The Effect of Single Heating on Soil Microbial Activity and Nutrient Cycling

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

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February 2020

Dedicated to My Beloved Parents

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Abstract

The effect of soil heating during forest fires (often >100 °C for several hours) on soil microbes and nutrient availability has been studied extensively. Less is known about the effect of fast-moving fires with low fuel load where soils reach 50 to 100 °C for a few minutes. In this thesis, the effect of heating soil to 60 °C within 1 h and then maintaining this temperature for 30 minutes was studied. Soils were rewetted after cooling to room temperature.

Heating of soils usually induces drying, but it is unclear if the effect of heating is only due to this water loss or if other factors are also important. An experiment included heated soils, constantly moist controls and air-dried soils which were dried at 30 °C to the same water content as the heated soils. Heating increased cumulative respiration and available N after rewetting about three-fold compared to the constantly moist control and the air-dried soils.

To assess how the effect of heating is influenced by amendment type or time between amendment application soil was amended with the same amount of total N and P as pea residue or inorganic N and P either eight or one day before heating. Heating only reduced respiration when residue was added one day before heating. Heating increased available N on day 10 in the unamended soil or with fertiliser by about 20% and in residue treatments about 10-fold, particularly when residue was added one day before heating.

To assess the effect of a second heating event, soils were heated once on day 8 (H8) or heated again 4, 8 and 16 days after the first heating event (H8-12, H8-16 and H8-24). Compared to unheated soil, cumulative respiration was about 10 and 20% higher in H8 and H8-12 and H8-16, but 30% higher in H8-24. The first heating increased available N and P by 25%. The second heating induced a further increase in available N and P compared to the first heating.

To assess the effect of plants, soil was planted with wheat for 4 weeks or left unplanted. The heating-induced increase in initial respiration rate, available N and P was greater in previously planted soil than unplanted soil.

The last chapter includes two experiments. In the first experiment, soils were constantly moist or exposed to a drying-rewetting event before heating. Prior drying and rewetting had no effect on the impact of heating on respiration and nutrient availability. In the second experiment, non-saline soil was salinised to $EC_{1:5}$ 1 and 4 ds m^{-1} (referred to as NS, S1 and S4). After one month and pea residue addition (10 g kg^{-1}), soil was incubated for 5 days, then heated. In moist soil, S4 reduced cumulative respiration but increased available N and P compared to NS and S1. Heating reduced cumulative respiration more in S4 than NS and S1. Compared to unheated treatments, available N in heated NS was up to ten-fold higher, but only three-fold in S4.

Declaration

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Acknowledgements

First and foremost I would like to express my sincere gratitude to my principal supervisor, Prof Petra Marschner. I was very fortunate to be her student. There are no words to thank her invaluable guidance, kindness and support during my PhD research. I thank her for helping me to obtain a deeper understanding of soil microbial activity and mechanisms behind soil nutrient cycling, which will be helpful for me to continue research after going back to my country. I am grateful to meet a great person like her.

I am also grateful to my co-supervisor, Dr Ashlea Doolette for her valuable suggestions and comments for my experiments and writing.

I would like to thank Mr. Colin Rivers for his help in collection of soil and technical assistance.

I am grateful to University of Adelaide for awarding me the Turner family scholarship and giving me this valuable opportunity to study at Adelaide University.

Many thanks go to my dear friends; Sonia, Keen, Monica, Gavers, Alamgir, Ha, Xuan Ivan, and Kennie. Your great help and company always encouraged me during my study here.

Most sincerely I would like to thank my parents for their love and encouragement through out of the journey.

Mihiri Seneviratne

February 2020

Publications arising from this thesis

1. Seneviratne, M., Doolette, A., Marschner, P., 2019. Impact of a short heating event followed by rewetting on soil respiration and nutrient availability is not only due to soil drying during heating. Biology and Fertility of Soils 55, 553–564.

2. Seneviratne, M., Marschner, P., 2019. Soil respiration and nutrient availability after short heating followed by rewetting differ between first and second heating and are influenced by the interval between heating events. Soil Biology and Biochemistry107537.

3. Seneviratne, M., Doolette, A., Marschner, P., 2020. Amendment type and time of addition influence the effect of short-term heating on soil respiration and nutrient availability. Journal of Soil Science and Plant Nutrition 1-8.

4. Seneviratne, M., Alamgir M., Marschner, P., 2020. Impact of heating and rewetting on soil respiration and nutrient availability is enhanced by prior growth of plants. Journal of Soil Science and Plant Nutrition 1-8.
5. Seneviratne, M., Marschner, P., 2020. Soil respiration and nutrient availability after heating are influenced by salinity but not by prior drying and rewetting. Biology and Fertility of Soils https://doi.org/10.1007/s00374-020-014450.

Chapter 1

Introduction and Literature Review

1.1 Introduction

One third of the earth's land surface is an arid or semi-arid environment (Schimel et al., 2007). In these regions, soils can be exposed to high temperatures (50-80°C) and drought (<300 mm annual rainfall) (Sun et al., 2018; Beringer et al., 2016). High soil temperatures (> 50 °C) can occur as result of solar irradiation (diurnal heating) or fire which subsequently can also dry soils.

It is generally accepted that if there are no other limiting factors, microbial activity increases between 0°C and about 40°C (Richardson et al., 2012; Zhou et al., 2014). During forest fires, soil temperatures range between 100 and 500 °C for minutes or hours (Soto 1991). Such high temperatures can kill a proportion of the microbial biomass and change nutrient availability (Barcenas- Moreno et al., 2009; DeLuca and Zouhar, 2000). However, less is known about how short exposure (<2 h) to temperatures between 50 and 100 °C, which can occur in fast-moving fires with low fuel load (Scotter, 1970; Stoof et al., 2013) influences soil microbes and nutrient availability.

