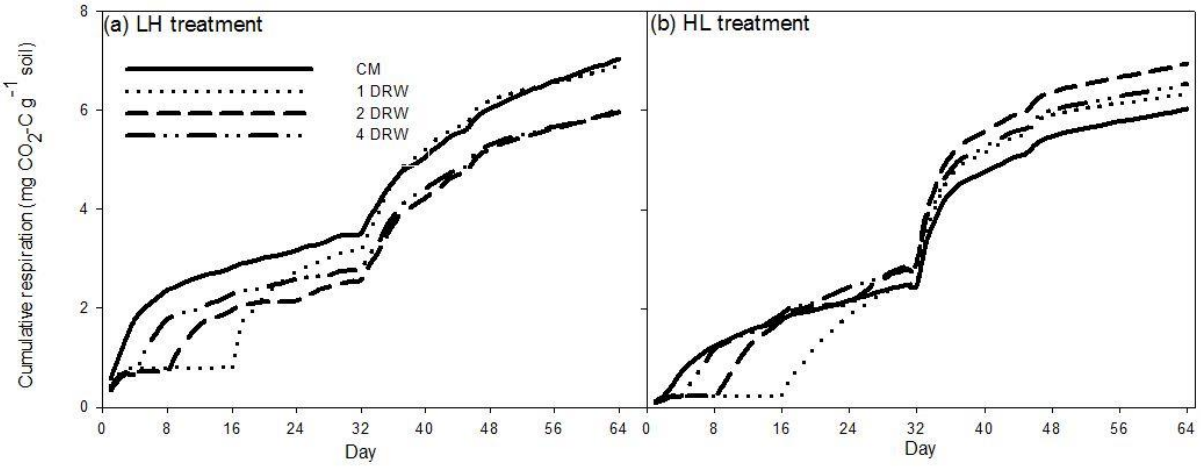
(a) LH treatment
(b) HL treatment
CM
1 DRW
2 DRW
4 DRW
Cumulative respiration (mg CO_2-C g^{-1} soil)
Day
Day
0
2
4
6
8
0
8
16
24
32
40
48
56
64

Figure S1.

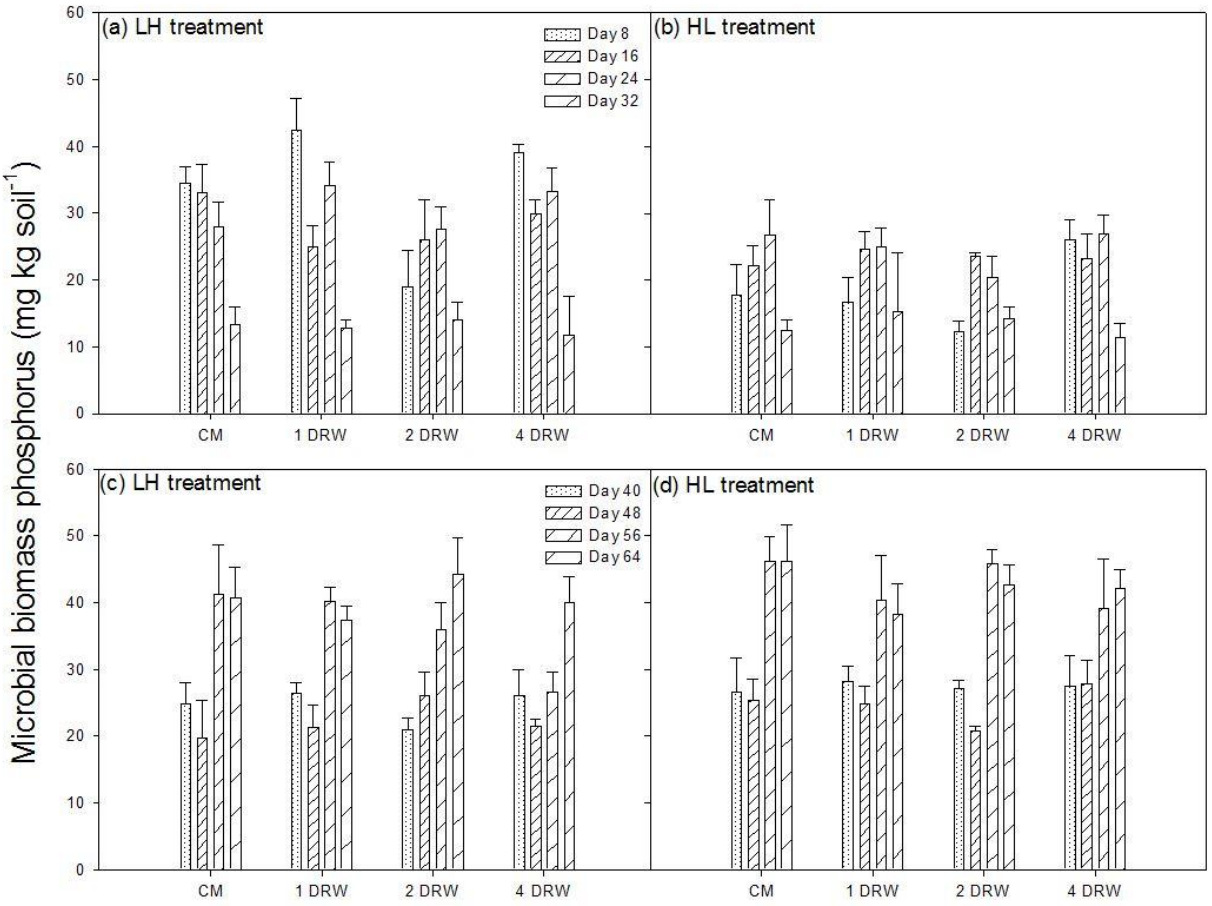
(a) LH treatment
(b) HL treatment
(c) LH treatment
(d) HL treatment
Day 8
Day 16
Day 24
Day 32
Day 40
Day 48
Day 56
Day 64
Microbial biomass phosphorus (mg kg soil-1)
0
10
20
30
40
50
60
CM
1 DRW
2 DRW
4 DRW

Figure S2.

CHAPTER 3

Residue addition combined with rewetting of dry soil – effect of timing of residue addition on soil respiration, microbial biomass, nutrient availability and legacy effect

Statement of Authorship

Title of Paper	Residue addition combined with rewetting of dry soil–effect of timing of residue addition on soil respiration, microbial biomass, nutrient availability and legacy effect
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted w ork w ritten in manuscript style
Publication Details	Zhang Y, Marschner P (2017) Residue addition combined with rewetting of dry soil–effect of timing of residue addition on soil respiration, microbial biomass, nutrient availability and legacy effect. Geoderma 299, 83-90.

Principal Author

Name of Principal Author (Candidate)	Yanchen Zhang		
Contribution to the Paper	Performed the experiment, analyses of soils, data analysis and interpretation, and manuscript writing.		
Overall percentage (%)	70%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	26/08/2018

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Petra Marschner		
Contribution to the Paper	Supervised development of the work, data interpretation, manuscript evaluation and correction and acted as the corresponding author.		
Signature		Date	24/08/2018

Geoderma 299 (2017) 83–90

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Residue addition combined with rewetting of dry soil – Effect of timing of residue addition on soil respiration, microbial biomass, nutrient availability and legacy effect

Yanchen Zhang, Petra Marschner*

School of Agriculture, Food and Wine, The University of Adelaide, South Australia 5005, Australia

ARTICLE INFO

Keywords:
Drying and rewetting
High C/N residue
Legacy effect
Low C/N residue
Microbial biomass
N availability
P availability

ABSTRACT

Harvest residues may be incorporated into soil just after a rainfall event in the field. The aim of this experiment was to determine the effect of residue addition upon rewetting on microbial activity, biomass and nutrient availability. A loamy soil was exposed to two wet-dry cycles with 5 days moist and 5 days dry each. Residues with high C/N ratio (H) or low C/N ratio (L) were added in eight residue treatments at different rates (10 or 20 g kg^{-1} soil) and timing (day 0 or day 10, before rewetting): 20 g kg^{-1} soil of H, L or a 50:50 mix of H and L was added on day 0 or day 10; 10 g kg^{-1} of H or L was added on day 0 followed by 10 g kg^{-1} of L or H on day 10 giving treatments HL and LH. Microbial biomass C, N and P and available N and P were measured on days 1, 5, 10, 11, 15 and 20. In the first 10 days, microbial biomass, N and P availability were higher with L than with H and higher at higher residue addition rate. Respiration rates decreased during the dry period and were very low prior to rewetting on day 10. Available N and P concentrations on day 11 were similar as on day 1 suggesting that if residues are added upon rewetting, nutrient release is not greater than if residues are added to moist soil. However, in the treatment where L had been added only on day 0, rewetting of dry soil induced N release from partially decomposed L residue left in the soil from the first period. When H was added to moist soil (day 0), MBN on day 1 and 5 was higher than in unamended soil. But when H was added on day 10, MBN increased only on day 11 indicating that with H microbial utilisation of residue N may be restricted if addition of residue is combined with rewetting. Microbial biomass C and MBN changed little during the first dry period, but decreased during the second dry period. This suggests that prior exposure to drying and rewetting reduced the ability of microbes to survive in dry soil even in treatments where residues were added upon rewetting. From day 11 to 20, MBN, available N and P were lower in LH than in HL, indicating that the second residue had a strong effect and thus the legacy effect was weaker than if the soil was moist throughout the experiment. We conclude that addition of residue upon rewetting enhanced microbial C and N uptake and reduces the legacy effect of the previously added residue.

1. Introduction

Decomposition of plant residues is influenced by residue properties such as C/N ratio. Nutrient availability during decomposition is determined by the C/nutrient ratio of plant residues. It is well-known that residues with low C/N ratio (< 20) and low lignin concentration are decomposed rapidly and lead to net N mineralisation whereas those with high C/N ratio induce net N immobilisation (Hadas et al., 2004). Previously, we showed that nutrient availability and microbial biomass after residue addition are also influenced by the properties of the previously added residue, referred to as legacy effect (Marschner et al., 2015; Nguyen et al., 2016). As a result of the legacy effect, nutrient availability after the second residue addition was lower in low C/N following high C/N residue than in low after low C/N residue. On the other hand, when the second amendment was high C/N residue, nutrient availability was higher in high following low C/N residue than in high after high C/N.

Soil water content is one of the most important environmental factors influencing decomposition and microbial activity in general (Thomsen et al., 1999). At low water content, thin water films restrict diffusion of nutrients to microbes and may draw water out of cells thus reducing microbial activity (Schimel et al., 2007). Rapid rewetting of dry soil is another stress for microbes because the sudden increase in water availability can lead to cell burst (Linn and Doran, 1984). On the

* Corresponding author at: School of Agriculture, Food and Wine, The University of Adelaide, South Australia 5000, Australia.
E-mail address: petra.marschner@adelaide.edu.au (P. Marschner).

http://dx.doi.org/10.1016/j.geoderma.2017.03.028
Received 12 November 2016; Received in revised form 28 March 2017; Accepted 29 March 2017

other hand, rewetting increases nutrient availability for the surviving microbes which, in turn, causes a flush of respiration (Birch, 1958) which is accompanied by an increase in microbial biomass C and N (Bottner, 1985; Wu and Brookes, 2005).

To date, most studies on the effect of drying and rewetting have been carried out in unamended soil (Fierer and Schimel, 2003; Shi and Marschner, 2014). The greater substrate availability in amended soil could increase or prolong the rewetting flush which, in turn, may also influence the legacy effect. In a recent experiment, one to four drying and rewetting events between the first and second residue addition did not influence the legacy effect (Zhang and Marschner, 2016). In the field, residue addition may coincide with rewetting, e.g. when harvest residues are incorporated into soil just after or during a rainfall event. In such a case, rewetting could increase nutrient release from the yet undecomposed residues as well as microbial nutrient uptake. Nutrient release upon rewetting in residue amended soil may depend on amount and C/N ratio of the residue and occurrence of dry-wet cycles relative to residue amendment. The aim of this experiment was to determine the effect of residue addition upon rewetting on microbial activity, biomass and nutrient availability and on the legacy effect. We hypothesised that (i) when residues are added at rewetting of dry soil nutrient availability will be greater than if residues are added to moist soil, and (ii) the legacy effect will be less pronounced if dry soil is rewetted when the second residue is added.

2. Material and methods

2.1. Soil and plant residues

A loamy soil was collected in the early 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 > 80 years. This site is in a semi-arid area and has a Mediterranean climate with cool, wet winters. The hot, dry summers can be interrupted by short, heavy rainfall events. 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 transect in three 2 × 2 m plots which were at least 10 m apart. In each sampling plot, after removal of plants and surface litter, five samples of topsoil (0–10 cm) were taken and sieved to < 2 mm followed by air-drying in a fan-forced oven at 40 °C. Soil from all sampling points was pooled and thoroughly mixed before subsamples were taken for the experiment. The soil has the following properties: 22% sand, 60% silt, 18% clay, maximum water holding capacity (WHC) 371 g kg^{-1}, pH (1:5) 5.6, electrical conductivity (EC) (1:5) 0.1 dS m^{-1}, total organic C 17 g kg^{-1}, total organic N 1.5 g kg^{-1}, bulk density 1.4 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, and mature wheat straw (*Triticum aestivum* L.) as high C/N ratio residue (Table 1). These two plant species were used because they are commonly grown 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 residue (young faba bean, L) had 5 to 10 times higher total N, total P, available N and P than high C/N ratio residue (wheat straw) (Table 1), and consequently lower C/N ratio and C/P ratios than high C/N residue (mature wheat straw, H). Water extractable organic C (WEOC) concentration and EC were nearly two-fold higher in L than in H. The residues had a similar pH and total organic C content.

2.2. Experimental design

Before the start of the experiment, the soil was pre-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 after rewetting. This water content was based on previous studies with this soil where microbial activity was maximal at 50% WHC (Marschner et al., 2015).

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 ($n = 4$). Different letters indicate significant differences between low and high C/N residue ($p \leq 0.05$).

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]	6.2	6.3
EC (mS m^{-1})[1]	10.2	5.6

[1] Measured in a 1:10 material to water extract.

During the 20-day experiment, soil was exposed to two wet-dry cycles with 5 days moist and 5 days dry each (Fig. 1). Residues with high C/N ratio (H) or low C/N ratio (L) were added either on day 0 or on day 10 in eight residue treatments. The total amount of residue added was 20 g kg^{-1} in all treatments, but residue was added either once or twice. In HL10 and LH10, residue was added twice: in HL10, 10 g kg^{-1} H was added on day 0 and 10 g kg^{-1} L on day 10. In LH10, 10 g kg^{-1} L was added on day 0 followed by 10 g kg^{-1} H on day 10. In L20-1, H20-1 and HL20-1, 20 g kg^{-1} residue was added on day 0 at the start of the first wet-dry cycle, either as only L (L20-1), only H (H20-1) or a 1:1 mixture of H and L (HL20-1). In L20-2, H20-2 and HL20-2, 20 g kg^{-1} residue was added on day 10 at the start of the second wet-dry cycle, either as only L (L20-2), only H (H20-2) or a 1:1 mixture of H and L (HL20-2). The HL10 and LH10 treatments were set up to study the effect of residue addition rate (10 g kg^{-1} compared to 20 g kg^{-1} in 20-1 and 20-2 treatments). The treatments also allowed studying the combined influence of rewetting and residue addition on respiration, microbial biomass and nutrient availability. For each amendment, residues were thoroughly mixed into the soil in a small plastic bag. Unamended soil was mixed in a similar manner.

At the start of the experiment, residue amended soil or unamended soil (30 g dry soil) 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}. To start a dry period, the soil was dried within 3 days using three pouches with silica gel (8 g/pouch) as described in Shi and Marschner (2014). The silica pouches were replaced by dry silica pouches daily during the dry period. After three days, a water content of 2% of WHC was reached and the soil remained dry for another two days until the end of the dry period. Rapid rewetting was carried out as described in Shi and Marschner (2014) by adding reverse osmosis (RO) water in a circular motion. In treatments that were amended on day 10 (end of the first dry period), residues were added after rewetting by emptying the soil from cores into a small plastic bag and mixing in the residues. Then the soil was returned to the core and bulk density was adjusted. Unamended soils were treated in a similar manner.

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 23–25 °C. Soil moisture was maintained at 50% of WHC by checking the water content every few days by weight and adding (RO) water if necessary. Soil respiration was measured daily. Soil cores were destructively sampled on day 1 (one day after the first residue addition), 5 (end of first moist period), 10 (end of first dry period), day 11 (one day after rewetting and in some treatments, residue addition), 15 (end of second moist period) and 20 (end of second dry period). Soil samples were analysed for available N and P, microbial

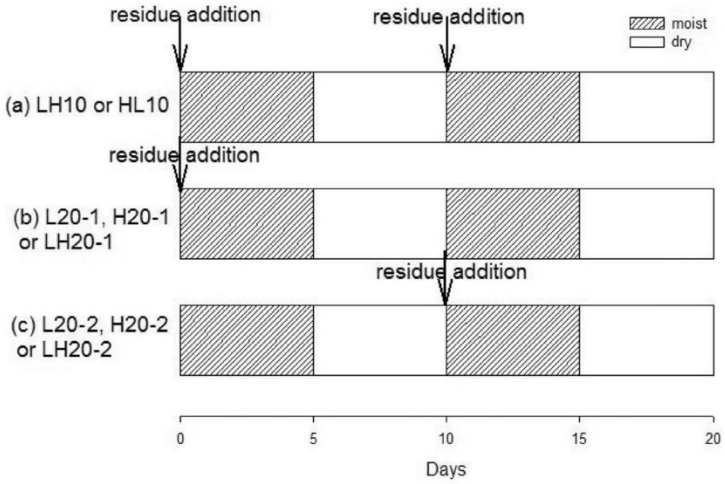

Fig. 1. Schematic diagram of experimental design. Soil amended with low followed by high C/N residue and with high followed by low C/N residue at the rate of 10 g kg^{-1} soil (a, LH10, HL10), or once as L, H or LH at the rate of 20 g kg^{-1} soil at the start of the experiment (b, L20-1; H20-1 or LH20-1) or upon rewetting at the start of the second moist period (c, L20-2; H20-2 or LH20-2).

biomass C, N and P. For each sampling and treatment there were four replicates, giving a total of 192 cores.

2.3. Measurements

Soil texture was determined according to the rapid textural analysis (Kettler et al., 2001). Soil maximum water holding capacity was measured in a sintered glass funnel connected to a 100 mm water column ($\psi_m = -10$ 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 h shaking at 25 °C. Total organic carbon of soil and plant residues was measured according to Walkley and Black (1934) and total nitrogen was measured using the Kjeldahl method (McKenzie and Wallace, 1954) followed by colorimetric measurement. 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 phosphovanado-molydate method (Hanson, 1950). Water extractable organic carbon was determined by shaking 1 g residue with 30 ml RO water for 1 h. The extract was then centrifuged at 3000 rpm for 10 min and filtered through a Whatman#42 filter paper. Organic C in the extract was determined after $K_2Cr_2O_7$ and H_2SO_4 oxidation by titration with acidified $(NH_4)_2Fe(SO_4)_2{\cdot}6H_2O$ (Anderson and Ingram, 1993).

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 to refresh the headspace with a fan 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 t1 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 2 M KCl at a 1:5 (w/v) soil to extractant ratio. Ammonium-N was measured using nitroprusside/dichloro-*S*-triazine after Willis et al. (1996). Nitrate-N was determined with VCl_3 and *N*-(1-naphthyl)ethylenediamine dihydrochloride and measured colorimetrically at 540 nm 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 by the molybdenum blue method (Murphy and Riley, 1962).

Microbial biomass C and N were determined by chloroform fumigation-extraction with 0.5 M K_2SO_4 at 1: 4 (w/v) soil to extractant ratio. Organic C concentration in the extract was measured by titration with 0.033 M acidified $(NH_4)_2Fe(SO_4)_2{\cdot}6H2O$ after dichromate oxidation (Anderson and Ingram, 1993). The chloroform-labile C concentration is the difference between fumigated and non-fumigated soil, which was multiplied by 2.64 to calculate microbial biomass C (Vance et al., 1987). Microbial biomass N was calculated as the difference in NH_4^+ concentration between fumigated and non-fumigated samples divided by 0.57 to convert ammonium to microbial biomass N as suggested by Moore et al. (2000). Microbial biomass P 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. 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 replicates for each treatment and sampling time. Data was tested for homogeneity and equal variance. For measurements carried out repeatedly during the experiment, one-way repeated measures analysis of variance (ANOVA) was carried out to determine if there were significant differences in microbial biomass and nutrient availability between treatments in each sampling time. The interaction of treatment and time was significant ($p < 0.05$). Therefore Tukey's multiple comparison test at 95% confidence interval was used for each sampling time separately to determine which treatments differ from each other. One way ANOVA was also used to compare cumulative respiration in 5-day intervals and at the end of the experiment as well as for the properties of two plant residues. Statistical analyses were carried out with Genstat 15th edition (VSN Int. Ltd., UK).

3. Results

3.1. Respiration

From day 1–10, respiration rates were much higher in all soils that had been amended on day 0 (Fig. 2a, b) than in unamended soils

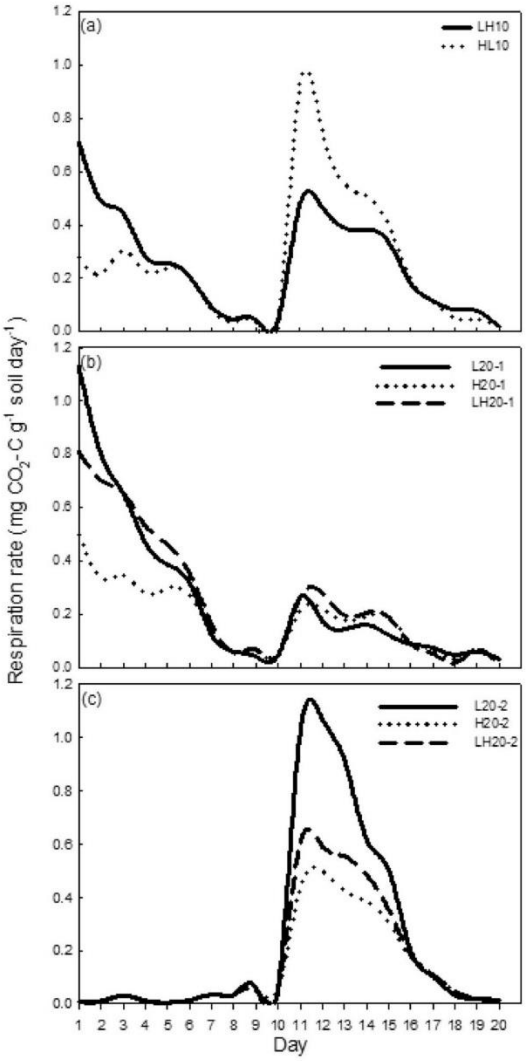

Fig. 2. Respiration rate over 20 days (n = 4, means ± SE) in treatments that received residues twice (a, LH10 and HL10) or once as L, H or LH at the rate of 20 g kg^{-1} soil on day 0 (b, L20-1; H20-1 or LH20-1) or upon rewetting on day 10 (c, L20-2; H20-2 or LH20-2).

(treatments 20–2) (Fig. 2c). In amended soils, the initial respiration rate was highest in L20-1 and lowest in HL10. Initial respiration rates depended on residue addition rate, they were higher in L20-1 than in LH10 and higher in H20-1 than in HL10. Respiration rates declined in the first 8 days (5 days moist and 3 days drying) with a sharper decline in treatments with L than with H. Rewetting on day 10 induced a flush of respiration that was greater in treatments where residues were added on day 10 (LH-10, HL-10, L20-2, H20-2, LH20-2) than in those without residue addition at rewetting. The flush was about 20% higher when 20 g kg^{-1} residue was added (L20-2, H20-2, LH20-2) compared to 10 g kg^{-1} of the same residue type in HL10 and LH10, e.g. L20-2 compared to HL10. In treatments that where amended on day 10, the flush was about two-fold higher with L compared to H whereas residue types did not differ in treatments that were amended only on day 0 (L20-1, H20-1, LH20-1).

Cumulative respiration in the first 10 days was lowest in unamended soils (treatments 20–2) (Fig. 3). In amended soils, cumulative respiration from day 0 to 5 (moist soil) was lowest in HL-10 and highest in L20-1. Within the same residue type (H or L), cumulative respiration in the periods day 0–5 and 6–10 was about 25% higher when 20 g kg^{-1} had been added on day 0 compared to 10 g kg^{-1}. Cumulative respiration in the first 5 days after rewetting (days 11–15) was greater than in the previous five dry days with the smallest increase in treatments that were not amended on day 10. In treatments with residue addition on day 10, the increase in cumulative respiration compared to the previous five dry days was greater when L was added on day 10 (HL10, L20-2, LH20-2) than with H addition (LH10, H20-2). Cumulative respiration during the second dry period (day 16 to 20) was much lower than in the previous moist period. Total cumulative respiration from day 0 to 20 was higher in treatments that received residues twice (LH10 and HL10) or L residues on day 0 (L20-1, LH20-1) and lowest in H20-2.

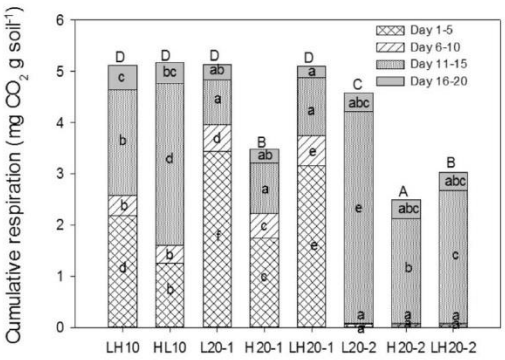

Fig. 3. Cumulative respiration from day 1–20 in 5-day intervals in treatments that received residues twice (a, LH10 and HL10) or once as L, H or LH at the rate of 20 g kg^{-1} soil on day 0 (b, L20-1; H20-1 or LH20-1) or upon rewetting on day 10 (c, L20-2; H20-2 or LH20-2) (n = 4, means ± SE). For each 5-day period (day 1–5, day 6–10, day 11–15 and day 16–20), bars with different lower case letters indicate significant differences in each period (five days) among treatments; bars with different upper case letters indicate significant differences in total cumulative respiration among treatments ($p \leq 0.05$).

3.2. Microbial biomass

Microbial biomass C (MBC) on day 1 was highest in L20-1 and low in all treatments that were not amended on day 0 (L20-2, H20-2, LH20-2) (Fig. 4a, Table S1). At the same residue addition rate it was about four times higher with L than H residue. MBC was more than two-fold higher when L or H were added at 20 g kg^{-1} (L20-1, H20-1) than at 10 g kg^{-1} (HL10, LH10). In treatments with L added on day 0 (L20-1, LH20-1, LH10), MBC was lower on day 10 (end of dry period) than on day 1. MBC was higher on day 10 than day 1 in treatments with H added on day 1 (H20-1, HL10) and did not change over time in the other treatments. On day 11 (one day after rewetting), MBC was highest in L20-2 and lowest in H20-1 (Fig. 4b). From day 10 to 11, MBC increased in most treatments except in LH10 where it remained unchanged and in H20-1 where it was about 75% lower on day 11 than day 10. On days 15 and 20, MBC was lowest in H20-1. On day 15 (end of moist period), MBC was higher in H20-2, L20-2 and LH20-2 than in the treatments that had received the same residue type on day 0 (H20-1, L20-1 and LH20-1). In these treatments, MBC was higher with both H and L added (HL) compared to only H addition. Differences among treatments were smaller at the end of the dry period (day 20) than at the end of the moist period (day 15). MBC decreased from day 11 to day 20 (end of second dry period) in treatments where L was added on day 10 (HL10, L20-2) or only on day 0 (L20-1). However, in treatments with H added on day 10 (LH10, H20-2, LH20-2), MBC increased during the moist period (day 11 to 15) and then decreased to day 20. Microbial biomass C on day 11 in L20-2, H20-2 and LH20-2 was

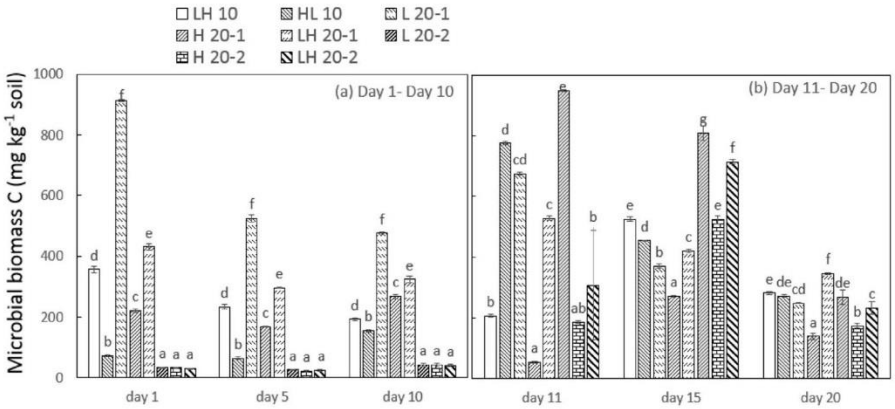

Fig. 4. Microbial biomass C concentration in the first (a, days 1, 5, 10) and the second wet-dry period (b, days 11, 15, 20) in treatments that received residues twice (a, LH10 and HL10) or once as L, H or LH at the rate of 20 g kg^{-1} soil on day 0 (b, L20-1; H20-1 or LH20-1) or upon rewetting on day 10 (c, L20-2; H20-2 or LH20-2) (n = 4, means ± SE). Bars with different lower case letters indicate significant differences among treatments at each sampling time ($p \leq 0.05$).

similar to L20-1, H20-1 and LH20-1 on day 1, respectively.

In the first 10 days, microbial biomass N (MBN) and P (MBP) were lowest in treatments without residue addition on day 0 (L20-2, H20-2, LH20-2) (Fig. 5a, c). On day 1, MBN was higher in treatments with L (LH10, L20-1, LH20-1) than those with H added on day 0 (HL10, H-20-1) (Fig. 5a, Table S2). It was about 20% higher in treatments with 20 than with 10 g kg^{-1}. In residue amended treatments, MBN decreased by about 25% from day 1 to the end of the moist period (day 5) and again to day 10 (end of dry period), except in L20-1 where it did not change from day 5 to day 10. Rewetting induced a more than two-fold increase in MBN on day 11 compared to day 10 (Fig. 5b). On day 11, MBN was highest in L20-2 and low in treatments with only H added (H20-1 and H20-2). It was about 25% lower in LH20-1 and LH20-2 compared to L20-1 and L20-2 and in these treatments higher where residue was added on day 10 compared to those amended on day 0. From day 11 to day 15, MBN increased nearly two-fold in L20-1 and H20-2, but decreased two-fold in L20-2 and LH20-2. On days 15 and 20, MBN was highest in L20-1 where it was about two-fold than HL10 and 30% higher than in L20-2. MBN remained low in treatments with H (H20-1, H20-2, LH20-1, LH20-2 and LH10). In all treatments MBN was lower on day 20 (end of dry period) than on day 11. MBN on day 11 was higher in L20-2 and LH20-2 than in L20-1 and LH20-1 on day 1 and similar in H20-1 and H20-2.

Microbial biomass P in residue amended soils in the first 10 days was always highest in L20-1 and low in treatments with H (HL10, H20-1, LH20-1)(Fig. 5c, Table S3). In the H treatments, MBP did not change much with time, but in L20-1 it was 25% lower at the end of the moist period (day 5) than on day 1 whereas it was 25% higher on day 5 than day 1 in LH20-1. Drying the soil from day 5 to day 10 had little effect on MBP. Rewetting the soil on day 10 increased MBP to day 11 only in treatments where it was accompanied by residue addition (HL10, LH10, L20-2, H20-2, LH20-2), but not in only rewetted treatments (L20-1, H20-1, LH20-1)(Fig. 5d). The increase was greater in HL10 than LH10. In the second period, MBP was higher in treatments with L (HL10,

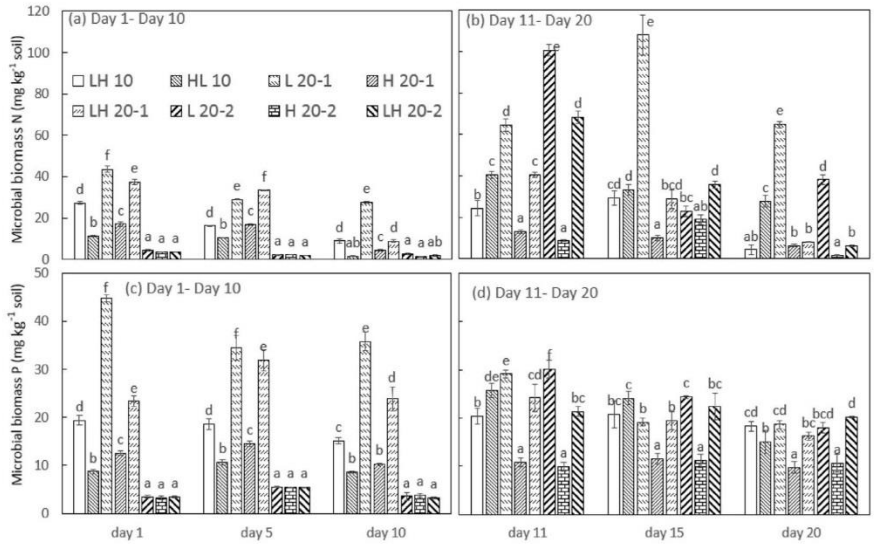

Fig. 5. Microbial biomass N and microbial biomass P concentration in the first (a, days 1, 5, 10) and the second wet-dry period (b, days 11, 15, 20) in treatments that received residues twice (a, LH10 and HL10) or once as L, H or LH at the rate of 20 g kg^{-1} soil on day 0 (b, L20-1; H20-1 or LH20-1) or upon rewetting on day 10 (c, L20-2; H20-2 or LH20-2) (n = 4, means ± SE). Bars with different lower case letters indicate significant differences among treatments at each sampling time ($p \leq 0.05$).

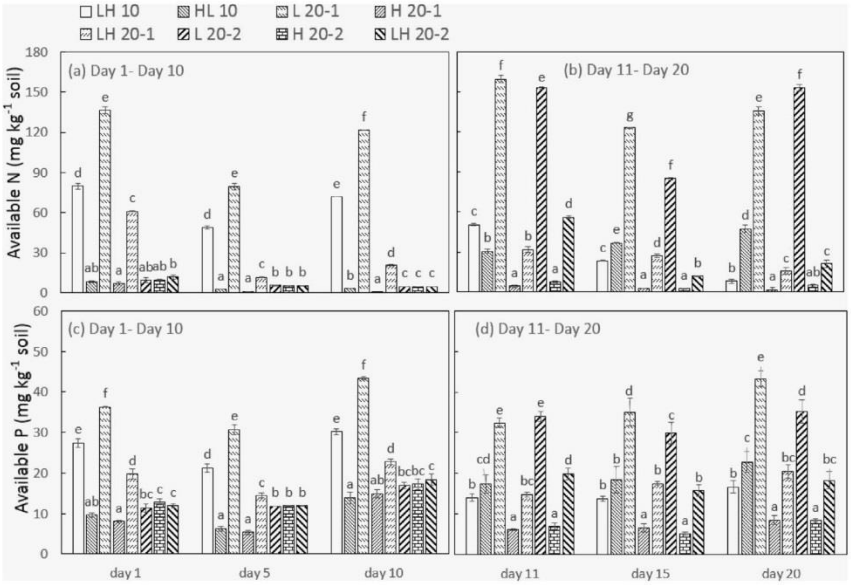

Fig. 6. Available N and available P concentration in the first (a, days 1, 5, 10) and the second wet-dry period (b, days 11, 15, 20) in treatments that received residues twice (a, LH10 and HL10) or once as L, H or LH at the rate of 20 g kg^{-1} soil on day 0 (b, L20-1; H20-1 or LH20-1) or upon rewetting on day 10 (c, L20-2; H20-2 or LH20-2)(n = 4, means ± SE). Bars with different lower case letters indicate significant differences among treatments at each sampling time ($p \leq 0.05$).

LH10, L20-1, L20-2, HL20-1, HL20-2) than those with only H. There was little difference in MBP between treatments that received 20 g kg^{-1} on day 0 and day 10 or between HL10 and LH10. Compared to MBP one day after residue addition at 20 g kg^{-1}, MBP on day 11 was lower in L20-2 than on day 1 in L20-1, but similar in H20-2 and LH20-2 on day 11 as in H20-1 and LH20-1 on day 1.

3.3. Available N and P

In the first 10 days, available N and P were low in treatments without residue addition on day 0 (L20-2, H20-2, LH20-2) and those that received only H (HL10, H20-1) (Fig. 6). Available N and P were about 30% higher in L20-1 than LH10. Available P was about two-fold higher and available N was up to five-fold higher in L20-1 than HL20-1. Available N decreased in the moist period (day 1 to day 5), but then increased from day 5 to day 10 (dry period) in treatments with L added on day 0 (LH10, L20-1, LH20-1) (Fig. 6a, Table S4). Rewetting increased available N in most treatments that received L at least once with a greater increase when residue was added at rewetting (HL10, L20-2, LH20-2) than in only rewet soils (L20-1, LH20-1) (Fig. 6b). In the second period, available N was higher in L20-1 and L20-2 than in the other treatments and it was low in treatments that received only H (H20-1, H20-1). Available N on day 11 was lower in LH20-1 and LH20-2 than L20-1 and L20-2. During the second moist period (day 11–15), available N decreased by at least 20% in L20-1, L20-2 and LH20-2, but then increased again during the dry period (day 15–20). Available N on day 11 was higher in LH10 than HL10, but on days 15 and 20, it was higher in HL10 than LH10. Available N was similar in L20-2, H20-2 and LH20-2 on day 11 as in L20-1, H20-1 and LH20-1 on day 1.

Available P did not change from day 1 to day 5 in treatments without residue addition or only H added on day 0 (HL 10, H20-1, H20-2) (Fig. 6c, Table S5). But available P decreased by about 20% during this period in LH10, L20-1 and LH20-1. Available P increased from day 5 to 10 in all treatments by at least 30%. Available P on day 11 was 30–50% lower than on day 10 in most treatments except in L20-2 where it increased nearly two-fold and in LH20-2 where it remained unchanged (Fig. 6d). Available P on day 11 was about two-fold higher in L20-1 and L20-2 than in the other treatments. It was lowest when H had been added once (H20-1 and H20-2). Available P changed little in the following moist and dry period. It was similar in L20-2, H20-2 and LH20-2 on day 11 as in L20-1, H20-1 and LH20-1 on day 1.

4. Discussion

This study confirmed that rewetting of dry soil results in a flush of respiration and nutrient availability, but it also showed that rewetting can increase nutrient release from previously added low C/N residue at high addition rate. Further, the results indicate that if the second residue addition is combined with rewetting, the effect of the previously added residue on nutrient availability may be reduced.

4.1. First 10-day period (day 1–10)

In the first five days when the soil was moist, respiration, microbial biomass and N and P availability decreased in the following order L > LH > H which is in agreement with previous studies (Marschner et al., 2015; Nguyen et al., 2016). The higher N, P and WEOC concentration of L compared to H makes it more readily decomposable and allows greater nutrient uptake by microbes. The lower respiration, nutrient availability and microbial biomass in LH-10 and HL-10 compared to L20-1 and H20-1 can be explained by the lower amount of residue and thus substrate added on day 0 (10 g kg^{-1} in the former compared to 20 g kg^{-1} in L20-1, H20-1).

Compared to L20-1, only half of the amount of L was added with LH20-1. Nevertheless, initial respiration rates of LH20-1 were only 20% lower than in L20-1 and cumulative respiration over 20 days was similar in L20-1 and LH20-1. Microbial biomass C on days 1 and 5 was, as expected based on the amounts of L added, twice as high in L20-1 than LH20-1. However, MBN and MBP on day 5 were similar in LH20-1 and L20-1, whereas available N in LH20-1 was 80% lower than in L20-1. These results suggest that the low C/N residue in LH-20 was preferentially decomposed and that N released during decomposition was taken up by microbes. Greater than expected decomposition and nutrient availability have been reported in residue mixes and are

referred to as synergistic interaction (Gartner and Cardon, 2004). Synergistic interaction has been explained by nutrient transfer between microbes decomposing different residues, changes in microbial community composition and priming (Gartner and Cardon, 2004).

Respiration rates were low in dry soil and cumulative respiration in the 5-day dry period was 75% lower than in the previous moist period. But MBC, MBN, MBP were similar at the end of the dry period (day 10) as at the end of the moist period (day 5). This suggests that microbes reduced activity as the soil dried but they survived the short dry period. The higher P availability in dry soil compared to moist soil found here has been reported before and was explained by the methods used to determine available P which involve addition of an extractant, thus rewetting. The addition of extractant induces release of microbial P and previously occluded P (Bramley and Barrow, 1992; Turner and Haygarth, 2003).

4.2. Second 10-day period (day 11–20)

In agreement with other studies, rewetting induced a flush of respiration (Baumann and Marschner, 2013; Shi and Marschner, 2014; Wu and Brookes, 2005). But the flush was two to three-fold greater if residues were added at rewetting. Thus, cumulative respiration in the moist period (day 11–15) in L20-2 and H20-2 was greater than in L20-1 and H20-1. In treatments with L, rewetting also induced an increase in MBC and MBN within one day compared to the end of the previous dry period. Osmolytes accumulated by microbes in dry soil such as glycine betaine, proline and quaternary ammonium compounds are N-rich (Ashraf and Foolad, 2007; Warren, 2013) and can be released upon rewetting. Therefore, the higher MBN in rewet soil could be due to decomposition of released osmolytes as well as of the low C/N residue.

The increase in MBC, MBN, MBP, available N and P in HL10 from day 10 to day 11 can be explained by the addition of L upon rewetting. In LH10 where H was added on day 10, MBC did not change from day 10 to day 11. On the other hand, available N and P decreased in LH10 from day 10 to day 11 whereas MBN and MBP increased, indicating immobilisation of N and P released from L during the first 10 days. Thus, when a smaller amount of residue was added twice (10 g kg^{-1}), microbial N and P uptake was greater when residue addition was combined with rewetting than when the same residue was added to moist soil on day 0, e.g. day 11 in HL10 compared to day 1 in LH10. Therefore, the first hypothesis (when residues are added at rewetting of dry soil nutrient availability will be greater than if residues are added to moist soil) can neither be confirmed nor declined.

Available N and P concentrations on day 11 were similar as on day 1 suggesting that if residues are added upon rewetting, nutrient release is not greater than if residues are added to moist soil. However, available N and P on day 11 were similar in L20-1 and L20-2 although in L20-1, N had already been released in the first period. This indicates that in L20-1 rewetting of dry soil induced N release from partially decomposed L residue left in the soil from the first period. Based on cumulative respiration and without taking a priming effect into account, about 50% of the previously added residue remained in the soil at the end of the first period. The finding that MBN on day 11 was greater in L20-2 than L20-1 suggests that more N was released upon rewetting from freshly added residue than from the previously residue and this additional N was taken up by microbes. In H20-1 and H20-2, available N was low, which was also the case in the first 10 days in H20-1. Thus, the low N concentration in H limited net N release even if rewetting on day 10 increased respiration rates and MBC compared to the previous dry period.

In treatments with L, the decrease in MBC from day 11 to day 15 was accompanied by high cumulative respiration. Thus, the decrease in MBC from day 11 to day 15 is likely due to depletion of easily available substrates. On the other hand, MBC increased from day 11 to day 15 in H20-1, H20-2 and LH10 where MBC was low on day 11. The delayed increase in MBC in treatments with H indicates that it takes longer to decompose H which can be explained by its high C/N ratio and low WEOC concentration. This increase in MBC was not accompanied by an increase in MBN and MBP, suggesting that the low N and P availability limited microbial N and P uptake.

At the end of the second moist period (day 15) MBC and MBN were higher than at the end of the first moist period (day 5). In unamended soil, microbial biomass decreased with increasing number of dry-rewetting cycles (Baumann and Marschner, 2013; Mikha et al., 2005; Wu and Brookes, 2005). In this study, the negative impact of the first drying and rewetting on microbes was likely compensated by nutrient release upon rewetting from residues left in the soil or added with rewetting. Microbial biomass C did not change during the first dry period (day 5–10) in most treatments except for HL10 and H20-1 where it increased. But MBC decreased in all treatments in the second dry period (day 15–20). The decrease in MBN was also more pronounced in the second than the first period. This indicates that the prior exposure to drying and rewetting reduced the ability of microbes to survive in dry soil even in treatments where residues were added upon rewetting.

4.3. Total cumulative respiration and legacy effect

In treatments with a single residue addition, total cumulative respiration over 20 days was lower when residues were added upon rewetting (L20-2, H20-2, HL20-2) than when they were added at the start (L20-1, H20-1, HL20-1). In the latter, residues could be decomposed over 20 days whereas they were decomposed over only 10 days in L20-2, H20-2 and HL20-2. High respiration rates were maintained longer when residues were added upon rewetting than when they were added to moist soil at the start of the experiment, and cumulative respiration in the moist period after addition was greater in L20-2 and H20-2 (day 11–15) than in L20-1 and H20-1 (day 1–5). But this greater respiration from day 11 to 15 could not compensate for the low respiration in L20-2 and H20-2 in the first 10 days. Total cumulative respiration did not differ between LH20-1 and LH20-2 or in treatments where at least a proportion of residues was added at the start of the experiment (HL10, LH10). This suggests that the timing of the mixing of H and L does not influence decomposition. However, it does affect when the bulk of CO_2 is released. Most of the CO_2 was released in the first moist period in LH20-1, but in the second moist period in HL10. In LH10, similar amounts of CO_2 were released in the first and second period, likely because decomposition of H in the second period was enhanced by nutrients released during decomposition of L left in the soil after the first period (Marschner et al., 2015).

In agreement with our previous studies on the legacy effect where the soil was maintained moist throughout (Marschner et al., 2015; Nguyen et al., 2016), MBC in the second period was similar in HL10 and LH10. This can be explained by simultaneous decomposition of H and L. For MBN, available N and P we can confirm the second hypothesis (the legacy effect will be less pronounced if dry soil is rewetted when the second residue is added). In our previous legacy experiments, MBN, available N and P after the second addition were similar in HL and LH (Marschner et al., 2015; Nguyen et al., 2016). But in the present study, MBN, available N and P from day 11 to 20 were lower in LH than in HL, indicating that the second residue had a stronger effect than expected and thus the legacy effect was weaker than if the soil was moist throughout the experiment. This suggests that the residue added upon rewetting is preferentially decomposed even in LH where the freshly added H is more difficult to decompose than the L residue left in the soil. It is likely that rewetting induces a rapid release of nutrients from the residues which in turn stimulates colonisation by microbes and subsequent decomposition.

5. Conclusion

The effect of rewetting on decomposition was influenced by timing and amount of residue addition. When large amounts of residues were

added initially (20 g kg^{-1}), rewetting of dry soil enhanced immediate nutrient release from partially decomposed residues in the soil, but delayed N uptake into the microbial biomass. Adding the same residue amount upon rewetting had little effect on nutrient availability but enhanced microbial biomass C and N. For the field this suggests that rewetting enhances decomposition of residues which can be beneficial if a crop can rapidly take up the released nutrients. On the other hand, rewetting events may also reduce the C sequestration potential of organic amendments.

In this experiment, moist and dry periods were short as they may occur during a hot summer with frequent rainfall events. Future experiments could investigate the effect of longer moist and dry periods. Use of ^{13}C and/or ^{15}N labelled residues would allow studying the fate of C and N.

Acknowledgement

Yanchen Zhang received a scholarship from The University of Adelaide.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.geoderma.2017.03.028.

References

Anderson, J., Ingram, J., 1993. Colorimetric determination of ammonium. In: Tropical Soil Biology and Fertility, A Handbook of Methods, second ed. CAB International, Wallingford, UK, pp. 73–74.
Ashraf, M., Foolad, M.R., 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ. Exp. Bot. 59 (2), 206–216.
Baumann, K., Marschner, P., 2013. Effects of salinity on microbial tolerance to drying and rewetting. Biogeochemistry 112 (1–3), 71–80.
Birch, H., 1958. The effect of soil drying on humus decomposition and nitrogen availability. Plant Soil 10 (1), 9–31.
Bottner, P., 1985. Response of microbial biomass to alternate moist and dry conditions in a soil incubated with ^{14}C- and ^{15}N- labelled plant material. Soil Biol. Biochem. 17, 329–337.
Bramley, R., Barrow, N., 1992. The reaction between phosphate and dry soil. II. The effect of time, temperature and moisture status during incubation on the amount of plant available P. J. Soil Sci. 43 (4), 759–766.
Butterly, C.R., Marschner, P., McNeill, A.M., Baldock, J.A., 2010. Rewetting CO_2 pulses in Australian agricultural soils and the influence of soil properties. Biol. Fertil. Soils 46 (7), 739–753.
Cavagnaro, T., Jackson, L., Six, J., Ferris, H., Goyal, S., Asami, D., Scow, K., 2006. Arbuscular mycorrhizas, microbial communities, nutrient availability, and soil aggregates in organic tomato production. Plant Soil 282 (1–2), 209–225.
Fierer, N., Schimel, J.P., 2003. A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. Soil Sci. Soc. Am. J. 67 (3), 798–805.
Gartner, T.B., Cardon, Z.G., 2004. Decomposition dynamics in mixed-species leaf litter. Oikos 104 (2), 230–246.
Hadas, A., Kautsky, L., Goek, M., Kara, E.E., 2004. Rates of decomposition of plant residues and available nitrogen in soil, related to residue composition through simulation of carbon and nitrogen turnover. Soil Biol. Biochem. 36 (2), 255–266.
Hanson, W., 1950. The photometric determination of phosphorus in fertilizers using the phosphovanado-molybdate complex. J. Sci. Food Agric. 1 (6), 172–173.
Isbell, R., 2002. The Australian Soil Classification. Vol. 4 CSIRO publishing.
Kettler, T., Doran, J.W., Gilbert, T., 2001. Simplified method for soil particle-size determination to accompany soil-quality analyses. Soil Sci. Soc. Am. J. 65 (3), 849–852.
Kouno, K., Tuchiya, Y., Ando, T., 1995. Measurement of soil microbial biomass phosphorus by an anion exchange membrane method. Soil Biol. Biochem. 27 (10), 1353–1357.
Linn, D., Doran, J., 1984. Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. Soil Sci. Soc. Am. J. 48 (6), 1267–1272.
Marschner, P., Hatam, Z., Cavagnaro, T., 2015. Soil respiration, microbial biomass and nutrient availability after the second amendment are influenced by legacy effects of prior residue addition. Soil Biol. Biochem. 88, 169–177.
McKenzie, H., Wallace, H.S., 1954. The Kjeldahl determination of nitrogen: a critical study of digestion conditions-temperature, catalyst, and oxidizing agent. Aust. J. Chem. 7 (1), 55–70.
Mikha, M.M., Rice, C.W., Milliken, G.A., 2005. Carbon and nitrogen mineralization as affected by drying and wetting cycles. Soil Biol. Biochem. 37 (2), 339–347.
Moore, J., Klose, S., Tabatabai, M., 2000. Soil microbial biomass carbon and nitrogen as affected by cropping systems. Biol. Fertil. Soils 31 (3–4), 200–210.
Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31–36.
Nguyen, T.T., Ngo, H.T.T., Marschner, P., 2016. Legacy effect of previous residue addition—influence of length of the moist period between residue additions on soil respiration, microbial biomass and nutrient availability. Biol. Fertil. Soils 1–11.
Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88 (6), 1386–1394.
Setia, R., Marschner, P., Baldock, J., Chittleborough, D., Smith, P., Smith, J., 2011. Salinity effects on carbon mineralization in soils of varying texture. Soil Biol. Biochem. 43 (9), 1908–1916.
Shi, A., Marschner, P., 2014. Drying and rewetting frequency influences cumulative respiration and its distribution over time in two soils with contrasting management. Soil Biol. Biochem. 72, 172–179.
Thomsen, I.K., Schjønning, P., Jensen, B., Kristensen, K., Christensen, B.T., 1999. Turnover of organic matter in differently textured soils: II. Microbial activity as influenced by soil water regimes. Geoderma 89 (3), 199–218.
Turner, B.L., Haygarth, P.M., 2003. Changes in bicarbonate-extractable inorganic and organic phosphorus by drying pasture soils. Soil Sci. Soc. Am. J. 67 (1), 344–350.
Vance, E., Brookes, P., Jenkinson, D., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19 (6), 703–707.
Walkley, A., Black, I.A., 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci. 37 (1), 29–38.
Warren, C.R., 2013. High diversity of small organic N observed in soil water. Soil Biol. Biochem. 57, 444–450.
Willis, R.B., Montgomery, M.E., Allen, P.R., 1996. Improved method for manual, colorimetric determination of total Kjeldahl nitrogen using salicylate. J. Agric. Food Chem. 44 (7), 1804–1807.
Wu, J., Brookes, P., 2005. The proportional mineralisation of microbial biomass and organic matter caused by air-drying and rewetting of a grassland soil. Soil Biol. Biochem. 37 (3), 507–515.
Zhang, Y., Marschner, P., 2016. Drying and rewetting after the first residue addition has little effect on nutrient availability and microbial activity after the second residue addition. Eur. J. Soil Biol. 77, 68–76.

Supplementary data

Table S1. Microbial biomass C concentration in the first (days 1, 5, 10) and the second wet-dry period (days 11, 15, 20) in treatments that received residues twice (LH10 and HL10) or once as L, H or LH at the rate of 20 g kg^{-1} soil on day 0 (L20-1; H20-1 or LH20-1) or upon rewetting on day 10 (L20-2; H20-2 or LH20-2) (n=4, means ± SE). Different letters indicate significant differences in a treatment over time ($P \leq 0.05$).

Microbial biomass C	Day 1	Day 5	Day 10	Day 11	Day 15	Day 20
LH10	356±11d	234±6b	193±5a	207±6a	525±6e	282±4c
HL10	73±3a	65±5a	156±4b	775±6e	454±3d	272±4c
L20-1	913±3f	524±11d	475±4c	676±5e	370±6b	248±3a
H20-1	221±7d	168±1c	268±7e	54±2a	270±4e	139±11b
LH20-1	430±9d	296±2a	323±9b	528±7e	419±7d	345±2c
L20-2	33±2a	27±3a	42±7a	948±2d	808±23c	268±24b
H20-2	33±1ab	21±2a	42±6b	186±4c	523±13d	174±8c
LH20-2	30±1a	25±3a	41±4a	308±108b	715±8c	232±21b

Table S2. Microbial biomass N concentration in the first (days 1, 5, 10) and the second wet-dry period (days 11, 15, 20) in treatments that received residues twice (LH10 and HL10) or once as L, H or LH at the rate of 20 g kg^{-1} soil on day 0 (L20-1; H20-1 or LH20-1) or upon rewetting on day 10 (L20-2; H20-2 or LH20-2) (n=4, means ± SE). Different letters indicate significant differences in a treatment over time ($P \leq 0.05$).

Microbial biomass N	Day 1	Day 5	Day 10	Day 11	Day 15	Day 20
LH10	27.3±0.8c	16.2±0.1b	8.9±1.2a	24.2±3.8c	29.4±3.4c	4.2±2.4a
HL10	11.1±0.4b	10.4±0.0b	1.5±0.3a	40.4±1.7e	33.1±2.7d	27.6±2.7c
L20-1	43.6±1.4b	28.9±0.2a	27.7±0.4a	64.4±2.9c	107.9±9.7d	64.7±1.4c
H20-1	17.1±1.1e	16.8±0.4e	4.4±0.4a	13.0±0.8d	9.9±1.3c	6.3±0.5b
LH20-1	37.5±1.2cd	33.4±0.1c	8.9±0.8a	40.3±1.3d	28.7±4.4b	7.9±0.1a
L20-2	4.5±0.4a	2.1±0.1a	2.7±0.3a	100.6±2.8d	22.8±2.5b	38.0±2.2c
H20-2	3.5±0.1b	2.1±0.2ab	1.3±0.1a	8.5±0.4c	18.7±2.2d	1.5±0.3a
LH20-2	3.5±0.1ab	2.0±0.0a	1.8±0.3a	68.3±3.1d	35.6±1.7c	6.2±0.4b

Table S3. Microbial biomass P concentration in the first (days 1, 5, 10) and the second wet-dry period (days 11, 15, 20) in treatments that received residues twice (LH10 and HL10) or once as L, H or LH at the rate of 20 g kg^{-1} soil on day 0 (L20-1; H20-1 or LH20-1) or upon rewetting on day 10 (L20-2; H20-2 or LH20-2) (n=4, means ± SE). Different letters indicate significant differences in a treatment over time ($P \leq 0.05$).

Microbial biomass P	Day 1	Day 5	Day 10	Day 11	Day 15	Day 20
LH10	19.4±1.1b	18.6±1.0b	15.1±0.7a	20.3±1.7b	20.8±2.8b	18.3±1.1ab
HL10	8.7±0.5a	10.6±0.6a	8.6±0.1a	25.7±1.5c	24.1±1.4c	15.0±2.5b
L20-1	44.7±0.7d	34.4±2.6c	35.8±2.0c	29.1±0.9b	19.1±0.8a	18.6±0.8a
H20-1	12.6±0.4c	14.5±0.6d	10.2±0.3ab	10.8±0.9abc	11.6±1.1bc	9.7±1.2a
LH20-1	23.3±1.1bc	31.9±2.2d	23.8±2.4bc	24.1±2.9c	19.4±1.8ab	16.2±0.8a
L20-2	3.5±0.3a	5.6±0.0a	3.6±0.8a	30.1±1.8d	24.3±0.3c	18.0±1.1b
H20-2	3.2±0.3a	5.4±0.0a	3.8±0.4a	9.8±0.9b	11.1±1.4b	10.6±1.8b
LH20-2	3.5±0.2a	5.4±0.1a	3.2±0.2a	21.3±1.0b	22.4±1.8b	20.2±0.2b

Table S4. Available N concentration in the first (days 1, 5, 10) and the second wet-dry period (days 11, 15, 20) in treatments that received residues twice (LH10 and HL10) or once as L, H or LH at the rate of 20 g kg^{-1} soil on day 0 (L20-1; H20-1 or LH20-1) or upon rewetting on day 10 (L20-2; H20-2 or LH20-2) (n=4, means ± SE). Different letters indicate significant differences in a treatment over time ($P \leq 0.05$).

Available N	Day 1	Day 5	Day 10	Day 11	Day 15	Day 20
LH10	79.7±1.9e	49.0±1.3c	72.0±0.1d	50.5±0.9c	23.2±0.1b	7.9±1.6a
HL10	8.1±0.5b	2.5±0.0a	2.9±0.1a	30.6±1.7c	36.8±0.4d	47.3±2.7e
L20-1	136.3±2.5c	80.0±2.4a	121.4±0.1b	159.6±2.8d	122.9±0.3b	135.8±3.3c
H20-1	6.8±1.3d	0.8±0.0ab	0.1±0.0a	4.0±1.1c	2.4±0.0bc	1.4±1.5ab
LH20-1	61.1±0.3f	11.4±0.1a	20.5±0.7c	31.8±2.1e	27.1±1.5d	15.5±2.3b
L20-2	9.2±1.7b	5.3±0.1a	4.4±0.1a	153.0±1.0d	84.9±0.9c	153.1±2.6d
H20-2	9.5±0.5d	5.2±0.0b	4.4±0.1b	7.3±0.5c	2.1±0.0a	4.8±1.0b
LH20-2	11.5±1.8b	5.2±0.0a	4.5±0.1a	56.2±1.1d	11.5±0.5b	20.1±2.8c

Table S5. Available P concentration in the first (days 1, 5, 10) and the second wet-dry period (days 11, 15, 20) in treatments that received residues twice (LH10 and HL10) or once as L, H or LH at the rate of 20 g kg^{-1} soil on day 0 (L20-1; H20-1 or LH20-1) or upon rewetting on day 10 (L20-2; H20-2 or LH20-2) (n=4, means ± SE). Different letters indicate significant differences in a treatment over time ($P \leq 0.05$).

Available P	Day 1	Day 5	Day 10	Day 11	Day 15	Day 20
LH10	27.4±1.0d	21.3±1.0c	30.2±0.6e	14.0±0.9a	13.8±0.6a	16.7±1.6b
HL10	9.6±0.5ab	6.3±0.6a	13.9±1.5bc	17.5±2.3c	18.5±3.2cd	22.9±2.6d
L20-1	36.3±0.2c	30.7±1.3a	43.4±0.5d	32.4±1.1ab	35.1±3.3bc	43.2±2.0d
H20-1	8.0±0.3bc	5.4±0.6a	14.9±1.0d	6.2±0.2a	6.6±1.0ab	8.5±1.1c
LH20-1	19.8±1.1c	14.4±0.7a	22.8±0.7d	14.8±0.6a	17.5±0.5b	20.6±1.6c
L20-2	11.4±1.1a	11.8±0.0a	16.9±0.8b	34.0±1.2d	29.9±2.7c	35.2±2.8d
H20-2	12.7±0.9c	11.9±0.1c	17.3±1.3d	7.0±0.8b	5.2±0.5a	8.5±0.5b
LH20-2	11.9±0.5a	11.9±0.1a	18.4±1.4bc	19.9±1.4c	15.8±1.4b	18.4±2.1bc

CHAPTER 4

Previous and current water content influence soil respiration, microbial biomass and nutrient availability after rewetting of dry soil

Yanchen Zhang and Petra Marschner

School of Agriculture, Food and Wine, The University of Adelaide, South Australia, 5005, Australia

Zhang Y, Marschner P (2018) Previous and current water content influence soil respiration, microbial biomass and nutrient availability after rewetting of dry soil (in preparation)

Statement of Authorship

Title of Paper	Previous and current water content influence soil respiration, microbial biomass and nutrient availability after rewetting of dry soil
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☑ Unpublished and Unsubmitted w ork w ritten in manuscript style
Publication Details	Zhang Y, Marschner P (2018) Previous and current water content influence soil respiration, microbial biomass and nutrient availability after rewetting of dry soil (in preparation)

Principal Author

Name of Principal Author (Candidate)	Yanchen Zhang
Contribution to the Paper	Performed the experiment, analyses of soils, data analysis and interpretation, and manuscript writing.
Overall percentage (%)	70%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 24/08/2018

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Petra Marschner
Contribution to the Paper	Supervised development of the work, data interpretation, manuscript evaluation and correction.
Signature	Date 24/08/2018

Previous and current water content influence soil respiration, microbial biomass and nutrient availability after rewetting of dry soil

Yanchen Zhang· Petra Marschner*

School of Agriculture, Food and Wine, The University of Adelaide, South Australia, 5005, Australia

*Corresponding author: Petra Marschner

Address: School of Agriculture, Food and Wine, The University of Adelaide, South Australia, 5000, Australia

Tel: +61 8313 7379

Fax: +61 83186511

E-mail address: petra.marschner@adelaide.edu.au

Abstract

In drying and rewetting experiments, soil is usually rewet to a water content optimal for microbial activity. But in the field, soil water content after rewetting of dry soils may vary. The aim of this study was to determine how current and previous water content after rewetting influences soil respiration, microbial biomass and nutrient availability with three rewetting events in unamended soils. Soil was exposed to two wet-dry cycles (5 days moist, 3 days dry) with rewetting soil to 50%, 30% or 10% WHC on days 0 and 8. A third rewetting was imposed with all treatments rewet to 50% WHC on day 16 and maintained at this water content for 7 days (day 23). In general, the effect of rewetting to 50% and to 30% WHC was similar. The flush of respiration after the first two rewetting events was more than two-fold higher with 50% than 10% WHC, and the second flush was about five times lower than the first. On day 9 (one day after the second rewetting), MBN was almost 10-fold higher in soil rewetted to 50% than to 10% WHC on both day 1 and day 9. Available N on day 9 was about two-thirds higher after rewetting to 50% than to 10% WHC. After rewetting of all treatments to 50% WHC on day 16, the flush was three-fold greater in soil previously rewet to 10% than soil rewet to 50% WHC previously. In soil previously rewet to 10% WHC compared to that rewet to 50% WHC, MBN and available P on days 17 and 23 were about two-fold higher whereas available N did not differ between treatments. The greater respiration and microbial biomass after the third rewetting event in soil previously rewet to 10% WHC compared to that rewet to 50% WHC can be explained by the greater amount of available substrate remaining after the first two rewetting events.

Keywords: Microbial biomass, nutrient availability, rewetting, soil respiration, water content

Introduction

Soil water content strongly influences microbial activity. In most soils, maximum aerobic microbial activity occurs at 50% to 70% of maximum water holding capacity (Linn and Doran 1984). Above this optimum microbial activity decreases due to low oxygen supply. At lower water content, microbial activity is reduced as a result of reduced soluble substrate diffusion, microbial mobility and low intracellular water potential (Stark and Firestone 1995).

Rewetting of dry soil can induce rapid, but short-lived flush of respiration and available nutrients (C, N and P) (Birch 1958). The respiration flush upon rewetting is thought to be due to increased substrate availability to the surviving microbes. Substrate after rewetting may come from the release of solutes previously accumulated by microbes in dry soil (Kim et al. 2012) and cell lysis (Bottner 1985; Wu and Brookes 2005). Further, aggregate breakdown upon rewetting can expose previously protected organic matter to microbes (Fierer and Schimel 2003; Van Gestel et al. 1993). Wu and Brookers (2005) showed that the respiration flush decreased after five drying and rewetting (DRW) cycles which could be explained by the lower substrate availability and microbial death. However, less is known about the effect of the amount of added water at rewetting.

In most previous DRW studies soil was rewet to a water content optimal for microbial activity (e.g., Schimel et al. 2007; Shi and Marschner 2014; Xiang et al. 2008; Zhang and Marschner 2016). However after a drought period in the field, soil may be rewet to water contents below optimal. Further, with intermittent rainfall, soil water content could vary among DRW events. The aim of this study was to determine (i) the effect of rewetting soil to low, medium or optimal water contents in two DRW cycles on soil respiration, microbial biomass and nutrient availability, and (ii) how previous rewetting water content influences microbial biomass and nutrient release after a third rewetting to optimal water content. The hypotheses

were that 1) in the first two cycles, soil respiration, microbial biomass and nutrient availability after rewetting will increase with rewetting water content, and 2) after the third rewetting to optimal water content, soil previously rewetted to higher soil water content will have lower soil respiration, microbial biomass and nutrient availability than soil rewetted to lower soil water content. This hypothesis is based on the assumption that at higher previous water content after rewetting, more available substrate was decomposed in the first two cycles, thus leaving less substrates for the third cycle compared to low previous water content.

Materials and methods

Soil

Soil was collected from 0 to 10 cm depth in Urrbrae, South Australia (Longitude 138°38′3.2″ E, Latitude 34°58′0.2″S). The area has a Mediterranean climate with hot, dry summers and cool, wet winters. The soil has been under permanent pasture for more than 80 years and is a silt loam with 22% sand, 60% silt and 18% clay. The soil is a Red-brown Earth according to Australian soil classification (Isbell 2002) and classified as Rhodoxeralf in US Soil Taxonomy. Some other main soil properties are as follows: water holding 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}.

Soil was collected along a randomly selected central transect 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 were taken at 0-10 cm depth and sieved to less than 2 mm followed by air-drying in a fan-forced oven at 40 °C. In southern Australia, top soils are often heated to 40 °C and even higher temperatures for a few hours a day on sunny summer days. Soils from all sampling points were mixed before starting the experiment.

Experimental design

Thirty grams of air-dry soil was filled into the PVC cores with 3.7 cm diameter and 5 cm height with a nylon mesh base (7.5 µm, Australian Filter Specialist), adjusted to a bulk density of 1.3 g cm^{-1}, and then placed into 1 L jars with gas-tight lids equipped with septa. At the start of the experiment, the soil was rewetted with reverse osmosis (RO) water to different soil water contents: 50%, 30% or 10% WHC (these water contents correspond to water potentials of -0.078, -0.32, -1.7MPa) by uniformly pipetting RO water onto the surface. The different levels of soil water content were maintained for 5 days. On day 6, all treatments were dried by placing bags containing silica gel in the jars for three days with daily replacement by bags with dry silica gel. A preliminary study showed that the soil (30 g) dries within 3 days with 24 g silica per jar (in three bags with 8 g per bag) with daily replacement by bags with dry silica (Zhang and Marschner 2016). The pouches that were removed were dried overnight in an oven. After three days of drying of soil, a water content of 2% WHC was reached. On day 8, the dried soil was exposed to a second rewet-dry cycle. Soil was rewetted to the same water content as at the start of the experiment (50%, 30% or 10% WHC), maintained at this water content for 5 days and then dried for 3 days with silica gel bags. After the two rewet-dry cycles, all treatments were rewetted to 50% WHC on day 16 (third rewetting) and maintained at 50% WHC for another 7 days (day 23). The jars were incubated in the dark at 22-23 °C. During the first and the second rewetting periods, soil water content (50%, 30% or 10% WHC) was maintained by checking the weight and adding RO water if necessary.

Soil respiration was measured daily. For determination of available N and P, microbial biomass N and P, soil cores were destructively sampled on days 1 and 9 (one day after first and second rewetting), day 17 (one day after the third rewetting to 50% WHC), and day 23 (7 days

after last rewetting). There were four replicates per treatment and sampling time (72 cores in total).

Measurements

Soil analyses were performed as described in Zhang and Marschner (2016). Briefly, soil texture was determined with rapid texture analysis (Chaudhari et al. 2008). Maximum water holding capacity of the soil was measured using a sintered glass funnel connected to a 100 mm water column (ψ_m = -10 kPa). 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 was measured according to Walkley and Black (1934) and total N was measured using the Kjeldahl method followed by colorimetric measurement as described in Bremmer and Mulvaney (1982).

Soil respiration was measured daily by quantifying the CO_2 concentration in the headspace of the 1L jars using a Servomex 1450 infra-red analyser (Servomex Group, Crowborough, UK) as described in Setia et al. (2011). Microbial biomass nitrogen (MBN) were determined by chloroform fumigation followed by extraction with 0.5 M K_2SO_4 at 1: 4 soil to extractant ratio (Vance et al. 1987). Microbial biomass N was calculated as the difference in NH_4^+ concentration between fumigated and non-fumigated samples divided by 0.57 which is the proportionality factor to convert ammonium to MBN suggested by Moore et al. (2000). Ammonium N determined as described below for available N.

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 Miranda et al. (2001). Available and microbial biomass P (MBP) were determined using anion exchange resin

following Kouno et al. (1995). For MBP, 1 ml hexanol was added. The P concentration in the extracts was determined colorimetrically according to Murphy and Riley (1962). Microbial biomass P is the difference in P concentration between fumigated and non-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).

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, one way repeated measures ANOVA was carried out in GenStat (GenStat for Windows, 15th edition, VSN Int. Ltd, UK, 2012). Tukey's multiple comparison test at 95% confidence interval was used for each sampling time separately to determine which treatments differ from each other.

Results

Respiration

From day 1 to day 6, respiration rate was higher in soil rewet to 50% and 30% WHC than that rewet to 10% WHC; it differed little between 50% and 30% WHC (Fig. 1a). Respiration rate declined in the first 8 days (5 days moist and 3 days drying) with a sharper decline at 50% and 30% WHC than at 10% WHC. The second rewetting to different water contents on day 8 induced a smaller flush in all treatments than the first rewetting. The maximum respiration rate on day 8 was about one fifth of that on day 1. As at the first rewetting, the flush was greater at 50 and 30% WHC than at 10% WHC. Then soil respiration rate declined and was not detectable

after day 14. On day 17 (one day after rewetting all treatments to 50% WHC), respiration rate in soil that rewet to 10% WHC previously was two to three-fold higher than soil that rewet to 30% or 50% WHC previously (Fig. 1b). Respiration rate then declined, but remained higher in soil that was rewetted to 10% WHC previously than the other two treatments until day 20. From day 21 to 23, respiration rate was similar and stable in all treatments.

Cumulative respiration from day 1 to day 8 and day 9 to day 16 was highest at 50% WHC and lowest at 10% WHC. Within each treatment, cumulative respiration was five to ten-fold higher after the first rewetting (day 1 to day 8) than after the second (9 to day 16) (Fig. 2a). Compared to soil rewet to 10% WHC, cumulative respiration in the first 16 days was three and four-fold higher in soil rewet to 30 and 50% WHC. Cumulative respiration from day 17 to day 23 was more than two-fold higher in soil that was rewetted to 10% WHC previously than in soil previously rewetted to 50% or 30% WHC (Fig. 2b).

Microbial biomass

On both days 1 and 9, MBN was highest in soil rewetted to 50% WHC, where it was about 20% higher than the soil rewetted to 30% WHC and nearly 10-fold higher than soil rewetted to 10% WHC (Fig. 3a). MBN on day 17 compared to day 9 was much lower in soil previously rewetted to 50 and 30% WHC, but it was about three-fold higher in soil rewet to 10% previously (Fig. 3b). MBN on day 17 and 23 was two-fold higher in soil previously rewetted to 10% than in soil rewetted to 30 and 50% WHC. MBP was about 4 mg/kg in all treatments and at all sampling times (data not shown).

Available N and P

On day 1, available N was slightly higher in soil rewetted to 50 % WHC than that rewetted to 10% WHC (Fig. 4a). Available N increased from day 1 to day 9, with the greatest increase in soil rewetted to 50% WHC. Compared to soil rewetted to 10% WHC, available N was about 60 and 10% higher in soil rewetted to 50% and 30%. Available N decreased from day 9 to day 17 in soil rewetted to 50% WHC in the first 16 days, but remained stable in the other two treatments (Fig. 4b). Available N did not differ among treatments on days 17 and 23.

Available P on days 1 and 9 after rewetting did not differ among treatments (Fig. 5a). Available P changed little from day 1 to day 9. But on days 17 and 23, after rewetting of all treatments to 50% WHC, available P was about two-fold higher in soil previously rewetted to 10% WHC than that rewetted to 30% or 50% WHC (Fig. 5b).

Discussion

This study showed that soil respiration and MBN after rewetting were influenced by both current and previous water content as well as the number of DRW events. In general, differences in measured parameters were small between 50 and 30% WHC. This indicates that microbes were not strongly limited by water availability at 30% WHC. In the following discussion, we will focus on differences between 10% and 50% WHC.

First two DRW events (day 0-16)

The first hypothesis (in the first two cycles, soil respiration, microbial biomass and nutrient availability after rewetting will increase with rewetting water content) can be confirmed with respect to respiration, MBN and available N for the comparison between 10% and 50% WHC.

In both rewetting events, the flush of respiration with 10% WHC was less than half of that at 50% WHC and respiration rates remained lower at 10% WHC during the moist periods. Further, MBN and available N one day after both rewetting events were lower at 10% WHC than at 50%. This indicates that at 10% WHC, soil water availability was limited microbial activity in general and N mineralisation in particular. At low water content, the water film around aggregates is thin and becomes disconnected, limiting substrate supply and reducing microbial activity (Manzoni et al. 2012; Schjønning et al. 2003; Skopp et al. 1990). A further reason for the smaller rewetting flush at 10% WHC could be that only the top of the soil in the cores was rewet. Most likely when rewetting soil to 10% WHC, the small amount of added water (37 g kg^{-1} compared to 186 g kg^{-1} to reach 50% WHC) remained in the top 1-2 cm, and therefore, soil at the bottom of the cores may have remained dry during the entire moist period. The soil near the top of the cores may have had a water content above 10% WHC. However, microbes in the moist layer represented only a small fraction of total microbes in the cores and their activity could not compensate for the low activity of the microbes in the drier soil.

Respiration rate declined during the five days in moist soil which can be explained by depletion of substrates released by soil disturbance during set-up of the cores (days 1-5) or by rewetting (days 9-12). The further decline after onset of drying (day 5-8) can be explained by reduced substrate diffusion in dry soil. The lower respiration flush after the second compared to the first rewetting is in agreement with previous DRW experiments (Fierer and Schimel 2002; Shi and Marschner 2014; Wu and Brookes 2005; Zhang and Marschner 2016). The lower flush with subsequent rewetting events has been explained by reduced substrate availability and microbial death (Harris 1981; Miller et al. 2005; Schimel et al. 2007; Wu and Brookes 2005).

Differences in available N between 10% and 50% WHC were more pronounced one day after the second rewetting (day 9) than the day after the first rewetting (day 1). The greater difference on day 9 is due to the strong increase in available N from day 1 to day 9 at 50%

WHC, whereas N availability changed little at 10% WHC. This is likely because in the soil maintained at 50% WHC from day 1 to day 5, more N could be mineralised than in that maintained at 10% WHC.

Third DRW event (day 17-23)

The third rewetting to 50% WHC in all treatments induced an about four-fold greater flush in soil previously rewetted to 10% WHC compared to that rewetted to 50% WHC and cumulative respiration from day 17 to 23 was about three-fold higher in the former. The greater flush in soil previously rewetted to 10% WHC was accompanied by higher MBN one and seven days after the third rewetting (day 17 and 23) compared to soil previously rewetted to 50% WHC. The greater flush and microbial biomass in soil previously rewetted to 10% WHC can be explained by greater substrate availability during the third DRW cycle compared to soil previously rewetted to 50% WHC. In soil rewetted to 10% compared to that rewetted to 50%, cumulative respiration in the first 16 days was 75% lower and MBN were about 60% lower on day 9. Thus, less organic C was decomposed during the first two cycles in soil rewetted to 10% and more remained available during the third cycle. As mentioned above, in soils rewetted to 10% WHC it is likely that only the soil close to the top of the cores was rewetted. And even there, water limited decomposition. For soil below this layer, the rewetting to 50% WHC on day 16 is likely to be the first rewetting event, resulting in high substrate availability from solutes released by cells, cell lysis and aggregate breakdown (Fierer and Schimel 2003; Halverson et al. 2000; Van Gestel et al. 1993).

Available N did not differ between treatments on days 17 and 23, but the higher MBN in soils previously rewetted to 10% indicates greater N mineralisation than in soils previously rewetted to 50% WHC. In contrast, treatments did not differ in MBP on days 17 and 23, but

available P was higher in soils previously rewetted to 10% WHC. This also indicates higher mineralisation compared to soils previously rewetted to 50% WHC. It is possible that MBP did not increase despite higher P availability because microbes were not P limited.

The finding that soil microbes are influenced by the current and previous water content is in agreement with Banerjee et al. (2016) who incubated soil at either static or changing water content (40-100% water-filled pore space). They found that bacterial community composition, expression of genes involved in N transformations and N_2O emissions differed among treatments not only while they were at different water content, but also after reaching the same water content.

Conclusion

This study showed that rewetting of dry soil to low water content induces only a small flush of respiration and thus little decomposition of organic matter. But if soil is then rewet to water content optimal for microbial activity, the respiration flush and microbial biomass are greater than in soil that had been rewet to optimal water content previously. This indicates that the previous water content should be considered when evaluating the impact of DRW on soil respiration, microbial biomass and nutrient availability.

In this study, dry and moist periods were relatively short and drying was rapid. To better understand the impact of DRW in the field, future experiments could use longer periods and more gradual drying.

Acknowledgement

Yanchen Zhang received the scholarship from The University of Adelaide.

References

Banerjee S, Helgason B, Wang L, Winsley T, Ferrari BC, Siciliano SD (2016) Legacy effects of soil moisture on microbial community structure and N_2O emissions. Soil Biol Biochem 95:40-50

Birch H (1958) The effect of soil drying on humus decomposition and nitrogen availability. Plant Soil 10:9-31

Bottner P (1985) Response of microbial biomass to alternate moist and dry conditions in a soil incubated with 14 C-and 15 N-labelled plant material. Soil Biol Biochem 17:329-337

Bremmer J, Mulvaney C (1982) Total N. Methods of Soil Analysis Part2. 2nd ed Agron Monog. 9:895-926

Butterly CR, Marschner P, McNeill AM, Baldock JA (2010) Rewetting CO_2 pulses in Australian agricultural soils and the influence of soil properties. Biol Fertil Soils 46:739-753

Chaudhari S, Singh R, Kundu D (2008) Rapid textural analysis for saline and alkaline soils with different physical and chemical properties. Soil Sci Soc Am J 72:431-441

Fierer N, Schimel JP (2002) Effects of drying–rewetting frequency on soil carbon and nitrogen transformations. Soil Biol Biochem 34:777-787

Fierer N, Schimel JP (2003) A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. Soil Sci Soc Am J 67:798-805

Halverson LJ, Jones TM, Firestone MK (2000) Release of intracellular solutes by four soil bacteria exposed to dilution stress. Soil Sci Soc Am J 64:1630-1637

Harris R (1981) Effect of water potential on microbial growth and activity. In: Parr J, Gardner W, Elliott L. (ed) Water potential relations in soil microbiology, Soil Sci Soc Am, Madison, pp 23-95

Isbell R (2002) The Australian soil classification. Vol 4. CSIRO publishing

Kim DG, Vargas R, Bond-Lamberty B, Turetsky M (2012) Effects of soil rewetting and thawing on soil gas fluxes: a review of current literature and suggestions for future research. Biogeosciences 9:2459

Kouno K, Tuchiya Y, Ando T (1995) Measurement of soil microbial biomass phosphorus by an anion exchange membrane method. Soil Biol Biochem 27:1353-1357

Linn D, Doran J (1984) Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. Soil Sci Soc Am J 48:1267-1272

Manzoni S, Taylor P, Richter A, Porporato A, Ågren GI (2012) Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytol 196:79-91

Miller AE, Schimel JP, Meixner T, Sickman JO, Melack JM (2005) Episodic rewetting enhances carbon and nitrogen release from chaparral soils. Soil Biol Biochem 37:2195-2204

Miranda KM, Espey MG, Wink DA (2001) A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric oxide 5:62-71

Moore J, Klose S, Tabatabai M (2000) Soil microbial biomass carbon and nitrogen as affected by cropping systems. Bio Fertil Soils 31:200-210

Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta 27:31-36

Schimel JP, Balser TC, Wallenstein M (2007) Microbial stress-response physiology and its implications for ecosystem function. Ecology 88:1386-1394

Schjønning P, Thomsen IK, Moldrup P, Christensen BT (2003) Linking soil microbial activity to water- and air-phase contents and diffusivities. Soil Sci Soc Am J 67:156-165

Setia R, Marschner P, Baldock J, Chittleborough D, Smith P, Smith J (2011) Salinity effects on carbon mineralization in soils of varying texture. Soil Biol Biochem 43:1908-1916

Shi A, Marschner P (2014) Drying and rewetting frequency influences cumulative respiration and its distribution over time in two soils with contrasting management. Soil Biol Biochem 72:172-179

Skopp J, Jawson M, Doran J (1990) Steady-state aerobic microbial activity as a function of soil water content. Soil Sci Soc Am J 54:1619-1625

Stark JM, Firestone MK (1995) Mechanisms for soil moisture effects on activity of nitrifying bacteria. Appl Environ Microbiol 61:218-221

Van Gestel M, Merckx R, Vlassak K (1993) Microbial biomass responses to soil drying and rewetting: the fate of fast- and slow-growing microorganisms in soils from different climates. Soil Biol Biochem 25:109-123

Vance E, Brookes P, Jenkinson D (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703-707

Walkley A, Black IA (1934) An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci 37:29-38

Willis RB, Montgomery ME, Allen PR (1996) Improved method for manual, colorimetric determination of total Kjeldahl nitrogen using salicylate. J Agric Food Chem 44:1804-1807

Wu J, Brookes P (2005) The proportional mineralisation of microbial biomass and organic matter caused by air-drying and rewetting of a grassland soil. Soil Biol Biochem 37:507-515

Xiang SR, Doyle A, Holden PA, Schimel JP (2008) Drying and rewetting effects on C and N mineralization and microbial activity in surface and subsurface California grassland soils. Soil Biol Biochem 40:2281-2289

Zhang Y, Marschner P (2016) Nutrient availability, soil respiration and microbial biomass after the second residue addition are influenced by the C/N ratio of the first residue added, but not by drying and rewetting between residue amendments. Eur J Soil Biol 77:68-76

Figures

Figure 1. Respiration rate (a) from day 1 to 16 in soil rewet twice (day 1 and 8) to 50%, 30% or 10% WHC, maintained moist for 5 days then dried for three days (day 5-8 and 13-16), and (b) from day 17 to day 23 after rewetting of all treatments to 50% WHC on day 16 (n=4, means ± SE).

Figure 2. Cumulative respiration (a) from day 1 to day 8 and from day 9 to day 16 in soil rewet twice (day 1 and 8) to 50%, 30% or 10% WHC, maintained moist for 5 days then dried for three days (day 5-8 and 13-16); and (b) from day 17 to day 23 after rewetting of all treatments to 50% WHC on day 17 (b) (n=4, means ± SE). For each interval, bars with different letters are significantly different, upper case letters in (a) indicate differences in total cumulative respiration from day 1 to 16 ($P \leq 0.05$).

Figure 3. Microbial biomass N concentration a) one day after rewetting (day 1 and 9) in soil rewet twice (day 1 and 8) to 50%, 30% or 10% WHC, maintained moist for 5 days then dried for three days (day 5-8 and 13-16); and (b) on days 17 and 23, one and seven days after rewetting of all treatments to 50% WHC on day 16 (n=4, means ± SE). At a given sampling time, bars with different letters are significantly different ($P \leq 0.05$).

Figure 4. Available N concentration a) one day after rewetting (day 1 and 9) in soil rewet twice (day 1 and 8) to 50%, 30% or 10% WHC, maintained moist for 5 days then dried for three days (day 5-8 and 13-16); and (b) on days 17 and 23, one and seven days after rewetting of all treatments to 50% WHC on day 16 (n=4, means ± SE). At a given sampling time, bars with different letters are significantly different ($P \leq 0.05$).

Figure 5. Available P concentration a) one day after rewetting (day 1 and 9) in soil rewet twice (day 1 and 8) to 50%, 30% or 10% WHC, maintained moist for 5 days then dried for three days (day 5-8 and 13-16); and (b) on days 17 and 23, one and seven days after rewetting of all treatments to 50% WHC on day 16 (n=4, means ± SE). At a given sampling time, bars with different letters are significantly different ($P \leq 0.05$).

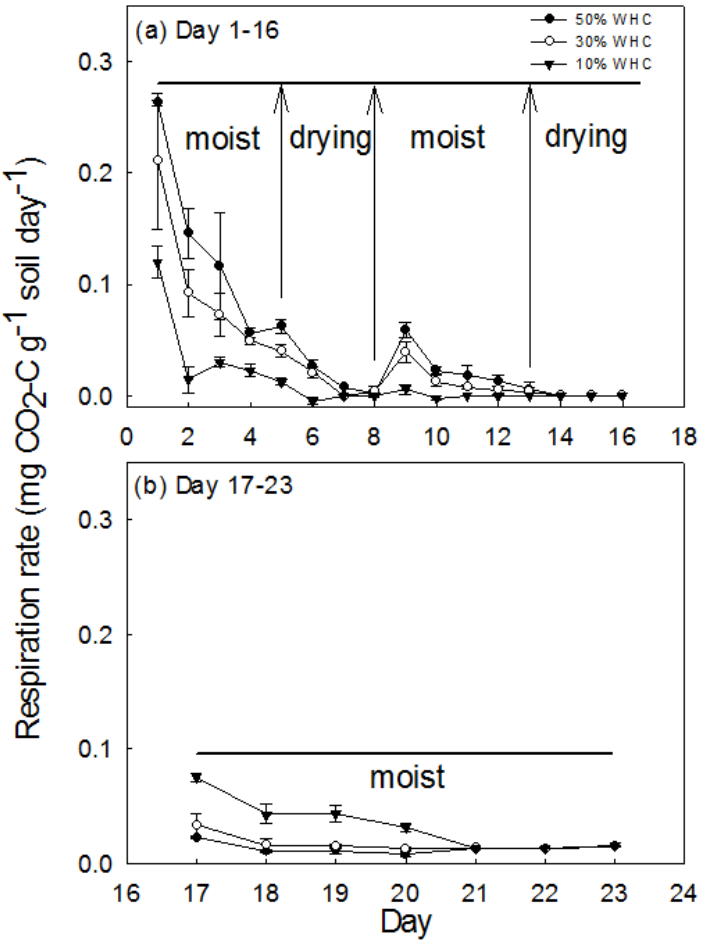
(a) Day 1-16
50% WHC
30% WHC
10% WHC
moist
drying
moist
drying
(b) Day 17-23
moist
Respiration rate (mg CO2-C g-1 soil day-1)
Day

Figure 1

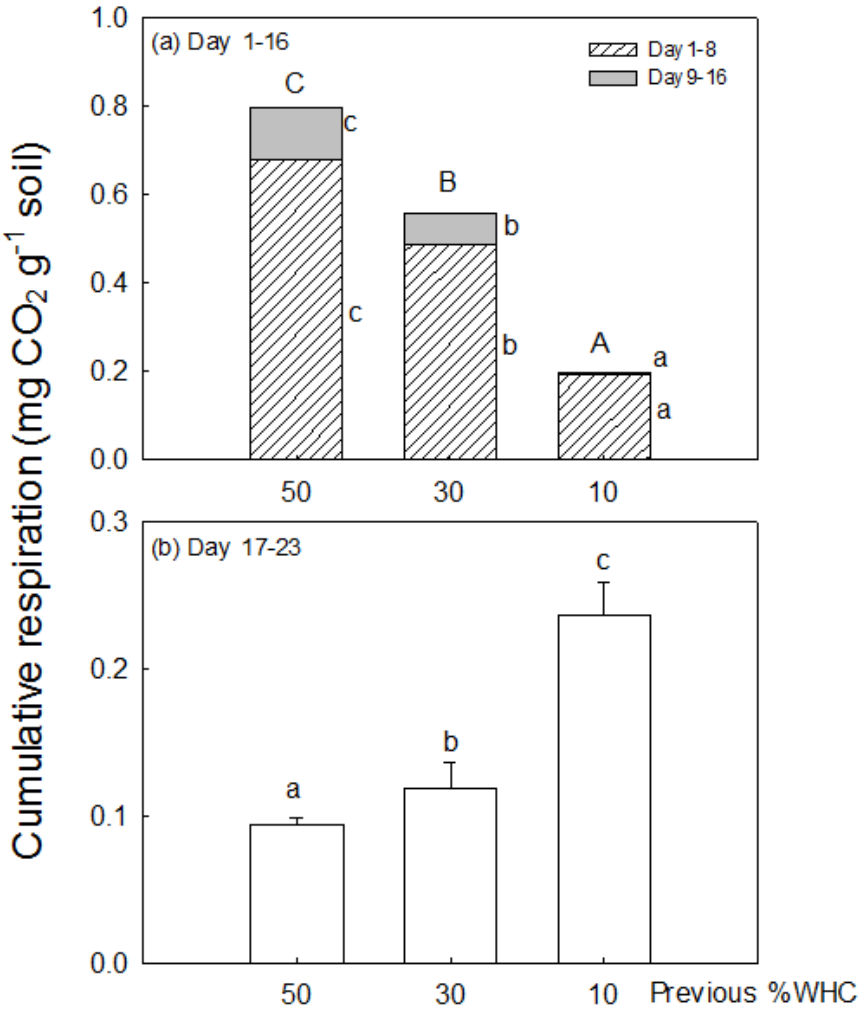
(a) Day 1-16
Day 1-8
Day 9-16
C
B
A
c
c
b
b
a
a
1.0
0.8
0.6
0.4
0.2
0.0
50
30
10
(b) Day 17-23
a
b
c
0.3
0.2
0.1
0.0
50
30
10
Previous %WHC
Cumulative respiration (mg CO2 g-1 soil)

Figure 2

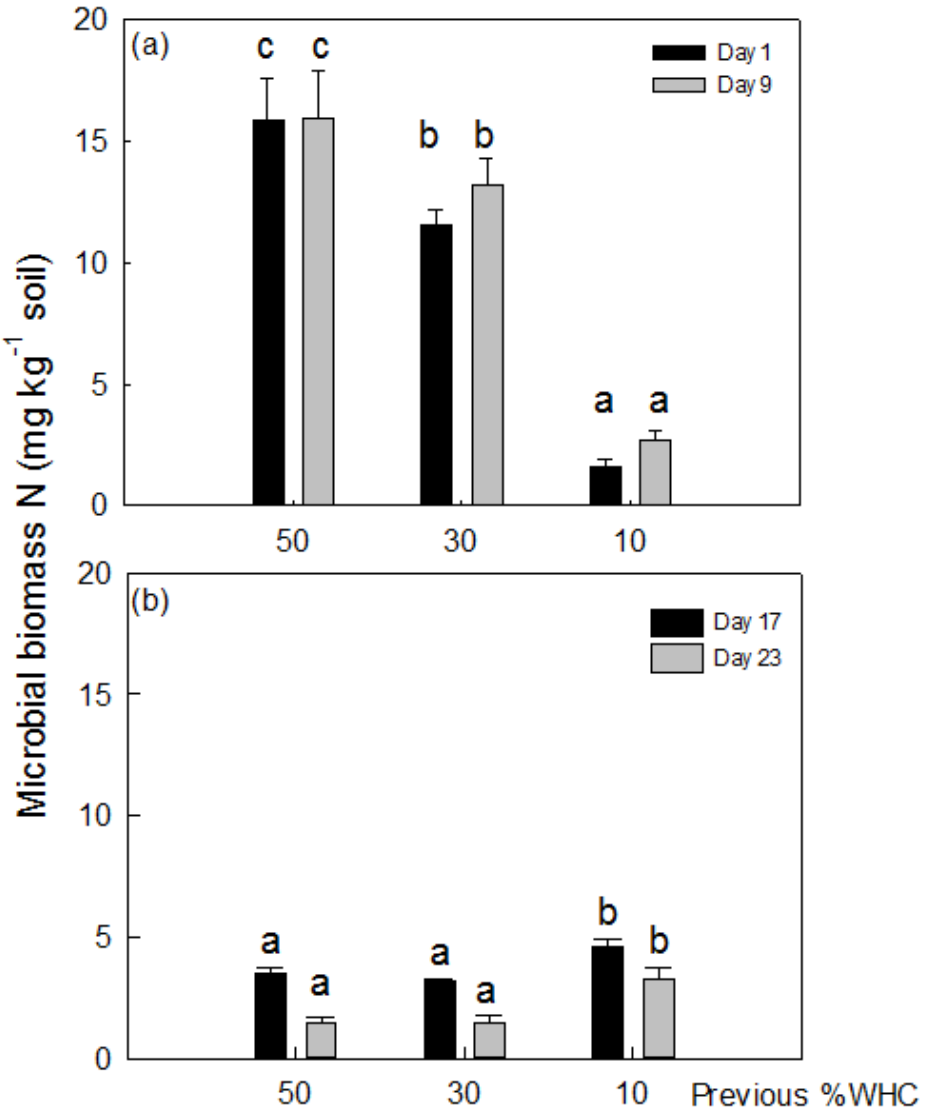
(a)
Day 1
Day 9
c c
b b
a a
20
15
10
5
0
50
30
10
(b)
Day 17
Day 23
a a
a a
b b
Microbial biomass N (mg kg-1 soil)
Previous %WHC

Figure 3

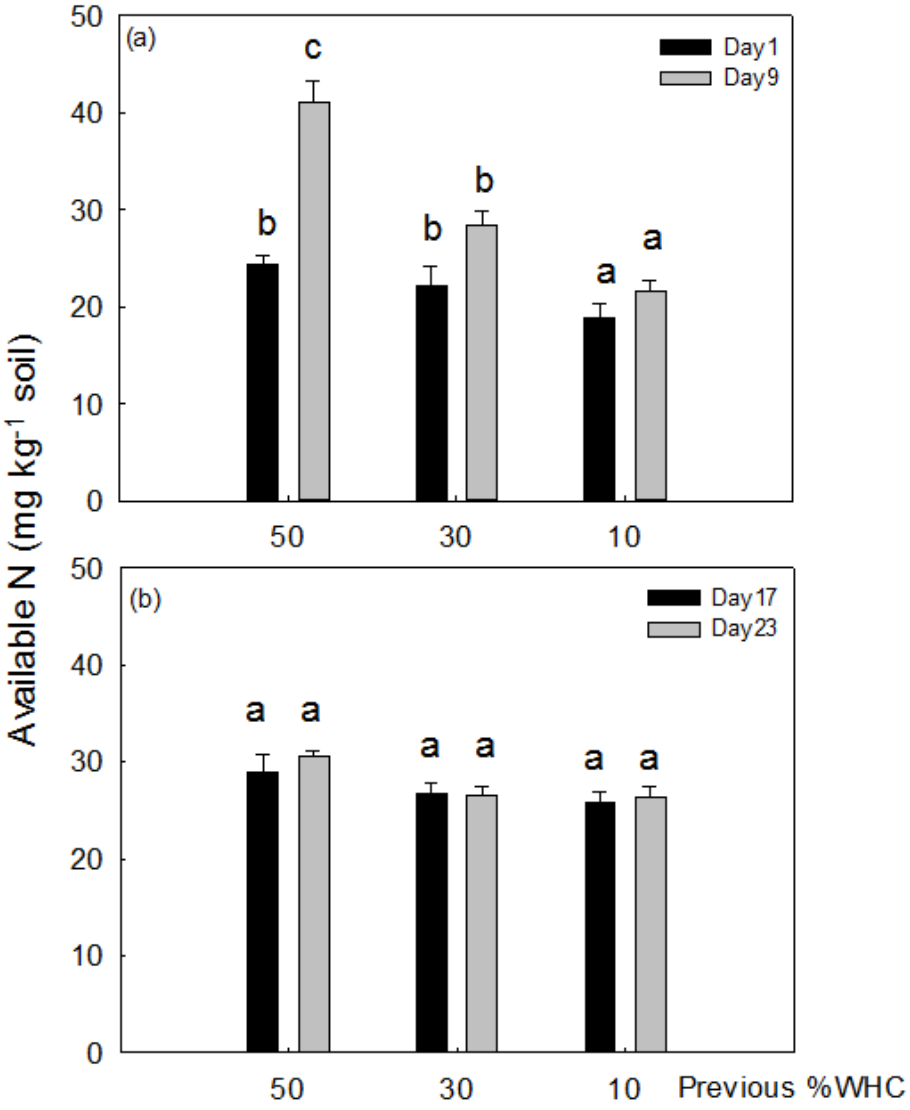
(a)
Day1
Day9
c
b
b
b
a
a
50
40
30
20
10
0
(b)
Day17
Day23
a
a
a
a
a
a
50
30
10
Previous %WHC
Available N (mg kg-1 soil)

Figure 4

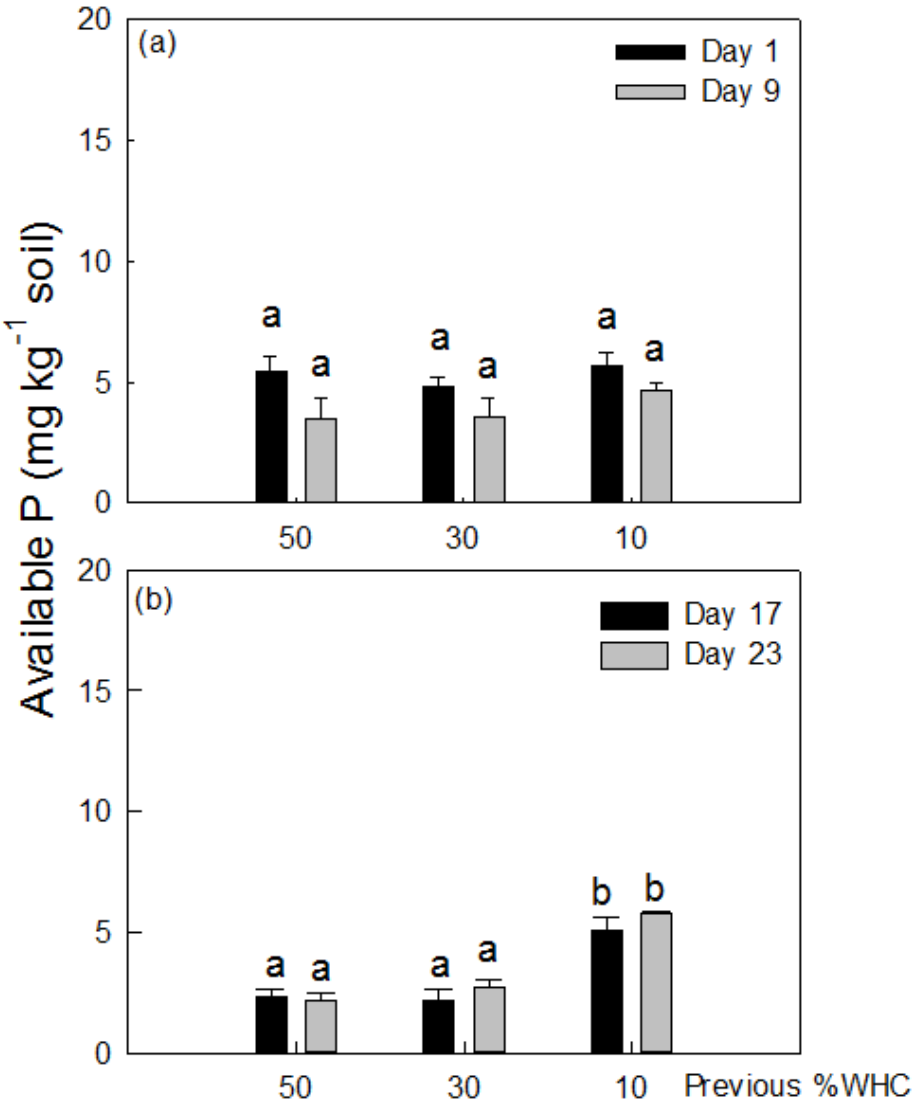
(a)
Day 1
Day 9
Available P (mg kg-1 soil)
20
15
10
5
0
a
a
a
a
a
a
50
30
10
(b)
Day 17
Day 23
a
a
a
a
b
b
Previous %WHC

Figure 5

CHAPTER 5

Respiration, microbial biomass and nutrient availability are influenced by previous and current soil water content in plant residue amended soil

Statement of Authorship

Title of Paper	Respiration, microbial biomass and nutrient availability are influenced by previous and current soil water content in plant residue amended soil
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Zhang Y, Marschner P (2018) Respiration, microbial biomass and nutrient availability are influenced by previous and current soil water content in plant residue amended soil. Journal of Soil Science and Plant Nutrition, 2018, 18(1), 173-187.

Principal Author

Name of Principal Author (Candidate)	Yanchen Zhang		
Contribution to the Paper	Performed the experiment, analyses of soils, data analysis and interpretation, and manuscript writing.		
Overall percentage (%)	70%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	26/08/2018

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Petra Marschner		
Contribution to the Paper	Supervised development of the work, data interpretation, manuscript evaluation and correction and acted as the corresponding author.		
Signature		Date	24/08/2018

Journal of Soil Science and Plant Nutrition, 2018, 18 (1), 173-187
RESEARCH ARTICLE

Respiration, microbial biomass and nutrient availability are influenced by previous and current soil water content in plant residue amended soil

Yanchen Zhang[1] , Petra Marschner[1*]

[1]*School of Agriculture, Food and Wine. The University of Adelaide, South Australia, 5005, Adelaide, Australia.*
**Corresponding author: petra.marschner@adelaide.edu.au*

Abstract:

The aim of this study was to determine how soil respiration, microbial biomass and nutrient availability after the first and second plant residue addition are influenced by soil water content and number of days between rewetting of dry soil and second residue addition. A loamy soil was amended with low C/N ratio (L) or high C/N ratio (H) plant residues and then maintained at 10% or 50% water holding capacity (WHC) for 10 days after which the soil at 10% WHC was rapidly rewetted to 50% WHC. A second residue with a different C/N ratio than the first was added one, two or five days after rewetting. Lower water content (10% WHC) during the first 10 days reduced respiration and microbial biomass. After day 10, MBN increased with number of days after rewetting. After the second residue addition, respiration rate in the first three to four days and available N after two days were higher when residue was added five days after rewetting compared to one day. But MBN was higher in treatments with residues added one day after rewetting compared to amendment after five days. The water content in the first 10 days had little effect on microbial biomass and nutrient availability after the second residue addition. We conclude that soil respiration, N mineralisation and immobilisation are influenced by the interval between plant residue additions, particularly in rewet soil.

Keywords: Microbial biomass, nutrient availability, plant residue addition, soil respiration, soil water content

1. Introduction

Global warming is predicted to result in increasing drought frequency and intensity, which will influence soil water content (IPCC, 2007). A key aspect of sustainable food production (Scotti *et al.*, 2015) is supply of nutrients to crops through decomposition of plant residues by soil microorganism (Power, 2010). Low soil water content limits plant residue decomposition as it restricts water availability and nutrient diffusion, and at very low water content, may draw water

out of the cells (Schimel *et al.*, 2007). Rapid rewetting of dry soil has been shown to result in a flush of respiration and available nutrients (Birch, 1958). The flush of available nutrients after rewetting of dry soil is due to a sudden increase in organic substrates which are then mineralised by the microbial biomass (Borken and Matzner, 2009).

Decomposition of organic residues is also influenced by residue properties such as C/N ratio (Heal *et al.*, 1997). Many studies have shown that residues with low C/N ratio are decomposed rapidly and lead to net N mineralization as they satisfy the N demands of microbes (Hadas *et al.*, 2004). However, in agricultural and natural ecosystems, plant residues with different C/N ratio may be added to soil over time. Studies in our group showed that nutrient availability and microbial biomass after the second residue addition are influenced by the properties of the previously added residue, which is referred as legacy effect (Marschner *et al.*, 2015; Nguyen *et al.*, 2016). For example, N availability after addition of low C/N residue is lower if it follows high C/N residue than if added to previously unamended soil. This legacy effect occurs because in the former case, soil microbes decompose not only the freshly added low C/N reside, but also the high C/N residue left in the soil from the previous amendment. Recently, Zhang and 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. On the other hand, respiration, microbial biomass and nutrient availability in plant residue amended soil were much lower at 10% water holding capacity than at 50% WHC (Zhang and Marschner, 2017). In Nguyen *et al.* (2016), the legacy effect decreased with time between first and second residue addition (10, 20 or 30 days) and in Zheng and Marschner (2017), the legacy effect decreased with amendment rate of the first residue. These results suggest that extent of the legacy effect is influenced by the amount of the first residue left in the soil when the second residue is added.

In the field, soil water content may vary between residue additions and residues can be added at different times after rewetting. The aims of this study were to determine how soil respiration, microbial biomass and nutrient availability after the first and second residue addition are influenced by (i) soil water content between first and second amendment (10 and 50% WHC), and (ii) number of days between rewetting of dry soil and second residue addition (1, 2, 5 days). We hypothesised that the legacy effect of the first residue added will be greater (i) when the soil water content between first and second residue addition is low, and (ii) if the second residue is added one day after rewetting compared to after two or five days.

2. Materials and Methods

2.1. Soil and plant residues

A loamy soil was collected in early 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 permanent pasture for more than 80 years. This site is in a semi-arid area and has a Mediterranean climate 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

transect 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. In southern Australia, top soils are often heated to 40 °C and higher temperatures on sunny summer days. Soil from all sampling points was 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 organic 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. Compared to H, L had five to ten times higher total N, total P, available N and P and two-fold higher water extractable C concentration, but lower C/N and C/P ratios (Table 1).

Table 1. Total organic C, N, P, C/N ratio and C/P ratio, available N and P, water-extractable C, and pH of low C/N (young faba bean) and high C/N (wheat straw) residues (n=4). Different lower case letters indicate significant differences among low and high C/N ratio residues ($P \leq 0.05$).

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

2.2. Experimental design

Before the start of the experiment, the air-dried soil was moistened to 50% of maximum WHC and incubated for 10 days at 22-23 °C in the dark at to activate the soil microbes and 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% WHC or dried in a fan-forced oven at 40 °C to 10 % WHC. The low water content (10% WHC) was selected because in Xue *et al*, 2016 with the same soil, respiration and microbial biomass were much lower than with 50% WHC, but still detectable. Half of the soil at each water content was amended with H at 10 g kg^{-1}, the other half with L. After 10 days, the soil at 10% WHC was rewet to 50% WHC, whereas the water content of the soil at 50% WHC was kept as

it was in the first 10 days. All soils were maintained at 50% until the end of the experiment. The second residue, which had a different C/N ratio than the first [L after H (HL) or H after L (LH)], was added 1, 2 and 5 days after rewetting. We refer to days after rewetting for simplicity although soil was not rewet in treatments that was kept at 50% WHC in the first 10 days. Treatment names refer to order in which residues were added and the water content in the first 10 days, i.e. LH-10 (low followed by high C/N residue at 10% WHC), LH-50 (low followed by high C/N residue at 50% WHC), HL-10 (high followed by low C/N residue at 10% WHC) and HL-50 (high followed by low C/N residue at 50% WHC). For each residue treatment, the second residue was added 1, 2, or 5 days after rewetting on day 10.

The residues were thoroughly mixed with the soil, then 30 g dry soil equivalent was filled into the PVC cores with 3.7 cm diameter and 5 cm height with 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 22–23 °C. Soil moisture (10% or 50% of WHC at the first 10 days and 50% of WHC after rewet) was maintained by checking the water content every few days by weighing and adding reverse osmosis (RO) water if necessary. Soil respiration was measured daily. Soil cores were destructively sampled two and 10 days after the first residue addition, one day before the second residue addition, and two and 10 days after the second residue addition. Soil samples were analysed for available N and P, microbial biomass N and P. For each sampling time and treatment there were four replicates, giving a total number of 176 cores.

2.3. Measurements

Soil analyses were carried out as described in Zhang and Marschner (2016). Briefly, soil texture was determined according to the rapid texture analysis (Chaudhari *et al.*, 2008; Kettler *et al.*, 2001). Maximum soil water holding capacity was measured using a sintered glass funnel connected to a 100 mm water column (ψ_m = -10 kPa). 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 and Black (1934) and total nitrogen was measured using the Kjeldahl method followed by colorimetric measurement as described in Bremmer and 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 phosphovanado-molydate method. Water extractable organic carbon was extracted 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). 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 Miranda *et al.*, (2001). Available P was extracted by the anion exchange resin method

(Kouno *et al.*, 1995), the P concentration was determined colorimetrically (Murphy and Riley, 1962).

Microbial biomass nitrogen (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). Microbial biomass N was calculated as the difference in NH_4^+ concentration between fumigated and non-fumigated samples divided by 0.57, which is the proportionality factor to convert ammonium to MBN 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. One-way ANOVA was used to compare the properties of two plant residues. For measurements carried out repeatedly during the experiment, repeated measures analysis of variance (ANOVA) was carried out in GenStat (GenStat for Windows, 15th edition, VSN Int. Ltd, UK, 2012). Two-way ANOVA with factors residue x moisture treatment was used for data in the first 10 days, two-way ANOVA with factors residue and moisture treatment x day of residue addition for data after the second residue addition. The interaction of residue treatment and time of the second residue addition was significant ($p<0.05$). Therefore, Tukey's multiple comparison test at 95% confidence interval was used for each sampling time separately to determine which treatments differ significantly from each other. In the results, only significant differences are reported.

3. Results

3.1. Respiration

In the first three days after the first residue addition, respiration rate was highest in LH-50 (low C/N young faba bean followed by high C/N wheat straw at 50% WHC) and lowest in HL-10 (high C/N wheat straw followed by low C/N young faba bean at 10% WHC) (Figure 1a). Within each residue treatment, it was lower with 10% WHC than 50% WHC, with greater differences in LH than HL. In LH, respiration rate declined in the first 6 days whereas in HL50, respiration rate increased from d2 to d5 and then decreased. Rewetting of the 10% WHC treatments on d10 induced a ten-fold increase in respiration rate on d11, after which respiration rate decreased until it was similar to that in 50% WHC treatments on d15.

After the second residue addition, respiration rate was influenced by timing of amendment and in LH, water content in the first 10 days (Figure 1b, c, d, e). In LH, respiration rate between the second and fourth day after amendment was highest when residue was added five days after rewetting (Figure 1b, c). When residue was added one day after rewetting, respiration rate decreased from d1 to d2 after which it remained stable until d6 and then decreased. In the treatment where the second residue was added two days after rewetting, respiration rate increased until d5 and then decreased.

In HL, respiration rate was highest on d1 when the second residue was added five days after rewetting (Figure 1d, e). Respiration rate declined from d2 and was little affected by when the residue was added from d4 onwards. Respiration rate was not influenced by water content in the first 10 days.

Cumulative respiration in the first 10 days was higher at 50% WHC than at 10% (Figure 2a). Only at 50% WHC it was higher in LH than HL. Cumulative respiration between rewetting and the second residue addition increased with number of days (Figure 2b, c, d). In the 10 days after the second residue addition, cumulative respiration was higher when residue was added five days was rewetting than if added after one or two days. Cumulative respiration after rewetting and residue addition was greater in soil kept at 10% WHC in the first ten days than with 50%. It was higher in HL than LH, with greater differences at 50% than 10% WHC.

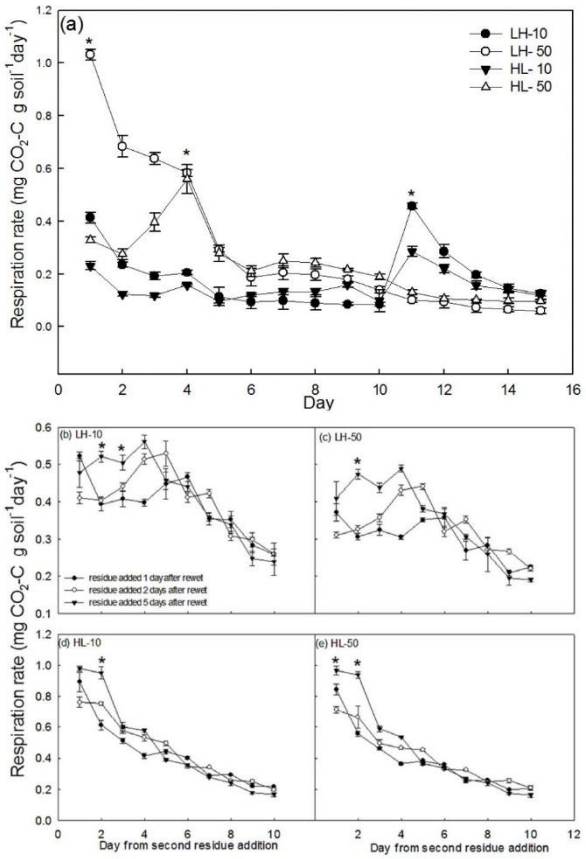

Figure 1. Respiration rate (n=4, means ± SE) from day 1 to 15 in treatments LH and HL at 10% or 50% WHC in the first 10 days (LH-10, LH-50, HL-10 and HL-50) (a); respiration rate in the 10 days after the second residue addition with residue added 1, 2 or 5 days after rewet in LH-10 (b), LH-50 (c), HL-10 (d) and HL-50 (e). (n=4, means ± SE). Asterisks were added where there was significant differences among treatments ($P \leq 0.05$).

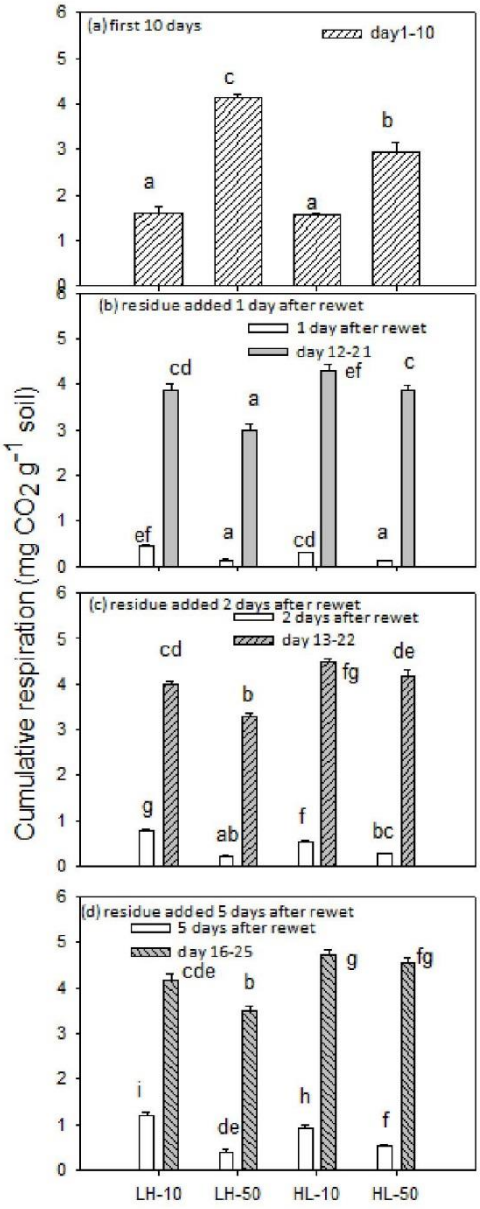

Figure 2. Cumulative respiration from day 1 to 10 in treatments LH and HL at 10% or 50% WHC (LH-10, LH-50, HL-10 and HL-50 (n=4, means ± SE) (a). Cumulative respiration after rewet before and after residue addition with residue added 1 (b), 2 (c) or 5 days after rewet (d).

3.2. Microbial biomass

Two days after the first residue addition, MBN was two-fold higher at 50% WHC than at 10%, but did not differ between residue treatments at a given water content (Figure 3a). From d2 to d10, MBN increased in LH, but decreased in HL (Figure 3b). On d10, MBN was up to ten-fold higher in LH than HL and it was higher at 50% WHC than at 10%. After rewetting and before the second residue addition, MBN in LH was lower after one day than after five days (Figure 3c). Time after rewetting had no consistent effect on MBN in HL. In general, MBN was higher in LH than HL, but was not influenced by the water content in the first ten days. Two days after the second amendment, MBN was highest when the second residue was added one day after rewetting (Figure 3d). In both residue treatments, MBN was lower with 50% WHC in the first ten days than with 10%. Ten days after the second amendment, MBN in most treatments except in HL-50, was two to five-fold higher if the second residue was added one day after rewetting than if it was added after five days (Figure 3e). There were no consistent differences in MBN between HL and LH.

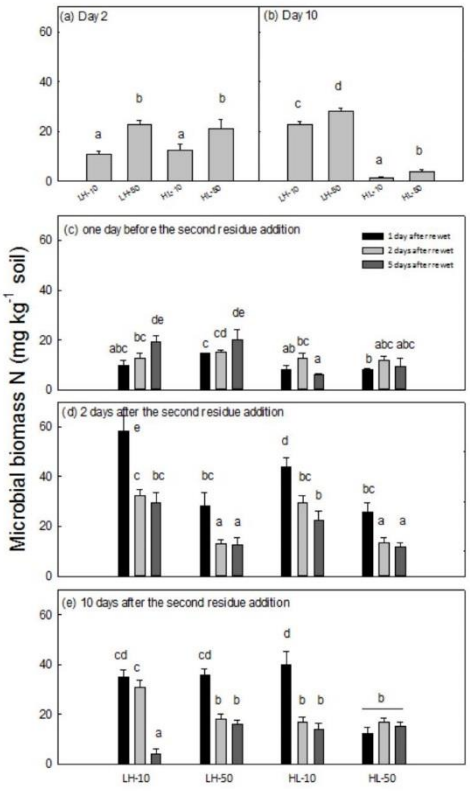

Figure 3. Microbial biomass N in treatments LH-10, LH-50, HL-10 and HL-50 on days 2 (a) and 10 (b), first day after rewet and before second residue addition (c), and 2 days (d) and 10 days after second residue addition (e) (n=4, means ± SE). Bars with different letters indicate significant differences among treatments at each sampling time ($P \leq 0.05$).

On d2 after the first residue addition, MBP was about ten-fold higher in LH than HL at both water contents (Figure 4a). From d2 to d10, MBP decreased in LH, but increased in HL. On d10, MBP was higher in LH-50 than in the other treatments (Figure 4b). After rewetting and before the second residue addition, MBP decreased with number of days after rewetting in LH-10, but did not change over time in the other treatments (Figure 4c). Residue treatment and water content in the first ten days had little effect on MBP before and after the second residue addition. The number of days between rewetting and second amendment did not influence MBP after the second residue addition.

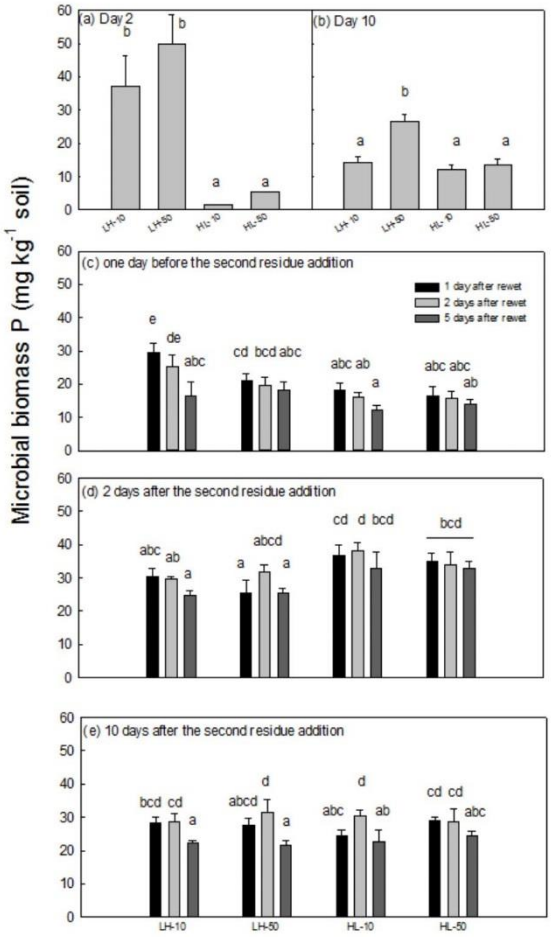

Figure 4. Microbial biomass P concentration in treatments LH-10, LH-50, HL-10 and HL-50 on days 2 (a), 10 (b), first day after rewet and before second residue addition (c), and 2 days (d) and 10 days after second residue addition (e) (n=4, means ± SE). At a given sampling time, bars with different letters are significantly different ($P \leq 0.05$).

3.3. Available N and P

Available N on d2 and d10 after the first residue addition was about two-fold higher in LH than HL (Figure 5 a, b). On d2, available N was slightly, but significantly higher at 10% WHC than at 50%. On d10, available N in LH was higher at 50% WHC than at 10%, but not in HL. After rewetting and before the second amendment, available N was three to four-fold higher in LH than HL. In LH, available N was lower one day after rewetting than five days after rewetting. After five days it was higher in soil that had been at 10% WHC in the first 10 days than the soil at 50% WHC (Figure 5c). In HL, available N was not influenced by the previous water content or time after rewetting. Two days after the second amendment, available N was about 40% higher in HL than LH (Figure 5d). In all treatments, available N was highest when the second residue had been added 5 days after rewetting. Available N was higher in HL-50 than HL-10, but water content in the first 10 days did not influence available N in LH. Ten days after the second residue addition, available N was higher in HL than LH, but there was no consistent effect of water content in the first ten days or number of days between rewetting and second residue addition (Figure 5e).

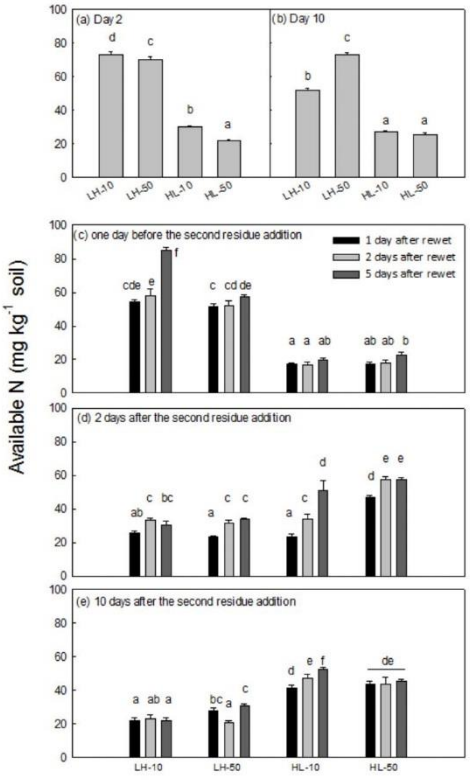

Figure 5. Available N concentration in treatments LH-10, LH-50, HL-10 and HL-50 on days 2 (a) and 10 (b), first day after rewet and before second residue addition (c), and 2 days (d) and 10 days after second residue addition (e) (n=4, means ± SE). At a given sampling time, bars with different letters are significantly different ($P \leq 0.05$).

Two and ten days after the first residue addition, available P was about two-fold higher in LH than HL (Figure 6a, b). Water content had little effect on available P except on d10 in LH where it was higher at 10% WHC than at 50%. After rewetting and before the second amendment, available P was more than two-fold higher in LH than HL (Figure 6c). Neither water content in the first ten days nor time after rewetting influenced available P. Two days after the second amendment, available P was highest in HL-50 (Figure 6d). The water content in the first ten days influenced available P only in HL where it was higher in HL-50 than HL-10. Ten days after the second residue addition, available P was about 20% higher in HL than LH (Figure 6e). Two and ten days after the second residue addition, available P was not influenced by the number of days between rewetting and residue addition.

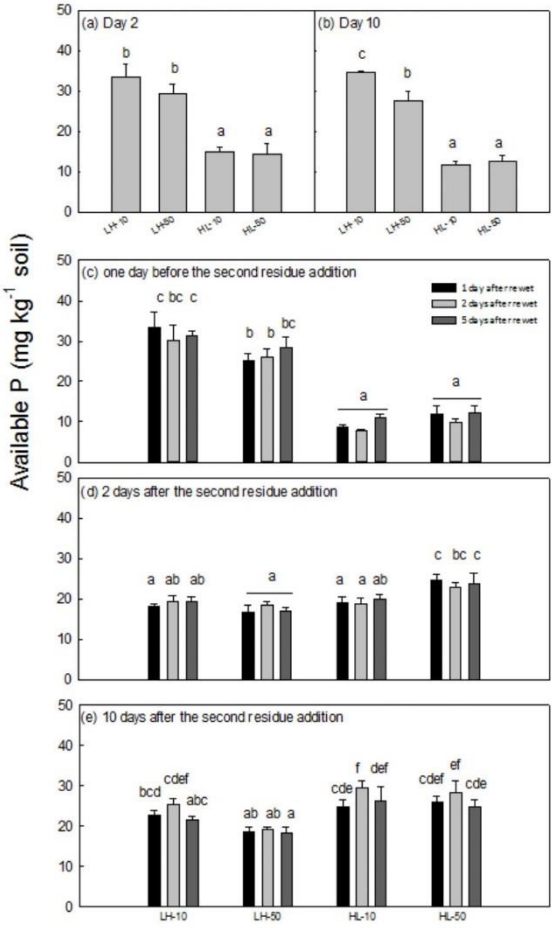

Figure 6. Available P concentration in treatments LH-10, LH-50, HL-10 and HL-50 on days 2 (a) and 10 (b), day after rewet and before second residue addition (c), and 2 days (d) and 10 days after second residue addition (e) (n=4, means ± SE). At a given sampling time, bars with different letters are significantly different ($P \leq 0.05$).

4. Discussion

In this study, the soil water content between the first and second plant residue addition influenced respiration whereas the time between rewetting and residue addition affected N availability. Microbial N uptake was influenced by both soil water content and time between rewetting and residue addition. However, neither soil water content between the first and second residue addition nor time between rewetting and residue addition influenced the legacy effect.

4.1. First ten days

Low water content (10% WHC) reduced cumulative respiration as well as MBN on d2 and d10 compared to 50% WHC. Reduced microbial activity at low soil water content is due to restricted water availability and disconnection of the water film around aggregates, which limits substrate diffusion to microbes (Schimel *et al*., 2007). However, cumulative respiration at 10% WHC was half of that at 50%, but not eliminated, which suggests that some microbial growth occurred at this low water content (Zhang and Marschner, 2017). Available N decreased from d2 to d10 in LH-10 indicating that N immobilisation exceeded N mineralisation at low water content. As expected from previous studies (Hadas *et al*., 2004; Marschner *et al*., 2015), the lower C/N ratio of L induced higher available N and P compared to H.

4.2. Between rewetting and second residue addition

The greater cumulative respiration after rewetting in the soil that had been at 10% WHC in the first 10 days compared to that at 50% WHC can be explained by the rewetting flush that has been documented in many previous studies (Schimel *et al*., 2007; Borken and Matzner, 2009; Shi and Marschner, 2014). However, the previous water content had little effect on nutrient availability and microbial biomass after rewetting.

The number of days between rewetting and residue addition influenced microbial biomass and N availability in LH-10. Five day after rewetting compared to one day, MBN and available N were higher. This suggests that with time after rewetting, more substrate became available allowing microbial N uptake and releasing available N from the low C/N residue added on d0. MBN increased from the first to the fifth day in both LH-10 and LH-50, thus was related to time after the first residue addition, not rewetting. However, available N increased with time only in LH-10. This suggests that rewetting induced N mineralisation beyond the requirement of the microbes (Mikha *et al*., 2005). In HL, time after rewetting did not influence microbial biomass or nutrient availability, probably because the low nutrient content of the H residues in the soil limited respiration, growth and nutrient mineralisation (Heal *et al*., 1997).

4.3. After second residue addition

Respiration rate in the first three to four days after the second residue addition were higher when residue was added five days after rewetting compared to one day, particularly in LH. This stimulated respiration suggests that microbes were more starved five days after rewetting (Zhang and Marschner, 2017) (15 days after the first amendment) and rapidly decomposed the freshly added residues. Cumulative respiration was greater in HL than LH which can be explained by the addition of easily decomposable L on day 10 in the former. The higher cumulative respiration in LH-10 and HL-10 compared to LH-50 and HL-50 shows that rewetting can stimulate respiration

for more than five days in residue amended soil. In a non-amended soil, the rewetting flush may be shorter-lived because of rapid depletion of available nutrients (Borken and Matzner, 2009).

Two days after the second residue addition, the previous water content influenced microbial N uptake in both residue treatments. Rewetting of the soil that had been at 10% WHC in the first ten days increased MBN compared to the soil maintained at 50% WHC. In LH, N taken up by microbes may have come from the L residue left in the soil from the first addition. In HL microbes likely mineralised the freshly added L. However, N availability was not influenced by the previous water content indicating that rewetting only increased microbial N uptake, not net N mineralisation. Residue type influenced available N and P only in soil that was at 50% in first ten days, where it was greater in HL than LH which can be explained by the addition of low C/N residue in HL two days before (Heal *et al.*, 1997). In the soil that was at 10% WHC in the first 10 days, it is likely that in LH sufficient L was left in the soil when H was added to induce net N and P mineralisation to a similar extent as in HL where L had just been added. The first hypothesis was that the legacy effect of the first residue added would be greater when the soil water content between first and second residue addition is low. A greater legacy effect would reduce the effect of the second residue added on the measured parameters. For example in LH, available N after the second residue addition should be higher in soil previously at 10% WHC than that kept at 50% WHC. However, the water content in the first 10 days had no consistent effect on the legacy effect. Therefore, we decline the hypothesis.

The number of days between rewetting and second amendment influenced MBN two days and 10 days after the second residue addition in all treatments. In treatments with residues added one day after rewetting, MBN was higher compared to amendment after five days. Since this occurred irrespective of the water content in the first 10 days, the higher MBN when residues were added one day after rewetting can be explained by the time between first and second residue addition: 10 compared to 15 days. A shorter interval between residue additions appears to stimulate N uptake by the microbial biomass. In N limiting conditions, the number of days between rewetting and residue addition may have little effect on MBN and available N. However, the number of days between rewetting and the second residue amendment did not influence legacy effect. Thus, the second hypothesis can be declined.

5. Conclusion

This study showed that in plant residue amended soil, N mineralisation and immobilisation are influenced by soil water content after the first residue addition and time between rewetting and second residue addition. The new finding is that two and 10 days after the second residue addition, MBN was higher in soil that was amended one day after rewetting than that amended after five days which indicates enhanced N immobilisation. Enhanced microbial N uptake may reduce plant N availability, but can also minimise N loss via leaching or volatilisation. Further, the immobilised N becomes plant available over time through microbial biomass turnover. This indicates that the interval between residue additions should be taken into account in farming systems. To better understand N dynamics in residue amended soil after rewetting and its impact on plant N uptake ^{15}N labelled residue should be used in future experiments.

Acknowledgements

Yanchen Zhang received a scholarship from The University of Adelaide.

References

Birch, H.F. 1958. The effect of soil drying on humus decomposition and nitrogen availability. Plant Soil. 10(1), 9-31.

Borken, W., Egbert M. 2009. Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. Global Change Biol. 15(4), 808-824.

Bremmer, J.M., Mulvaney, C.S. 1982. Total N. Methods of Soil Analysis. Part2. 2nd ed. Agron. Monog 9, 895-926.

Butterly, C.R., Marschner, P., McNeill, A.M., Baldock, J.A. 2010. Rewetting CO_2 pulses in Australian agricultural soils and the influence of soil properties. Biol fert soils. 46(7), 739-753.

Chaudhari, S.K., Singh, R., Kundu, D.K. 2008. Rapid textural analysis for saline and alkaline soils with different physical and chemical properties. Soil Sci Soc Am J. 72(2), 431-441.

Hadas, A., Kautsky, L., Goek, M., Kara, E.E. 2004. Rates of decomposition of plant residues and available nitrogen in soil, related to residue composition through simulation of carbon and nitrogen turnover. Soil Biol Biochem. 36(2), 255266.

Heal, O.W., Anderson, J.M., Swift, M.J. 1997. Plant litter quality and decomposition: an historical overview. Driven by nature: plant litter quality and decomposition. Ed. G. Cadisch. CABI.

IPCC, AR. 2007. Intergovernmental panel on climate change. IPCC Secretariat Geneva.

Isbell, R. 2002. The Australian soil classification, 4. CSIRO publishing.

Kettler, T.A., Doran, J.W., Gilbert, T.L. 2001. Simplified method for soil particle-size determination to accompany soil-quality analyses. Soil Sci Soc Am J. 65(3), 849-852.

Kouno, K., Tuchiya, Y., Ando, T. 1995. Measurement of soil microbial biomass phosphorus by an anion exchange membrane method. Soil Biol Biochem. 27(10), 1353-1357.

Marschner, P., Hatam, Z., Cavagnaro, T.R. 2015. Soil respiration, microbial biomass and nutrient availability after the second amendment are influenced by legacy effects of prior residue addition. Soil Biol Biochem. 88, 169-177.

Mikha, M.M., Rice, C.W., Milliken, G.A., 2005. Carbon and nitrogen mineralization as affected by drying and wetting cycles. Soil Biol Biochem. 37(2), 339-347.

Miranda, K.M., Espey, M.G., Wink, D.A. 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric oxide. 5(1), 62-71.

Moore, J.M., Klose, S., Tabatabai, M.A. 2000. Soil microbial biomass carbon and nitrogen as affected by cropping systems. Biol. Fert. Soils. 31(3-4), 200-210.

Murphy, J., Riley, J.P. 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica chimica acta. 27, 31-36.

Nguyen, T.T., Ngo, H.T.T., Marschner, P. 2016. Legacy effect of previous residue addition—influence of length of the moist period between residue additions on soil respiration, microbial biomass and nutrient availability. Biol. Fert. Soils. 1-11.

Nye, P.H. 1966. The effect of the nutrient intensity and buffering power of a soil, and the absorbing power, size and root hairs of a root, on nutrient absorption by diffusion. Plant Soil. 25(1), 81-105.

Power, A.G. 2010. Ecosystem services and agriculture: tradeoffs and synergies. Philosophical transactions of the royal society B: biological sciences. 365, 2959-2971.

Schimel, J., Balser, T.C., Wallenstein, M. 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology. 88(6), 1386-1394.

Scotti, R., Bonanomi, G., Scelza, R., Zoina, A., Rao, M.A. 2015. Organic amendments as sustainable tool to recovery fertility in intensive agricultural systems. J. soil sci. plant nutr. 15(2), 333-352.

Setia, R., Marschner, P., Baldock, J., Chittleborough, D., Smith, P., Smith, Jo. 2011. Salinity effects on carbon mineralization in soils of varying texture. Soil Biol. Biochem. 43(9), 1908-1916.

Shi, A., Marschner, P. 2014. Drying and rewetting frequency influences cumulative respiration and its distribution over time in two soils with contrasting management. Soil Biol. Biochem. 72, 172-179.

Vance, E.D., Brookes, P.C., Jenkinson, D.S. 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19(6), 703-707.

Walkley, A., Black, I.A. 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci. 37(1), 29-38.

Willis, R.B., Montgomery M.E., Allen P.R. 1996. Improved method for manual, colorimetric determination of total Kjeldahl nitrogen using salicylate. J. Agri. Food Chem. 44(7), 1804-1807.

Xue, R., Shen, Y., Marschner, P. 2016. Low soil water content during plant growth influences soil respiration and microbial biomass after plant removal and rewetting. J. Soil Sci. Plant Nutr. 16(4), 955-966.

Zhang, Y., Marschner, P. 2016. Nutrient availability, soil respiration and microbial biomass after the second residue addition are influenced by the C/N ratio of the first residue added, but not by drying and rewetting between residue amendments. Euro. J. Soil Biol. 77, 68-76.

Zhang, Y., Marschner, P. 2017. 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. J. Soil Sci. Plant Nutr. 17(3), 594-608.

Zheng, B., Marschner, P. 2017. Previous residue addition rate and C/N ratio influence nutrient availability and respiration rate after the second residue addition. Geoderma. 285, 217-224.

CHAPTER 6

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

Statement of Authorship

Title of Paper	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
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Zhang Y, Marschner P (2017) 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. Journal of Soil Science and Plant Nutrition, 2017, 17(3), 594-608.

Principal Author

Name of Principal Author (Candidate)	Yanchen Zhang		
Contribution to the Paper	Performed the experiment, analyses of soils, data analysis and interpretation, and manuscript writing.		
Overall percentage (%)	70%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	24/08/2018

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Petra Marschner		
Contribution to the Paper	Supervised development of the work, data interpretation, manuscript evaluation and correction and acted as the corresponding author.		
Signature		Date	24/08/2018

Journal of Soil Science and Plant Nutrition, 2017, 17 (3), 594-608
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

Yanchen Zhang, Petra Marschner*

School of Agriculture, Food and Wine, The University of Adelaide, South Australia, 5005, Australia.
**Corresponding author: petra.marschner@adelaide.edu.au*

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).

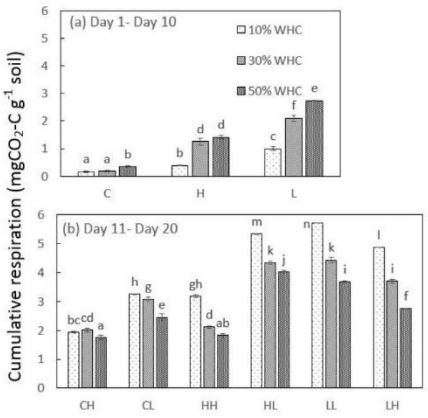

Figure 1. Cumulative respiration from day 0 to day 10 and from day 11 to day 20 in soil with different water contents (10%, 30% or 50% WHC) in the first 10 days and at 50% WHC from day 10 to day 20. The soil was unamended (C) or amended with high (H) or low (L) C/N ratio residue on day 0 followed by 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).

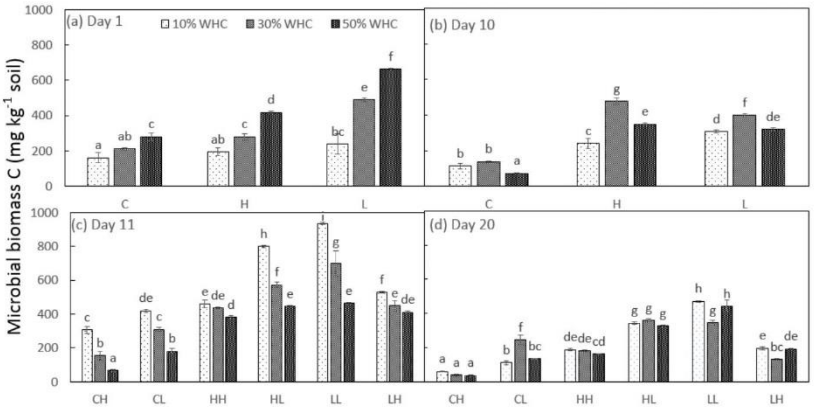

Figure 2. Microbial biomass C concentration on days 1 (a), 10 (b), 11 (c) and 20 (d) in soil with different water contents (10%, 30% or 50% WHC) in the first 10 days and at 50% WHC from day 10 to day 20. The soil was unamended (C) or amended with high (H) or low (L) C/N ratio residue on day 0 followed 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).

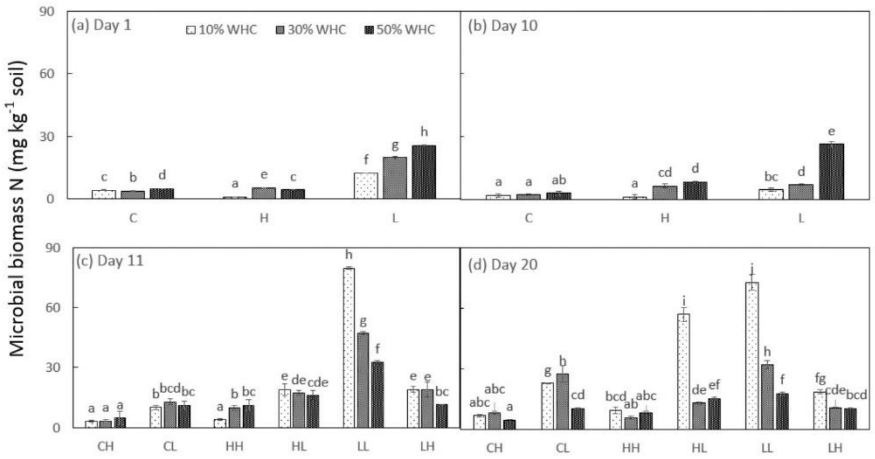

Figure 3. Microbial biomass N concentration on days 1 (a), 10 (b), 11 (c) and 20 (d) in soil with different water contents (10%, 30% or 50% WHC) in the first 10 days and at 50% WHC from day 10 to day 20. The soil was unamended (C) or amended with high (H) or low (L) C/N ratio residue on day 0 followed 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.

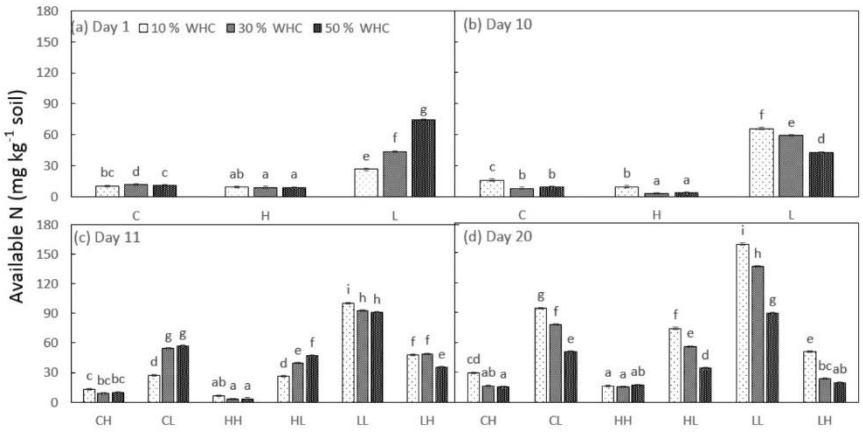

Figure 4. Available N concentration on days 1 (a), 10 (b), 11 (c) and 20 (d) in soil with different water contents (10%, 30% or 50% WHC) in the first 10 days and at 50% WHC from day 10 to day 20. The soil was unamended (C) or amended with high (H) or low (L) C/N ratio residue on day 0 followed by addition 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).

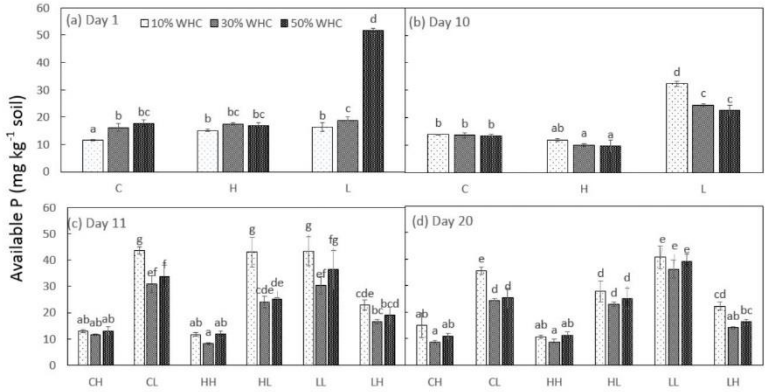

Figure 5. Available P concentration on days 1 (a), 10 (b), 11 (c) and 20 (d) in soil with different water contents (10%, 30% or 50% WHC) in the first 10 days and at 50% WHC from day 10 to day 20. The soil was unamended (C) or amended with high (H) or low (L) C/N ratio residue on day 0 followed by addition 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.

Acknowledgement

Yanchen Zhang received a scholarship from The University of Adelaide.

References

Brady, N., Weil, R. 2002. The nature and properties of soils. Upper Saddle River, Prentice Hall, NJ.

Bremmer, J., Mulvaney, C. 1982. Total N. Methods of Soil Analysis. Part 2. 2nd ed. Agron. Monog. 9, 895-926.

Butterly, C.R., Marschner, P., McNeill, A.M., Baldock, J.A. 2010. Rewetting CO_2 pulses in Australian agricultural soils and the influence of soil properties. Biol. Fert. Soils. 46, 739-753.

Cavagnaro, T., Jackson, L., Six, J., Ferris, H., Goyal, S., Asami, D., Scow, K. 2006. Arbuscular mycorrhizas, microbial communities, nutrient availability, and soil aggregates in organic tomato production. Plant Soil. 282, 209-225.

Chaudhari, S., Singh, R., Kundu, D. 2008. Rapid te tural analysis for saline and alkaline soils with different physical and chemical properties. Soil Sci. Soc. Am. J. 72, 431-441.

Chowdhury, N., Marschner, P., Burns, R. 2011. Response of microbial activity and community structure to decreasing soil osmotic and matricpotential. Plant Soil. 344, 241-254.

Geisseler, D., Horwath, W.R., Scow, K.M. 2011. Soil moisture and plant residue addition interact in their effect on extracellular enzyme activity. Pedobiologia. 54, 71-78.

Hadas, A., Kautsky, L., Goek, M., Kara, E.E. 2004. Rates of decomposition of plant residues and available nitrogen in soil, related to residue composition through simulation of carbon and nitrogen turnover. Soil Biol. Biochem. 36, 255-266.

Heal, O., Anderson, J., Swift, M. 1997. Plant litter quality and decomposition: an historical overview. Driven by nature: plant litter quality and decomposition. Ed. G. Cadisch. CABI.

Ilstedt, U., Nordgren, A., Malmer, A. 2000. Optimum soil water for soil respiration before and after amendment with glucose in humid tropical acrisols and a boreal mor layer. Soil Biol. Biochem. 32, 1591-1599.

Isbell, R., 2002. The Australian soil classification, 4. CSIRO publishing.

Kettler, T., Doran, J.W., Gilbert, T. 2001. Simplified method for soil particle-size determination to accompany soil-quality analyses. Soil Sci. Soc. Am. J. 65, 849-852.

Kouno, K., Tuchiya, Y., Ando, T. 1995. Measurement of soil microbial biomass phosphorus by an anion exchange membrane method. Soil Biol. Biochem. 27, 1353-1357.

Marschner, P., Hatam, Z., Cavagnaro, T. 2015. Soil respiration, microbial biomass and nutrient availability after the second amendment are influenced by legacy effects of prior residue addition. Soil Biol. Biochem. 88, 169-177.

Moore, J., Klose, S., Tabatabai, M. 2000. Soil microbial biomass carbon and nitrogen as affected by cropping systems. Biol. Fert. Soils. 31, 200-210.

Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta. 27, 31-36.

Nguyen, T.T., Ngo, H.T.T., Marschner, P. 2016. Legacy effect of previous residue addition—influence of length of the moist period between residue additions on soil respiration, microbial biomass and nutrient availability. Biol Fert. Soils. 52, 1047–1057

Power, A.G. 2010. Ecosystem services and agriculture: tradeoffs and synergies. Phil. trans. Royal Soc. B: Biological Sciences. 365, 2959-2971.

Schimel, J., Balser, T.C., Wallenstein, M. 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology. 88, 1386-1394.

Schwendener, C.M., Lehmann, J., de Camargo, P.B., Luizao, R.C., Fernandes, E.C. 2005. Nitrogen transfer between high-and low-quality leaves on a nutrient-poor Oxisol determined by 15 N enrichment. Soil Biol. Biochem. 37, 787-794.

Scotti, R., Bonanomi, G., Scelza, R., Zoina, A., Rao, M.A. 2015. Organic amendments as sustainable tool to recovery fertitlity in intensive africultural systems. J. Soil Sci. Plant Nutr. 15, 333-352

Setia, R., Marschner, P., Baldock, J., Chittleborough, D., Smith, P., Smith, J. 2011. Salinity effects on carbon mineralization in soils of varying texture. Soil Biol. Biochem. 43, 1908-1916.

Solomon, S., Qin, D., Manning, M., Chen, Z., Ma quis, M., Averyt, K., Tignor, M., Miller, H. 2007. Contribution of working group I to the fourth a sessment report of the intergovernmental panel on climate change, 2007. Cambridge University Press, Cambridge.

Vance, E., Brookes, P., Jenkinson, D. 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703-707.

Vrede, K., Heldal, M., Norland, S., Bratbak, G. 2002. Elemental composition (C, N, P) and cell volume of exponentially growing and nutrient-limited bacterioplankton. Appl. Environ. Microbiol. 68, 2965-2971.

Walkley, A., Black, I.A. 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci. 37, 29-38.

Willis, R.B., Montgomery, M.E., Allen, P.R. 1996. Improved method for manual, colorimetric determination of total Kjeldahl nitrogen using salicylate. J. Agri. Food Chem. 44, 1804-1807.

Wu, J., Brookes, P. 2005. The proportional mineral sation of microbial biomass and organic matter caused by air-drying and rewetting of a grassland soil. Soil Biol. Biochem. 37, 507-515.

Xue, R., Shen, Y., Marschner, P. 2016. Low soil water content during plant growth influences soil respiration and microbial biomass after plant removal and rewetting. J. Soil Sci. Plant Nutr. 16, 955-966

Zhang, Y., Marschner, P. 2016. Nutrient availability, soil respiration and microbial biomass after the second residue addition are influenced by the C/N ratio of the first residue added, but not by drying and rewetting between residue amendments. Euro. J. Soil Biol. 77, 68-76.

Zheng, B., Marschner, P. 2017. Previous residue addition rate and C/N ratio influence nutrient availability and respiration rate after the second residueaddition. Geoderma. 285, 217-224.

CHAPTER 7

Influence of previous and current water content on microbial biomass and nutrient availability is modulated by the order in which low and high C/N ratio residues are added

Yanchen Zhang and Petra Marschner

School of Agriculture, Food and Wine, The University of Adelaide, Glen Osmond, SA, 5064, Australia

Zhang Y, Marschner P (2018) Influence of previous and current water content on microbial biomass and nutrient availability is modulated by the order in which low and high C/N ratio residue are added (in preparation)

Statement of Authorship

Title of Paper	Influence of previous and current water content on microbial biomass and nutrient availability is modulated by the order in which low and high C/N ratio residue are added
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☑ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Zhang Y, Marschner P (2018) Influence of previous and current water content on microbial biomass and nutrient availability is modulated by the order in which low and high C/N ratio residue are added (in preparation)

Principal Author

Name of Principal Author (Candidate)	Yanchen Zhang
Contribution to the Paper	Performed the experiment, analyses of soils, data analysis and interpretation, and manuscript writing.
Overall percentage (%)	70%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 24/08/2018

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Petra Marschner
Contribution to the Paper	Supervised development of the work, data interpretation, manuscript evaluation and correction.
Signature	Date 24/08/2018

Influence of previous and current water content on microbial biomass and nutrient availability is modulated by the order in which low and high C/N ratio residue are added

Yanchen Zhang, Petra Marschner*

School of Agriculture, Food and Wine, The University of Adelaide, Glen Osmond, SA, 5064, Australia

*Corresponding author: Petra Marschner

Present address: School of Agriculture, Food and Wine, The University of Adelaide, Glen Osmond, SA, 5064, Australia

Tel: +61 83137379

Fax: +61 83186511

E-mail address: petra.marschner@adelaide.edu.au

Abstract

Soil water content is a key factor regulating microbial activity and water availability varies over time. However, little is known about how previous and current soil water content influence microbial activity and nutrient cycling in soils amended with residue differing in C/N ratio. The aim of this study was to determine how soil respiration, microbial biomass and nutrient availability after the first and second residue addition are influenced by water content after each residue addition and whether the influence is modulated by the order in which residues are applied. A loamy soil was incubated at 10% or 50% water holding capacity (WHC) in 10-day period with low (L) or high C/N ratio (H) residues added on day 0 and day 10. Cumulative respiration was lower at 10% WHC than 50% WHC in both 10-day periods. From day 11 to 20, cumulative respiration was lower in LH than HL in soil that was at 50% WHC in the first 10 days. In LH at 50% WHC from d11 to d20, previous low water content (10% WHC) enhanced N immobilisation. In HL with 10% WHC from d11 to d20, MBN, available N and P on d20 were higher in soil that was at 50% WHC in the first 10 days compared to that at 10%. We conclude that microbial biomass and nutrient availability after the second residue addition are influenced by both previous and current water content and that the influence is modulated by the order in which the residues are added.

Keywords

C/N ratio, microbial biomass, nutrient availability, residue addition, respiration, soil water content

1. Introduction

Soil water availability is critical for soil microbial activity and thus residue decomposition may change over time [1]. When soil is dry, water films around aggregates are thin which restricts nutrient diffusion to soil microbes and the low water potential may draw water out of cells, thus reducing microbial activity [2, 3]. Moreover, microbes may be influenced by current and past water content. Banerjee et al. [4] recently showed that bacterial community composition and expression of genes involved in N transformations and N_2O emissions differed among treatments not only while they were at different water content, but also after being adjusted to the same water content. They concluded that microbes are influenced by both past and current water content. Cavagnaro [5] found that previous water content influenced subsequent mycorrhizal colonization after rewetting of soil. Xue et al. [6] reported that the effect of low soil water content on microbial biomass and nutrient availability depended on whether low water content was imposed during or after plant growth.

The incorporation of organic fertilizers and amendments such as plant residues can help to maintain long-term sustainability and reduce environmental costs in agricultural systems [7]. Organic matter from plant residues can improve soil properties such as amount of plant available water and nutrients [8], thus providing nutrients for plant growth and improving agriculture productivity. The C/N ratio of plant materials can be used to predict net N immobilization and mineralization during decomposition [9]. Addition of residues with high C/N ratio (> 20) results in immediate net N immobilization in the microbial biomass [10], while amendment with low C/N ratio (< 20) residues leads to net N mineralization as they satisfy the nutrient demand of microbes [11]. Previously we showed that nutrient availability and microbial biomass after the second residue addition are influenced by the properties of the previously added residue, which is referred as legacy effect [12, 13]. For example, nutrient availability after the second residue addition was lower with low C/N residue (L) that followed

high C/N residue (H) than with L added to previously unamended soil. On the other hand, nutrient availability was higher with H following L than with H added to previously unamended soil. The legacy effect can be explained by microbes decomposing both the previously added and the freshly added residue together. Correspondingly, the legacy effect has been shown to depend on the amount of previously added residue left when the second residue is added [13, 14].

Recently, Zhang and Marschner [15] found that one to four drying and rewetting events between the first and second residue addition did not influence the legacy effect. On the other hand, the legacy effect of the previously added residue was reduced if the second residue addition was accompanied by rewetting of dry soil [16]. Residue addition may alleviate substrate limitation induced by low water content which could minimize differences between soil water contents. But the effect of residue addition may depend on their C/N ratio. We showed previously that water content between residue additions influences soil respiration and nutrient availability not only directly, but also after rewetting and the second residue addition [17]. In that study, soils were rewetted to 50% WHC after the second residue addition therefore only the effect of soil water content between the first and the second residue addition on microbial biomass and nutrient availability was evaluated. However, soil water content may also vary after the second residue addition. The aim of the present study was to determine how soil respiration, microbial biomass and nutrient availability after the first and second residue amendment are influenced by water content after each amendment and how the influence is modulated by the order in which the residues are applied. The first hypothesis was that the effect of the second residue addition on microbial biomass and nutrient availability is smaller in soil that was at low water content after the first addition than with high water content. This hypothesis was based on the assumption that at previous low water content, more of the first residue is left in the soil when the second residue is added than when the previous water content

was optimal for microbial activity. The second hypothesis was that the influence of the water content after the second amendment will be modulated by the order in which high and low C/N residues are added.

2. Materials and methods

2.1. Soil and plant residues

A loamy soil was collected in early 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 permanent pasture for more than 80 years. The area is now converting to wheat growing. The soil properties are: 22% sand, 60% silt, 18% clay, maximum water holding 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 organic N 1.5 g kg^{-1}, bulk density 1.3 g cm^{-3}, available P 10 mg P kg^{-1} and available N 15 mg N kg^{-1}. The site has a Mediterranean climate with cool, wet winters and hot, dry summers. The soil is a Red-brown Earth according to Australian soil classification [18] and classified as Rhodoxeralf in US Soil Taxonomy. Soil was collected along a randomly selected central transect in three 2 x 2 m plots which were about 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 was combined and mixed before starting the experiment.

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. Compared to H, L 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 (Table 1). The residues had a similar pH and total organic C content.

2.2. Experimental design

Before the start of the experiment, the air-dried soil was incubated for 10 days at 22-23 °C in the dark at 50% of WHC to activate the soil microbes and stabilise soil respiration. Microbial activity is maximal at 50% WHC in this soil [12]. After the pre-incubation, the soil was either kept at 50% WHC or dried in a fan-forced oven at 40 °C to 10% WHC. Half of the soil at each water content was amended with H, the other half with L at 10 g kg^{-1}. The soils were maintained at this water content for 10 days. Then soil water content was adjusted to 10 or 50% WHC or the water content was maintained. The treatments were 10-10, 10-50, 50-10 and 50-50, where the first value is the soil water content from day 0 to day 10 and the second value the soil water content after day 10. Soils were dried in a fan-forced oven at 40 °C or rewet rapidly, depending on treatment. The second residue which had a different C/N ratio than the first was added after the adjustment of water content on day 10. Soils were maintained at 10 or 50% WHC until the end of the experiment (day 20).

After adjustment of the soil water content on days 0 and 10, residues were thoroughly mixed with the soil in a small plastic bag, then 30 g dry soil equivalent was filled into PVC cores with 3.7 cm diameter and 5 cm height with a nylon mesh base (7.5 µm, Australian Filter Specialist), and packed to a bulk density of 1.3 g cm^{-3}. 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 22-23 °C. Soil water content (10 or 50% WHC during the experiment) was maintained by checking the water content every few days by weighing and adding reverse osmosis (RO) water if necessary. Soil

respiration was measured daily. Soil cores were destructively sampled on days 1, 10 (before adjustment of the water content and addition of the second residue), 11 and 20. The soil samples were analysed for available N and P, and microbial biomass N and P. For each sampling time and treatment there were four replicates, giving a total number of 192 cores.

2.3. Measurements

Soil analyses were carried out as described in Zhang and Marschner [16]. Briefly, soil texture was determined with rapid texture analysis [19]. Maximum soil water holding capacity was measured using a sintered glass funnel connected to a 100 mm water column (ψ_m = -10 kPa). Soil pH and EC were measured in a 1:5 (w/v) soil to RO water ratio after 1 hour shaking at 25°C. Total organic C of soil and plant residues was measured according to Walkley and Black [20] and total N was measured using the Kjeldahl method followed by colorimetric measurement as described in Bremmer and Mulvaney [21]. 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 phosphovanado-molydate method. Water extractable organic carbon was extracted 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. [22]. Microbial biomass nitrogen (MBN) was measured after chloroform fumigation-extraction with 0.5 M K_2SO_4 at 1: 4 soil to extractant ratio [23]. Ammonium-N was measured in the K_2SO_4 extract after Willis et al. [24]. 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. [25].

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 in the KCl extract was measured as described for MBN. Nitrate-N was determined as described in Miranda et al. [26]. Available and microbial biomass P (MBP) were determined using anion exchange resin following Kouno et al. [27]. For MBP, 1 ml hexanol was added. The P concentration in the extracts was determined colorimetrically according to Murphy and Riley [28]. Microbial biomass P is the difference in P concentration between fumigated and non-fumigated soil [27]. No correction factor was used for P because recovery of a P spike in this soil was 98% (Butterly, pers. comm.).

2.4. Statistical analysis

There were four replicate cores for each treatment and sampling time. Data was tested for homogeneity and equal variance. For cumulative respiration, available N and P and microbial biomass N and P carried out repeatedly during the experiment, two-way ANOVA (residue x water treatment) repeated measures analysis of variance was carried out in GenStat (GenStat for Windows, 15th edition, VSN Int. Ltd, UK, 2012). Time had a significant effect on measured parameters and the interaction of residue treatment and water content was significant ($p<0.05$). Therefore, Tukey's multiple comparison test at 95% confidence interval was used for each sampling time separately to determine which treatments differ from each other. One-way ANOVA was used to compare the properties of two plant residues.

3. Results

3.1. Cumulative respiration

Cumulative respiration in both 10-day periods was lower at 10% WHC than 50% WHC (Fig. 1). In the first 10 days, it was higher in LH than in HL. Cumulative respiration from d11 to d20 differed little between HL and LH in treatments that were at 10% WHC in the first 10 days. But in soil that had been at 50% WHC, cumulative respiration from d11 to d20 was lower in LH than HL.

3.2. Microbial biomass

On d1 and d10, MBN was lower at 10 than at 50% WHC (Fig. 2a, b). At both water contents, MBN was about three-fold higher in LH than HL. On d11 and d20, MBN was lower in LH than HL except on d20 in 10-50 where the residue treatments had similar MBN (Fig. 2c, d). In HL, MBN on d11 and d20 was about 50% lower at 10% than at 50% WHC, irrespective of the water content in the first 10 days. In LH, MBN on d11 and d20 was higher in 10-50 than 10-10, but did not differ between 50-10 and 50-50.

MBP on d1 and d10 was two to five times higher in LH than HL with greater differences on d1 than d10 (Fig. 3a, b). Water content did not influence MBP in HL. In LH, MBP was lower at 10% WHC than at 50% WHC with greater differences on d1 than d10. In LH, MBP decreased from d1 to d10 at 50% WHC. From d10 to d11, MBP increased three to four-fold in HL, but changed little in LH. Residue treatments differed in MBP on d11 only at 50% WHC; it was about 30% higher in HL than LH in 10-50, but twice as high in LH compared with HL in 50-50. MBP on d11 was lower in 10-10 than 10-50, particularly in HL. In soil that was at

50% WHC in the first 10 days, MBP was about 50% lower in 50-10 than 50-50 only in LH (Fig. 3c). On day 20, MBP differed between HL and LH only in moisture treatments with the same water content in both 10-day periods (Fig. 3d). In 10-10, MBP was about 20% higher in LH than HL. But in 50-50, MBP was 40% higher in HL than LH. In HL, MBP on d20 was higher at 50% WHC than at 10% with greater differences in soil that was at 50% WHC in the first 10 days. In contrast, MBP on d20 in LH was only influenced by the previous water content; it was about 25% higher in soil that had been at 10% WHC than that at 50% WHC in the first 10 days.

3.3. Available N and P

On d1 and d10, available N was two to four-fold higher in LH than HL, with greater differences on d10 (Fig. 4a, b). On d1, water content had little effect on available N. On d10, available N was higher at 50% WHC than at 10% WHC, particularly in LH. On d11, available N was low in all treatments (Fig. 4c). Available N on d11 was similar as on d10 in HL, but lower in LH. Residue treatments differed in available N on d11 only in 10-50 where it was about 30% higher in HL than LH. Current and previous water content had no effect on available N in LH. In HL, available N was higher at 50% WHC than at 10%, irrespective of the previous water content. Available N on d20 was similar in HL and LH except in 50-50 where it was two-fold higher in HL than in LH (Fig. 4d). Water content from d11 to d20 influenced available N only in soil that was at 50% WHC in the first 10 days where it was higher in 50-10 than 50-50. The previous water content only influenced available N on d20 at 10% WHC, it was higher in 50-10 than 10-10.

Available P on d1 and d10 was three to eight-fold higher in LH than HL, with greater differences on d1 (Fig. 5a, b). In HL, available P was not influenced by water content, but in

LH it was lower at 50% WHC than 10% WHC. Available P did not change from d10 to d11 in LH, but increased about three-fold in HL (Fig. 5c). Available P on d11 was higher in HL than LH only in moisture treatments where the water content had not changed on d10: 10-10 and 50-50. On d20, available P was higher in HL than LH only in soil that was at 50% WHC in the first 10 days (Fig. 5d). In HL, available P was lower at 50% WHC from d11 to d20 than at 10% WHC. In LH available P was lower in 50-50 than 50-10.

4. Discussion

This study showed that soil respiration, microbial nutrient uptake and nutrient availability after the second residue amendment are influenced by soil water content at that time, the previous water content as well as the C/N ratio of the first and second amendment. After the second amendment, the current water content had a stronger effect on these parameters than the previous water content. Effects of the current water content were greater in soil amended with L than with H. Therefore, the first hypothesis (the effect of the second residue addition on microbial biomass and nutrient availability is smaller in soil at low water content after the first addition than with high water content) can only be confirmed for 50% WHC after the second residue addition. The second hypothesis (the influence of the water content after the second amendment will be modulated by the order in which high and low C/N residues are added) can be confirmed.

4.1. Day 1-10

The higher cumulative respiration, MBN and MBP with L compared to H can be explained by the higher nutrient content and water-extractable C in L which makes it more

easily decomposable than H [11, 12]. Water content had little effect on microbial N and P uptake and N and P availability in soil amended with H because its low nutrient content limited N and P mineralization even at optimal water content. With L, respiration and microbial biomass were higher at 50% WHC than at 10% due to the higher water availability and improved diffusion at the higher water content [29]. The higher MBN on d1 at 50% than at 10% WHC indicates rapid N uptake by microbes. By day 10, available N was higher at 50% WHC than at 10% particularly with L, likely due to mineralisation of organic N in L. The effect of water content in soil amended with L on available P was opposite to that on available N. The higher P availability at 10% WHC is likely due to the lower microbial P uptake at this water content than at 50% WHC. Residues contain inorganic P [30, 31] that would be released after addition to the soil. In L, both available P and MBP decreased from d1 to d10 which can be explained by binding of P to soil particles [32]. The high MBP on d1 could be an artefact; hexanol may release P from residues [33]. It should be noted that P availability in soil with low water content may have been overestimated because available P determination involves addition of an extractant which can release P from aggregates when added to dry soil [34].

4.2. Day 11-20

In HL where soil was amended with L on day 10, cumulative respiration and MBN from day 11 to 20 were higher at 50% WHC than at 10% because of the improved diffusion [29], which is similar to the effect of water content in the first 10 days. On the other hand, available N on day 11 was not influenced by water content after the second residue addition. The lower N availability on day 20 at 50% WHC than at 10%, is likely due N immobilisation because MBN was higher at 50% than 10% WHC.

Also similar as in the first 10 days, the effect of the water content from day 11 to 20 was greater in HL than LH because on d10 L was added to the former whereas the slowly decomposable H was added in LH. In LH, available N on day 11 was less than half of that on day 10 prior to H addition, particularly at 50% WHC. Craswell [35] showed that the addition of straw enhanced N immobilization compared with unamended soil and increased with soil water content. However in the present study, immobilisation of N by the microbial biomass cannot explain this decrease in available N because MBN did not change from d10 to d11 at 10% WHC and halved at 50% WHC. The lower N availability on day 11 may be due to gaseous N loss induced by rewetting. Nitrogen availability differed little between day 10 and day 11 in HL, but the higher MBN indicates that after addition of L on day 10, N immobilisation prevented an increase in available N.

The effect of a previous low water content (10% WHC) on microbial biomass and nutrient availability at 50% WHC after d10 can be evaluated by comparing 10-50 with 50-50. Available N on d11 was similar in 10-50 and 50-50. On the other hand, MBN in LH on d11 and d20 was greater in 10-50 than 50-50. This suggests that the previous low water content enhanced N immobilisation compared to 50-50 where the water content was optimal in the first 10 days. As indicated by the lower cumulative respiration in the first 10 days, more L was left in the soil with 10% WHC on d10 than with 50% WHC. Thus, after addition of H on d10, microbes in 10-50 decomposed the remaining L together with the freshly added H, which led to net N immobilisation. In 50-50 on the other hand, N mineralisation and immobilisation after H addition on d10 were limited by the smaller amount of L left in the soil. In 10-50, cumulative respiration from day 11 to day 20 and MBN on d20 did not differ between HL and LH. In this treatment, a large proportion of the initially added residue was left in the soil when the second residue was added. Therefore after d10, residues added on d0 and d10 were decomposed together, quite similar to the situation when both residues are added simultaneously. This is in

agreement with our previous study that the legacy effect depend on the amount of previously added residue left when the second residue is added [13, 14]. In 50-50 on the other hand, cumulative respiration and MBN on d20 were lower in LH than HL. In this treatment, more of the initially added residue was decomposed in the first 10 days than in 10-50, thus less left in the soil from d11 t0 d20. Therefore in 10-50, cumulative respiration and MBN on d20 were mainly influenced by the second residue.

The effect of previous water content on microbial biomass and nutrient availability at low water content from d11 to d20 can be assessed by comparing 10-10 with 50-10. In LH, cumulative respiration was higher in 10-10 than 50-10, probably because more of first residue was left in the soil on d11 in the former. On d11 MBN, available N and P differed little between 10-10 and 50-10. But on d20 in HL, MBN, available N and P were higher in 50-10 than 10-10, suggesting that nutrients were released from L added on d10 to a greater extent in the soil that was at 50% WHC in the first days. This is likely due to the smaller amount of the initially added H left on d10 compared to 10-10. Thus, microbes decomposed primarily L, increasing not only MBN, but also available N and P.

5. Conclusion

After the second amendment in a given residue treatment, the current water content had a stronger effect on respiration, microbial biomass and nutrient availability than the previous water content. The influence of the previous water content on respiration and microbial biomass was stronger when the first amendment was low C/N residue because its decomposition was more strongly affected by water content than that of high C/N residue. In this study, the period between first and second residue addition was short as it may occur in

natural ecosystems in autumn. In future studies, longer periods between residue additions (several weeks to months) could be used.

Acknowledgement

Yanchen Zhang received a scholarship from The University of Adelaide.

References

[1] R. Harris, Effect of water potential on microbial growth and activity, in: J.F. Parr, W.R. Gardner, L.F. Elliott (Eds.), Water Potential Relations in Soil Microbiology, Am. Soc. Agron., Madison, WI, 1981, pp. 23-95.

[2] S. Manzoni, P. Taylor, A. Richter, A. Porporato, G.I. Ågren, Environmental and stoichiometric controls on microbial carbon-use efficiency in soils, New Phytol. 196 (2012) 79-91.

[3] J. Schimel, T.C. Balser, M. Wallenstein, Microbial stress-response physiology and its implications for ecosystem function, Ecology 88 (2007) 1386-1394.

[4] S. Banerjee, B. Helgason, L. Wang, T. Winsley, B.C. Ferrari, S.D. Siciliano, Legacy effects of soil moisture on microbial community structure and N_2O emissions, Soil. Biol. Biochem. 95 (2016) 40-50.

[5] T.R. Cavagnaro, Soil moisture legacy effects: Impacts on soil nutrients, plants and mycorrhizal responsiveness, Soil. Biol. Biochem. 95 (2016) 173-179.

[6] R. Xue, Y. Shen, P. Marschner, Low soil water content during plant growth influences soil respiration and microbial biomass after plant removal and rewetting, J. Soil Sci. Plant Nutr. 16 (2016) 955-966.

[7] J. Six, S.M. Ogle, R.T. Conant, A.R. Mosier, K. Paustian, The potential to mitigate global warming with no-tillage management is only realized when practised in the long term, Glob. Change Biol. 10 (2004) 155-160.

[8] J.W. Doran, Soil health and global sustainability: translating science into practice, Agric. Ecosyst. Environ. 88 (2002) 119-127.

[9] I. Trinsoutrot, S. Recous, B. Bentz, M. Linères, D. Chèneby, B. Nicolardot, Biochemical quality of crop residues and carbon and nitrogen mineralization kinetics under nonlimiting nitrogen conditions, Soil Sci. Soc. Am. J. 64 (2000) 918-926.

[10] N. Moritsuka, J. Yanai, K. Mori, T. Kosaki, Biotic and abiotic processes of nitrogen immobilization in the soil-residue interface, Soil. Biol. Biochem. 36 (2004) 1141-1148.

[11] A. Hadas, L. Kautsky, M. Goek, E.E. Kara, Rates of decomposition of plant residues and available nitrogen in soil, related to residue composition through simulation of carbon and nitrogen turnover, Soil. Biol. Biochem. 36 (2004) 255-266.

[12] P. Marschner, Z. Hatam, T. Cavagnaro, Soil respiration, microbial biomass and nutrient availability after the second amendment are influenced by legacy effects of prior residue addition, Soil. Biol. Biochem. 88 (2015) 169-177.

[13] T.T. Nguyen, T.R. Cavagnaro, H.T.T. Ngo, P. Marschner, Soil respiration, microbial biomass and nutrient availability in soil amended with high and low C/N residue–Influence of interval between residue additions, Soil. Biol. Biochem. 95 (2016) 189-197.

[14] B. Zheng, P. Marschner, Previous residue addition rate and C/N ratio influence nutrient availability and respiration rate after the second residue addition, Geoderma 285 (2017) 217-224.

[15] Y. Zhang, P. Marschner, Nutrient availability, soil respiration and microbial biomass after the second residue addition are influenced by the C/N ratio of the first residue added, but not by drying and rewetting between residue amendments, Eur. J. Soil Biol. 77 (2016) 68-76.

[16] Y. Zhang, P. Marschner, Residue addition combined with rewetting of dry soil–Effect of timing of residue addition on soil respiration, microbial biomass, nutrient availability and legacy effect, Geoderma 299 (2017) 83-90.

[17] Y. Zhang, P. Marschner, 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, J. Soil Sci. Plant Nutr. 17 (2017) 594-608.

[18] R. Isbell, The Australian soil classification, Vol. 4, CSIRO publishing, 2002.

[19] N. Chowdhury, P. Marschner, R. Burns, Response of microbial activity and community structure to decreasing soil osmotic and matric potential, Plant Soil 344 (2011) 241-254.

[20] A. Walkley, I.A. Black, An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method, Soil Sci. 37 (1934) 29-38.

[21] J. Bremmer, C. Mulvaney, Total N, Methods of Soil Analysis. Part2. 2nd ed. Agron. Monog. 9 (1982) 895-926.

[22] R. Setia, P. Marschner, J. Baldock, D. Chittleborough, P. Smith, J. Smith, Salinity effects on carbon mineralization in soils of varying texture, Soil. Biol. Biochem. 43 (2011) 1908-1916.

[23] E. Vance, P. Brookes, D. Jenkinson, An extraction method for measuring soil microbial biomass C, Soil. Biol. Biochem. 19 (1987) 703-707.

[24] R.B. Willis, M.E. Montgomery, P.R. Allen, Improved method for manual, colorimetric determination of total Kjeldahl nitrogen using salicylate, J. Agri. Food Chem. 44 (1996) 1804-1807.

[25] J. Moore, S. Klose, M. Tabatabai, Soil microbial biomass carbon and nitrogen as affected by cropping systems, Biol. Fertil. Soils 31 (2000) 200-210.

[26] K.M. Miranda, M.G. Espey, D.A. Wink, A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite, Nitric Oxide 5 (2001) 62-71.

[27] K. Kouno, Y. Tuchiya, T. Ando, Measurement of soil microbial biomass phosphorus by an anion exchange membrane method, Soil. Biol. Biochem. 27 (1995) 1353-1357.

[28] J. Murphy, J.P. Riley, A modified single solution method for the determination of phosphate in natural waters, Anal. Chim. Acta 27 (1962) 31-36.

[29] D. Or, B.F. Smets, J. Wraith, A. Dechesne, S. Friedman, Physical constraints affecting bacterial habitats and activity in unsaturated porous media–a review, Adv. Water Resour. 30 (2007) 1505-1527.

[30] R.C. Granstedt, R.C. Huffaker, Identification of the leaf vacuole as a major nitrate storage pool, Plant Physiol. 70 (1982) 410-413.

[31] M.J. McLaughlin, A. Alston, J. Martin, Phosphorus cycling in wheat pasture rotations. II. The role of the microbial biomass in phosphorus cycling, Soil Res. 26 (1988) 333-342.

[32] P. Nye, The effect of the nutrient intensity and buffering power of a soil, and the absorbing power, size and root hairs of a root, on nutrient absorption by diffusion, Plant Soil 25 (1966) 81-105.

[33] M.J. McLaughlin, A. Alston, J. Martin, Measurement of phosphorus in the soil microbial biomass: a modified procedure for field soils, Soil. Biol. Biochem. 18 (1986) 437-443.

[34] R. Bramley, N. Barrow, The reaction between phosphate and dry soil. II. The effect of time, temperature and moisture status during incubation on the amount of plant available P, J. Soil Sci. 43 (1992) 759-766.

[35] E. Craswell, Some factors influencing denitrification and nitrogen immobilization in a clay soil, Soil. Biol. Biochem. 10 (1978) 241-245.

Table 1. Total organic C, N, and P, C/N ratio, C/P ratio, available N and P, water-extractable C, and pH of low C/N (young faba bean) and high C/N (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 organic C (g kg^{-1})	92^{b}	54^{a}
pH (1:10)	6.2	6.3

Figure 1. Cumulative respiration in treatments LH and HL from day 1 to 10 at 10% and 50% WHC (n=4, means ± SE) (a) and from day 11 to 20 (b) in different moisture treatments (treatment names: the first number represents water content from day 1 to 10, the second number represents water content from day 11 to 20 (n=4, means ± SE) (b). Within each period, bars with different letters are significantly different ($P \leq 0.05$).

Figure 2. Microbial biomass N in treatments LH and HL on day 1 (a) and day 10 (b) at 10% and 50% WHC, and day 11 (c) and day 20 (d) in different moisture treatments (for treatment names see Figure 1) (n=4, means ± SE). For each sampling time, bars with different letters are significantly different ($P \leq 0.05$).

Figure 3. Microbial biomass P in treatments LH and HL on day 1 (a) and day 10 (b) at 10% and 50% WHC, and day 11 (c) and day 20 (d) in different moisture treatments (for treatment names see Figure 1) (n=4, means ± SE). For each sampling time, bars with different letters are significantly different ($P \leq 0.05$).

Figure 4. Available N concentration in treatments LH and HL on day 1 (a) and day 10 (b) at 10% and 50% WHC, and day 11 (c) and day 20 (d) in different moisture treatments (for treatment names see Figure 1) (n=4, means ± SE). For each sampling time, bars with different letters are significantly different ($P \leq 0.05$).

Figure 5. Available P concentration in treatments LH and HL on day 1 (a) and day 10 (b) at 10% and 50% WHC, and day 11 (c) and day 20 (d) in different moisture treatments (for treatment names see Figure 1) (n=4, means ± SE). For each sampling time, bars with different letters are significantly different ($P \leq 0.05$).

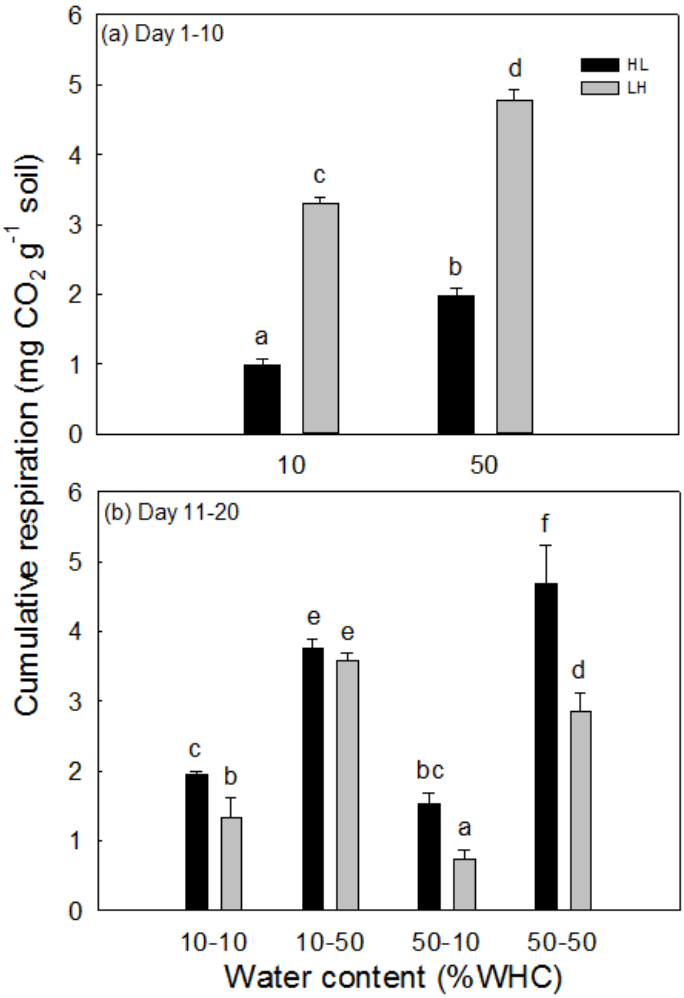
(a) Day 1-10
HL
LH
a
c
b
d
10
50
(b) Day 11-20
c
b
e
e
bc
a
f
d
10-10
10-50
50-10
50-50
Water content (%WHC)
Cumulative respiration (mg CO2 g-1 soil)
0
1
2
3
4
5
6

Figure 1.

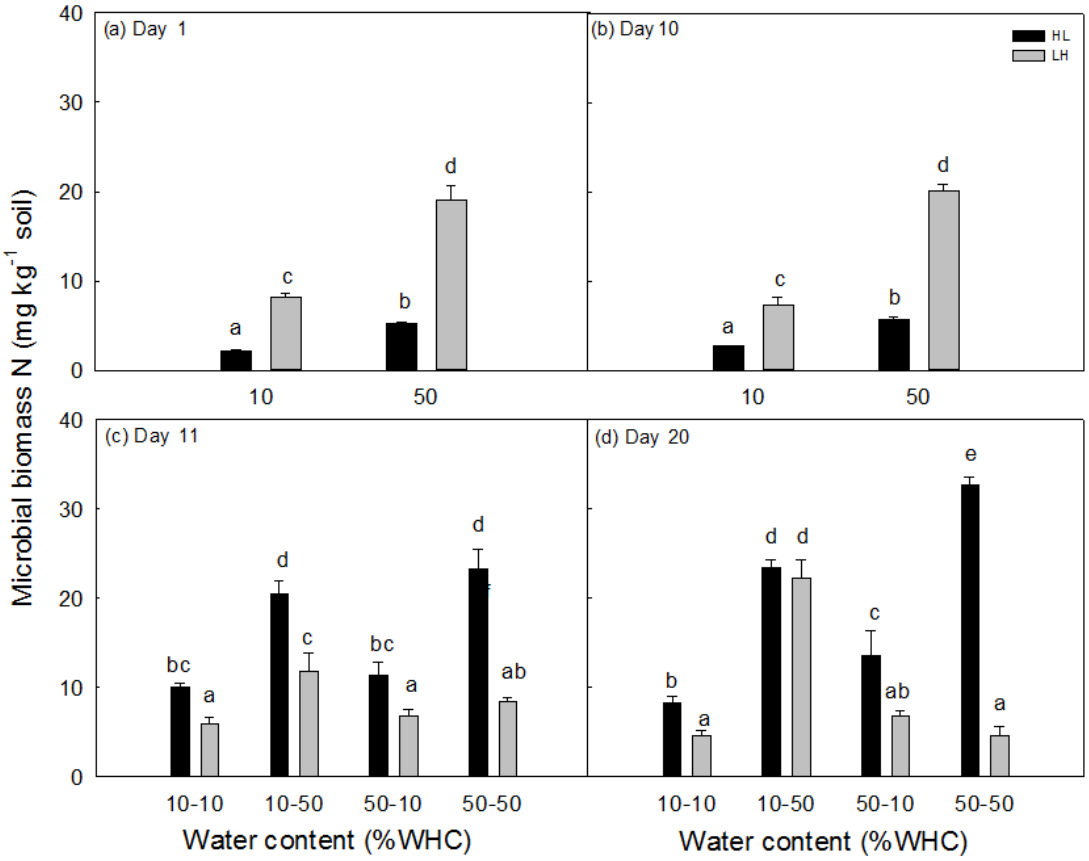
(a) Day 1
(b) Day 10
(c) Day 11
(d) Day 20
HL
LH
Microbial biomass N (mg kg-1 soil)
Water content (%WHC)
10-10
10-50
50-10
50-50
10
50

Figure 2.

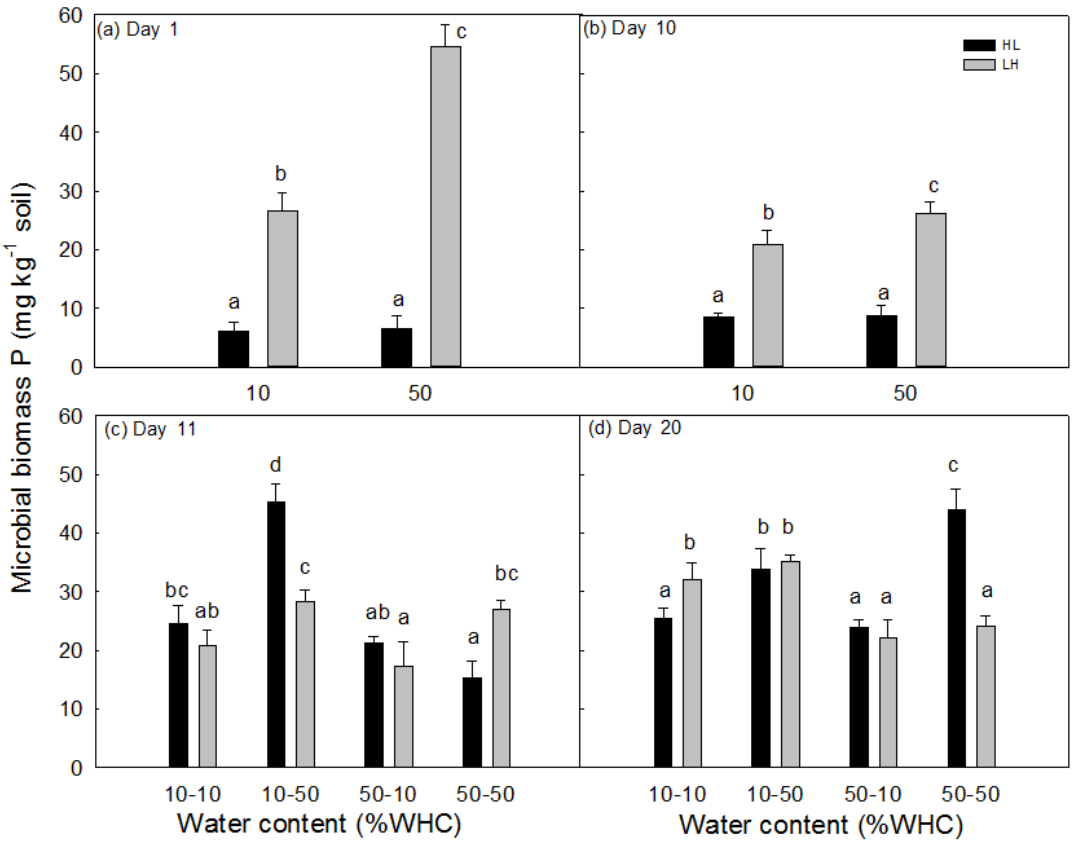
(a) Day 1
(b) Day 10
(c) Day 11
(d) Day 20
HL
LH
Microbial biomass P (mg kg-1 soil)
Water content (%WHC)
10-10
10-50
50-10
50-50
10
50

Figure 3.

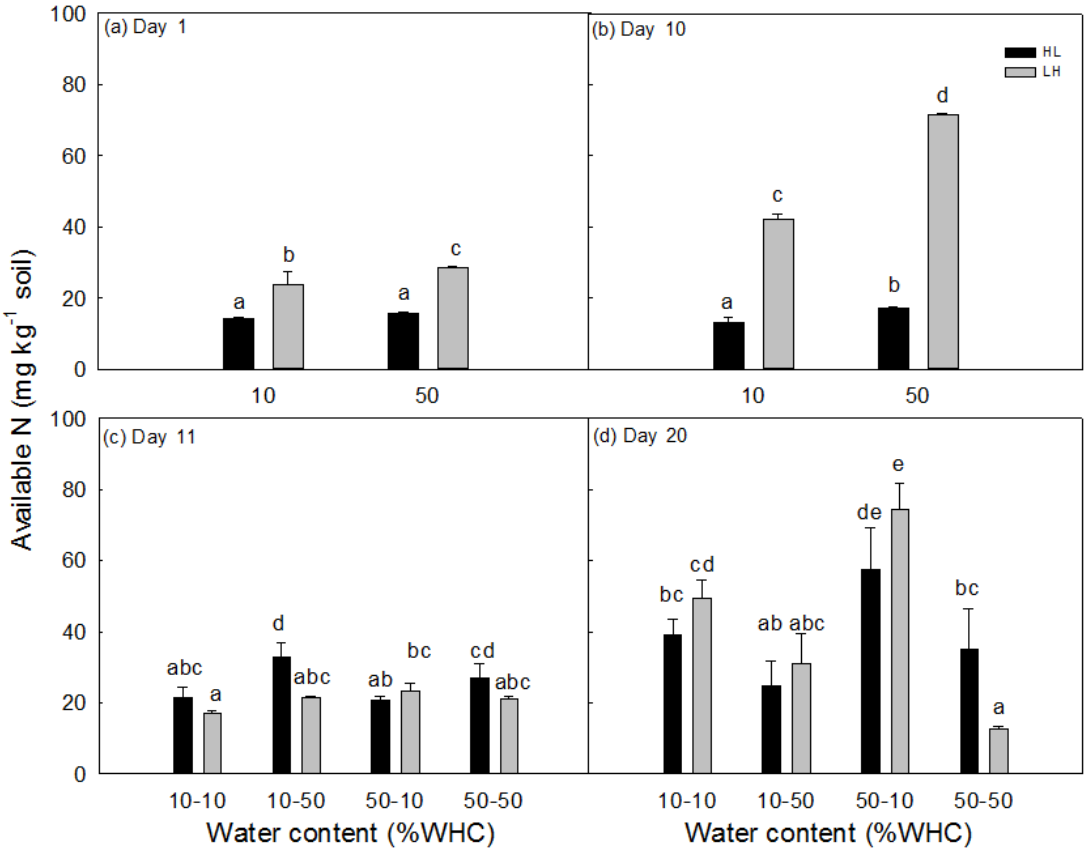
(a) Day 1
(b) Day 10
(c) Day 11
(d) Day 20
HL
LH
Available N (mg kg-1 soil)
Water content (%WHC)
10
50
10-10
10-50
50-10
50-50
a
b
c
d
e
ab
abc
bc
cd
de

Figure 4.

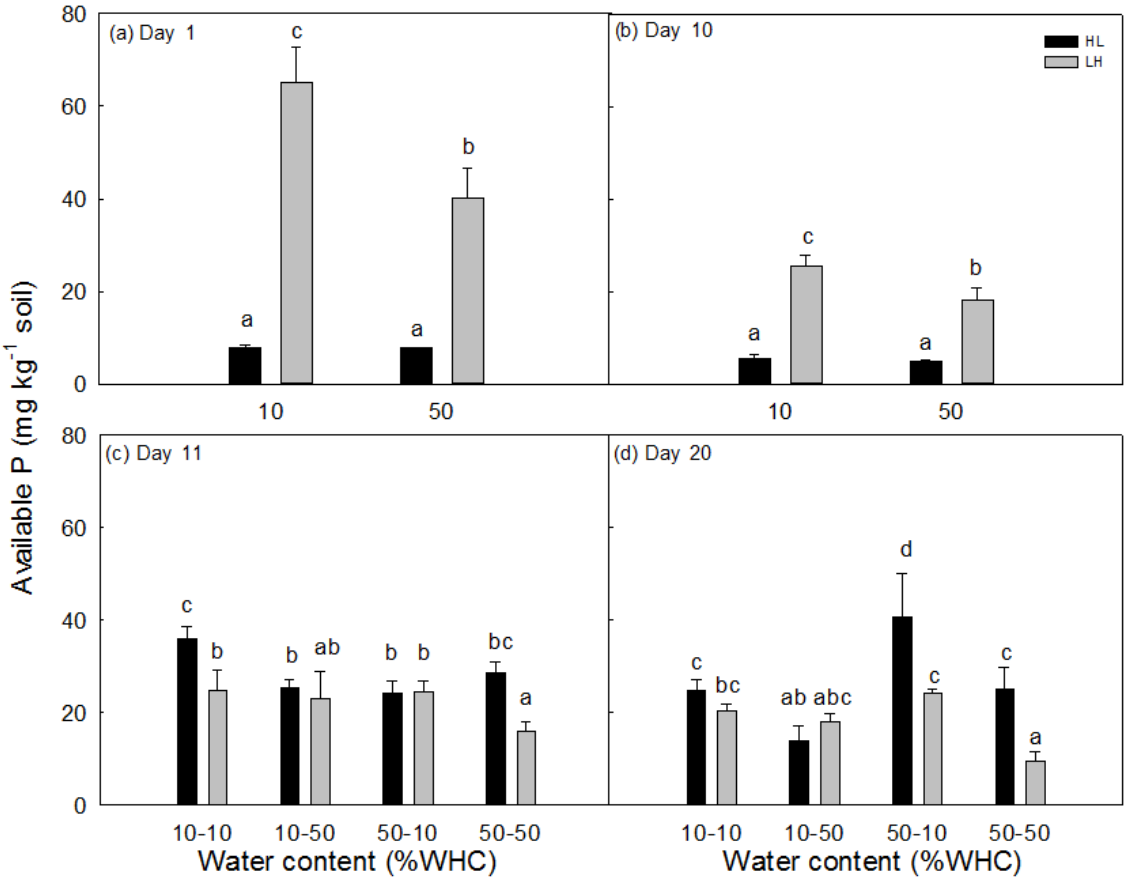
(a) Day 1
(b) Day 10
(c) Day 11
(d) Day 20
HL
LH
Available P (mg kg-1 soil)
Water content (%WHC)
10-10
10-50
50-10
50-50
10
50

Figure 5.

CHAPTER 8

Conclusions and future research

Conclusions

Soil water content varies over time and regulates microbial activity (Harris 1981). Microbial activity depends on soil water content and is maximal at a water content where the limiting effects of substrate diffusion and oxygen supply are equal (Skopp et al. 1990). Low soil water content limits decomposition as it restricts water availability and nutrient diffusion, and at very low water content, it may draw water out of the cells (Schimel et al. 2007). Further, when soil is dry, substrate diffusion is reduced, suppressing microbial activity and biomass (Stark and Firestone 1995; Yao et al. 2011). Rapid rewetting of dry soil induces a sudden increase in water potential which can cause microbial cell lysis, death or release of intracellular solutes (Bottner 1985; Halverson et al. 2000; Linn and Doran 1984). Soils may also undergo less dramatic changes in water content over time in the field.

Nutrient release during decomposition of plant residue is a complex process influenced by both internal and external factors (Parr and Papendick 1978; Scholes and Archer 1997; Swift et al. 1979). Decomposed plant residues may be single species or different species either added at the same time or sequentially (Hooper and Vitousek 1997).

The legacy effect is defined as nutrient availability and microbial biomass after the second residue addition are influenced by the C/N ratio of the previously added residue. In this project, we investigated how the drying-rewetting and previous lower water content influence soil respiration, microbial biomass and nutrient availability and the legacy effect. This project also studied how soil respiration, microbial biomass and nutrient availability influenced by both previous and current soil water content with or without organic amendment differing in C/N ratio.

The experiments in Chapters 2 and 3 investigated the effect of drying-rewetting on decomposition of residues differing in C/N ratio. In the experiment described in Chapter 2, soil

was amended twice (days 0 and 32) with plant residues with either high (H) or low (L) C/N ratio resulting in the treatments LH or HL. Between the first and the second residue addition the soil was maintained at 50% WHC or exposed to one, two or four DRW cycles. After the second residue addition all treatments were kept at 50% WHC until day 64. Only drying and rewetting cycles up to four cycles between residue additions stimulated decomposition of the freshly added low C/N ratio residue compared with other water regimes, but the effect was transient as moisture treatment did not influence available N and MBN from day 48 onwards (8 days after the second residue amendment). Therefore, the experiment showed that drying-rewetting influenced soil respiration, nutrient availability and microbial biomass in residue amended soils, but drying-rewetting between residue additions did not change the legacy effect of the first residue addition (Fig. 1).

However, the study described in Chapter 3 (residue addition upon rewetting on microbial activity, biomass and nutrient availability) showed that the effect of rewetting on decomposition was influenced by the timing of residue addition relative to rewetting. Soil was exposed to two wet-dry cycles with 5 days moist and 5 days dry each. Residues with high (H) or low C/N ratio (L) were added in eight residue treatments at different rates (10 or 20 g kg^{-1} soil) and timing (day 0 or day 10, before rewetting). Rewetting increased nutrient release from previously added low C/N residue when added at high rate. And compared to residue in constantly moist soil, addition of residue upon rewetting enhanced microbial C and N uptake and reduced the legacy effect of the previously added residue.

Chapters 2 and 3 showed that drying-rewetting only slightly influenced the legacy effect of the first residue addition. Therefore, in Chapter 4, we investigated how current and previous water content after rewetting influences soil respiration, microbial biomass and nutrient availability in unamended soils. Soil was exposed to two wet-dry cycles with rewetting soil to 50%, 30% or 10% WHC on days 0 and 8. All treatments were rewet to 50% WHC on

day 16 and maintained at this water content for 7 days. Respiration and microbial biomass after day 16 were greater in soil previously rewet to 10% WHC compared to that rewet to 50% WHC. The higher respiration and microbial biomass after rewetting to 10% WHC can be explained by low water availability in the first 16 days. Therefore, more available substrate remained after the first two rewetting events compared to rewetting to 30 or 50% WHC during this time (Fig. 2).

To further investigate the effect of drying and rewetting on nutrient release, the experiment described in Chapter 5 was carried out. The aim was to determine the effect of soil water content and number of days between rewetting of dry soil and second residue addition on soil respiration, microbial biomass and nutrient availability after the first and second residue addition. In this study, soil was amended with residues on day 0 and 10. The soil was maintained at 10% or 50% WHC for 10 days after which the soil at 10% WHC was rapidly rewetted to 50% WHC. The second residue which had a different C/N ratio than the first one was added one, two or five days after rewetting. Two and ten days after the second residue addition, MBN was higher in soil amended one day after rewetting than when the second residue was added five days after rewetting indicating enhanced N immobilization. However, neither previous soil water content nor time between rewetting and the second residue addition influenced the legacy effect (Fig. 1).

In Chapters 6 and 7, the effect of previous and current soil water content on soil respiration, microbial biomass and nutrient availability in soil amended with residue differing in C/N ratios was determined. In the experiment described in Chapter 6, residues were added on days 0 and 10. There were three soil water contents (10, 30 or 50% WHC) from day 1 to day 10; and after sampling on day 10, soil water content was adjusted to 50% WHC and residues added. Therefore, the water content treatments were 10-50, 30-50 and 50-50. Water content between the first and second residue amendment affected the extent of the legacy effect

after the second residue addition. Compared to 50% WHC, 10% WHC after the first residue addition reduced residue decomposition, particularly the low C/N residue and therefore enhanced its legacy effect after addition of high C/N residue (Fig. 3).

In Chapter 7, the effect of water content after each amendment on soil respiration, microbial biomass and nutrient availability was studied. In this experiment, soil was amended with residues also on day 0 and day 10 and the soil was at 10 or 50% WHC from day 1 to day 10, but unlike the experiment described in Chapter 6, the water content from day 10 to 20 was either 10 or 50 % WHC, not only 50%. Therefore, the water content treatments were 10-10, 10-50, 50-10 and 50-50. After the second amendment in a given residue treatment, the current water content had a stronger effect on respiration, microbial biomass and nutrient availability than the previous water content. Further, the influence of the previous water content on respiration and microbial biomass was stronger when the first amendment was low C/N residue because its decomposition was more strongly affected by water content than that of high C/N residue (Fig. 4).

In summary, the experiments showed that drying-rewetting cycles between first and second residue addition influenced soil respiration, microbial biomass and nutrient availability but had little effect on the legacy effect. However, the addition of residue upon rewetting compared to residue addition several days after rewetting enhanced microbial C and N uptake and reduced the legacy effect of the previously added residue. Further, a low soil water content between the first and the second residue amendment reduced the decomposition of the first residue and therefore resulted in a greater amount of the first residue left in the soil when the second residue was added. If a large amount of previously added residue is left in the soil when the second residue is added, the legacy effect is strong, particularly if low C/N residue is followed by high C/N residue.

Conceptual model

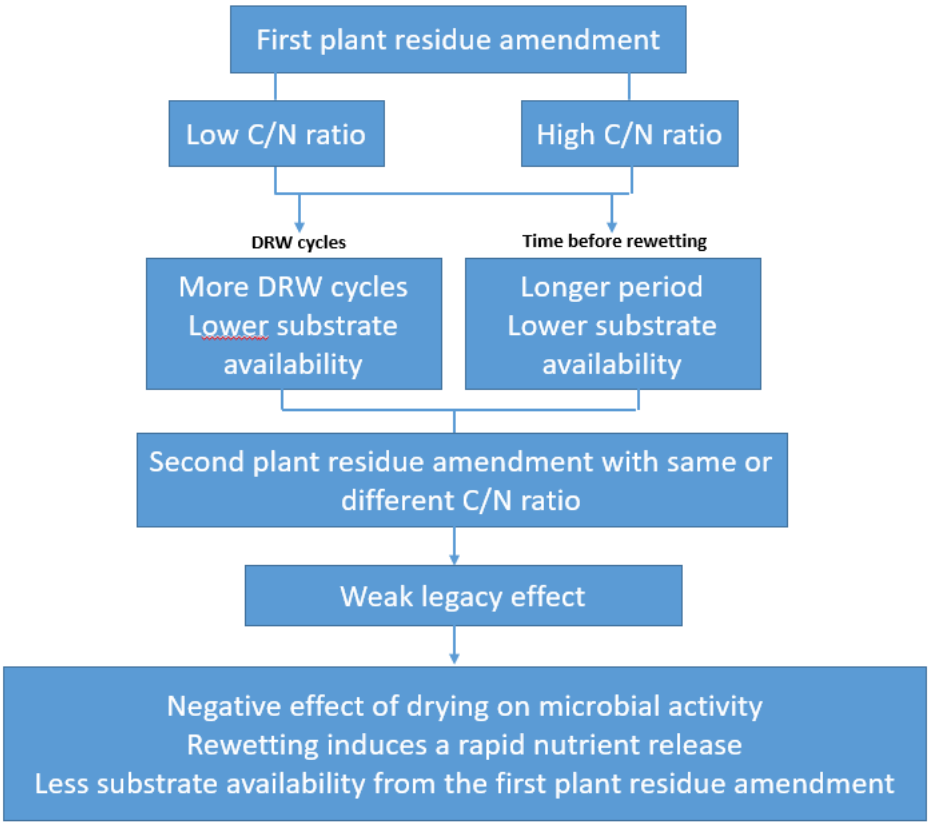

Fig. 1 Conceptual model of the influence of drying-rewetting on legacy effect in soil amended with plant residues

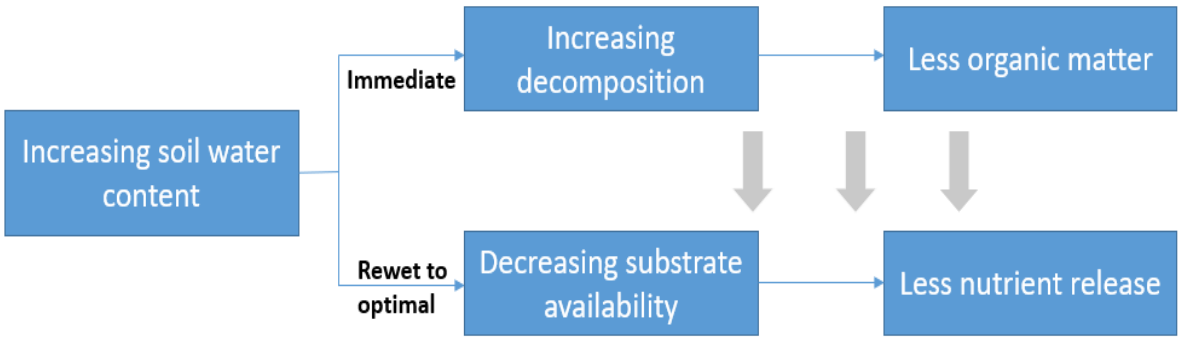

Fig. 2 Conceptual model of the influence of increasing soil water content on decomposition process in unamended soil

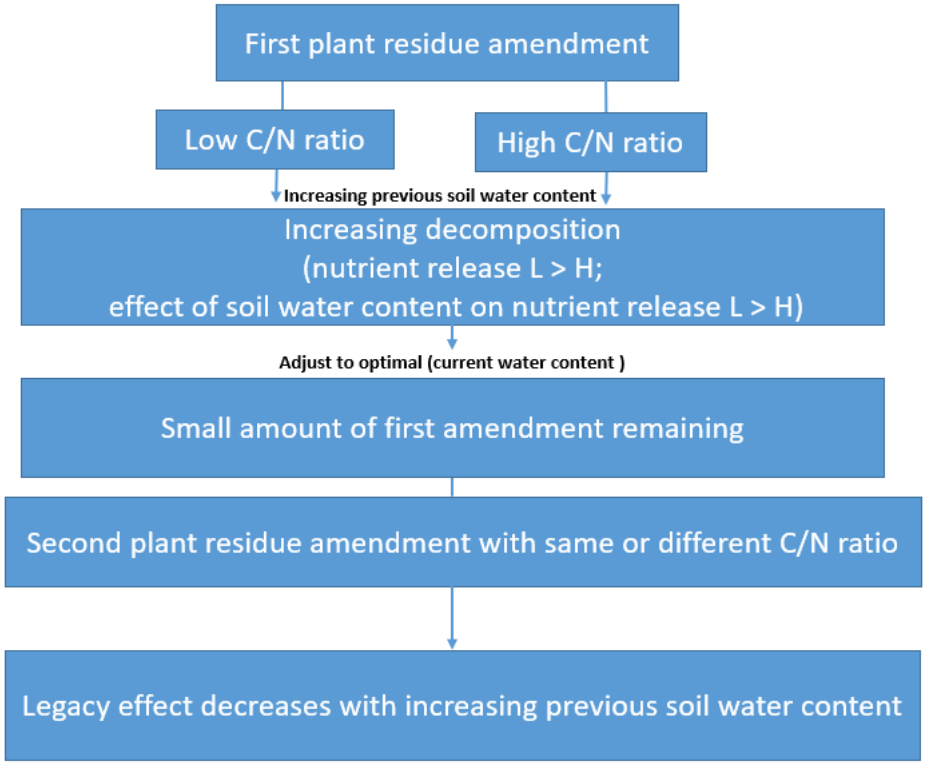

Fig. 3 Conceptual model of the influence of increasing previous soil water content on legacy effect in soil amended with plant residues

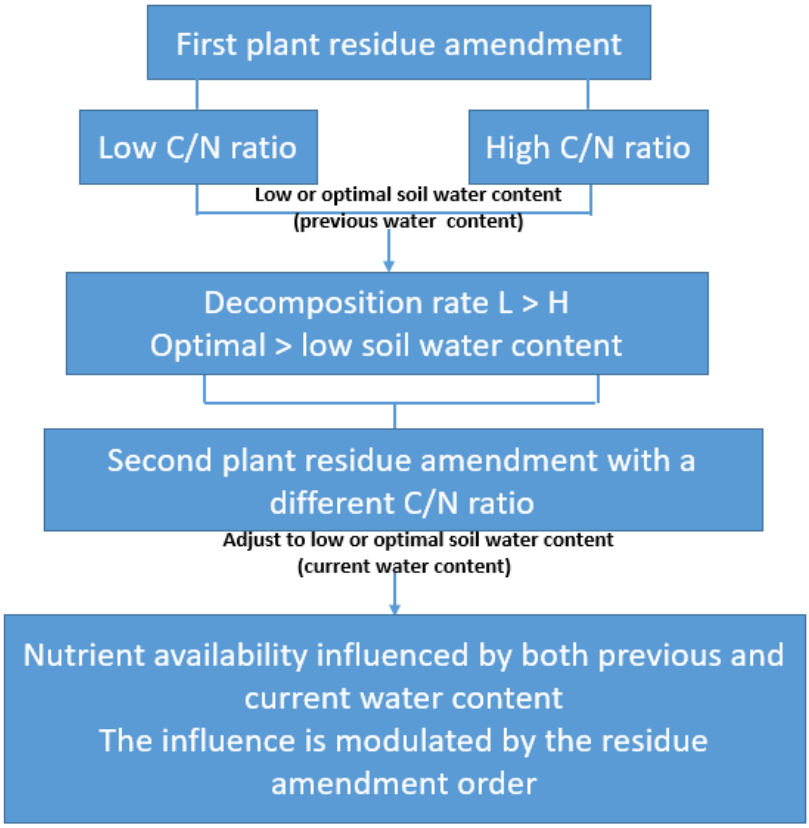

Fig. 4 Conceptual model of the influence of different water content after the second residue addition on legacy effect in soil amended with plant residues

Future research

The experiments in this thesis about drying-rewetting or different water regimes were quite short (about a month). The period between first and second residue addition was short as it may occur in natural ecosystems in autumn. However, in Mediterranean climate, soils may remain dry for weeks or months and soils may remain moist for several weeks. Such longer dry or moist periods may have a different effect on microbial biomass and nutrient cycling. Longer periods between residue additions (several weeks to months) would better reflect conditions in agriculture after harvest or green manuring.

In the current study, the rapid rate of drying in all incubations was different from the field where soils dry more slowly and may also be rewet more slowly. Slower drying may give microbes time to adjust to lower water availability which could reduce the negative impact of drying on microbial activity. However, the short-term drying-rewetting cycles and the rewetting events showed the general principles of how nutrient availability and microbial activity are influenced by soil water content. Future incubation experiments could be conducted mimicking changes in soil water content in the field or be conducted under field conditions. Field experiments could also include the effects of plant roots and soil animals on nutrient cycling.

The source of available nutrients and those in the microbial biomass was not determined in these experiments. For example, the source of available or microbial biomass nitrogen could be native soil organic matter, microbial biomass turnover (Mary et al. 1996) or added residues. And after the second residue addition, the nutrient source could be either the first or second residue. In our experiments, the priming effect of the second residue amendment on the first residue amendment may have been underestimated. For example, the second plant residue may increase the decomposition of the first plant residue remaining in the soil. To better understand C and N dynamics in residue amended soil under different soil moisture regimes and assess priming, ^{13}C and ^{15}N labelled residue should be used in future experiments.

Most C and N transformation processes involved in residue amended soils are mediated by soil microbes. However, in the current PhD project, the related microbial community ecology to the nutrient availability were not studied. Steps in N mineralisation, such as ammonification and nitrification, are mediated by specialized microbial groups (Hayatsu et al. 2008). It is also of interest to better understand the links between nutrient cycling processes and microbial communities in residue amended soils with variable soil water content. For example, the abundance of functional genes such as ammonia monooxygenase gene *amoA* and

the nitrite reductase genes *nirK* and *nirS* during the decomposition process with variable soil water content may allow better understanding of N cycling process in residue amended soils.

The experiments have shown increased nutrient availability after lower soil water content. However, the impact of this on plant nutrient availability remains unclear. In experiments with plants, the effect of sowing time after residue addition and changes in soil water content could be studied in a glasshouse or field trails. These experiments could also use ^{15}N labelled residue to investigate the source of N in plants.

References

Bottner P (1985) Response of microbial biomass to alternate moist and dry conditions in a soil incubated with ^{14}C- and ^{15}N-labelled plant material. Soil Biology and Biochemistry 17:329-337

Halverson LJ, Jones TM, Firestone MK (2000) Release of intracellular solutes by four soil bacteria exposed to dilution stress. Soil Science Society of America Journal 64:1630-1637

Harris RF (1981) Effect of water potential on microbial growth and activity. In: Water potential relations in soil microbiology (eds J.F. Parr, W.R. Gardner & E.T. Elliott), pp. 23-95. Special Publication No 9. Soil Science Society of America, Madison, WI.

Hayatsu M, Tago K, Saito M (2008) Various players in the nitrogen cycle: diversity and functions of the microorganisms involved in nitrification and denitrification. Soil Science and Plant Nutrition 54:33-45

Hooper DU, Vitousek PM (1997) The effects of plant composition and diversity on ecosystem processes. Science 277:1302-1305

Linn D, Doran J (1984) Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. Soil Science Society of America Journal 48:1267-1272

Mary B, Recous S, Darwis D, Robin D (1996) Interactions between decomposition of plant residues and nitrogen cycling in soil. Plant and Soil 181:71-82

Parr J, Papendick R (1978) Factors affecting the decomposition of crop residues by microorganisms. p. 100-129. *In* W.R.Oschwald (ed.) Crop residue management systems. ASA Spec. Publ.31. ASA, CSSA, and SSA, Madison, WI.

Schimel J, Balser TC, Wallenstein M (2007) Microbial stress-response physiology and its implications for ecosystem function. Ecology 88:1386-1394

Scholes R, Archer S (1997) Tree-grass interactions in savannas. Annual review of Ecology and Systematics 28:517-544

Skopp J, Jawson M, Doran J (1990) Steady-state aerobic microbial activity as a function of soil water content. Soil Science Society of America Journal 54:1619-1625

Stark JM, Firestone MK (1995) Mechanisms for soil moisture effects on activity of nitrifying bacteria. Applied and Environmental Microbiology 61:218-221

Swift MJ, Heal OW, Anderson JM (1979) Decomposition in terrestrial ecosystems. Blackwell Scientiic Publications Ltd., Oxford.

Yao SH, Zhang B, Hu F (2011) Soil biophysical controls over rice straw decomposition and sequestration in soil: the effects of drying intensity and frequency of drying and wetting cycles. Soil Biology and Biochemistry 43:590-599