Heating dries soil which reduces water availability to microbes as the water film around soil particles held more tightly in dry soil (Ilstedt et al., 2000). Some microbes can tolerate drying by the accumulation of osmolytes, production of exopolysaccharides or by dormancy (Chenu and Roberson, 1996; Jones and Lennon, 2010; Warren, 2016). Periods of drought can be interrupted by rainfall events which can pose a stress on microbes due to the sudden increase in water availability. This can kill a proportion of the microbial biomass (Bottner, 1985; Van Gestel et al., 1993). On the other hand, rewetting dry soils increases the substrate availability for surviving microbes via released osmolytes, dead microbial biomass and exposed organic matter through slaking of aggregates (Halverson et al., 2000; Denef et al., 2001; Warren, 2014). This induces a flush of respiration and nutrient availability after rewetting of dry soil (Birch, 1958; Franzluebbers et al., 2000; Fierer and Schimel, 2002; Mikha et al., 2005).

The response of microbes to soil temperature and water content is influenced by substrate availability. Generally, the availability of soil organic matter is low. Therefore the addition of organic substrates can increase microbial activity (Hadas et al., 2004; Ferreras et al., 2006; Flavel and Murphy, 2006). Organic substrates can be added via plants through root exudates, root death and litter incorporation (Walker et al., 2003; Marschner, 2012) which not only increases substrate availability for microbes but can also influence nutrient availability. This can change the effect of heating or rewetting on soil microbes due to greater substrate availability after stress (Xiang et al., 2008), but also because active microbes are usually more susceptible to stress than dormant ones (Van Gestel et al., 1993; Schimel et al., 2007).

This literature review will discuss the effect of several soil properties on soil microbes and nutrient availability before focussing on the effect of temperature, soil water content and substrate availability. It will then summarize the knowledge gaps that will be addressed in the thesis followed by an outline of the thesis.

1.2 Factors affecting microbial activity

A number of factors affect microbial activity such as soil water content, substrate and nutrient availability, salinity, heavy metals, organic and inorganic pollutants. In this literature review the influence of soil water content, salinity, substrate and nutrient availability on microbial activity will be discussed, because they are included in this PhD project. In the experiments, soils were exposed to 60 °C which dried the soil. Therefore the effects of drying and rewetting and high temperatures on microbial activity will be discussed in greater detail. (Section 1.3 and 1.4)

1.2.1 Soil water content

Soil water content strongly influences microbial activity because it is a medium for nutrient transport, cellular biochemical reactions and helps to maintain the turgor pressure inside cells. When dry soil is wetted, microbial activity increases with soil water content up to a point due to an increase in substrate availability. But any further increase in water content decreases microbial activity due to a limitation of oxygen availability (Linn and Doran, 1984)(Fig. 1).

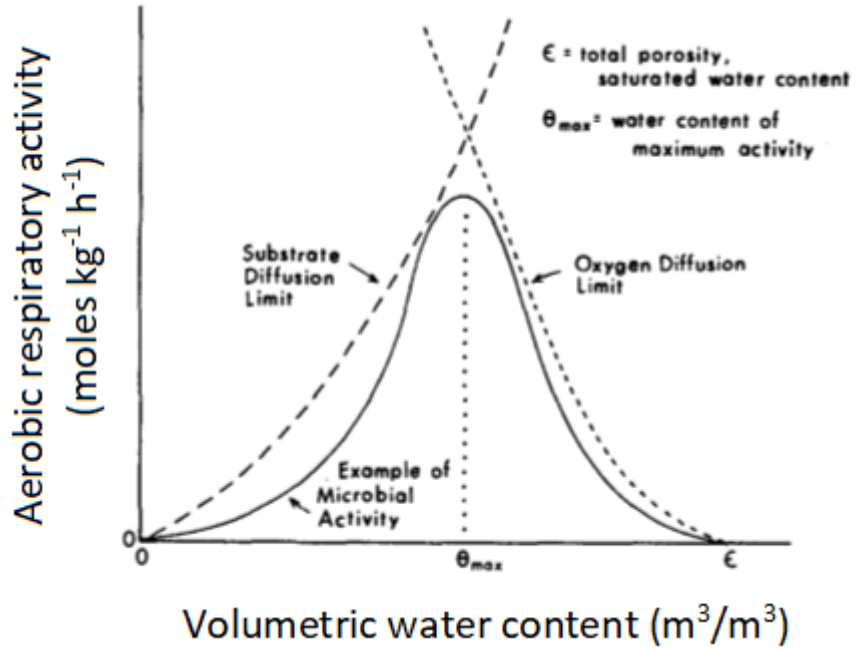

Fig. 1 Conceptual plot of microbial activity as a function of soil water content. (Skopp et al., 1990).

Soil water can be expressed as volumetric or gravimetric water content, percentage water holding capacity or water potential. Volumetric and gravimetric water content, and percentage water holding capacity indicate the amount of water in a soil while water potential is a measure of water availability. A soil water content equivalent to 50%-70% of maximum water holding capacity (WHC) is optimal for microbial activity such as ammonification, nitrification, organic matter decomposition and respiration (Greaves and Carter, 1920; Rixon and Bridge, 1968; Pal and Broadbent, 1975). When the soil water content is greater than 100% WHC (saturated soil), microbial activity is low due to limited oxygen availability. However, anaerobic microbial activity, e.g., denitrification increases at low oxygen availability (Mohn et al., 2000). The influence of drying and rewetting on microbial activity and nutrient availability will be discussed in greater detail below (Section 1.3).

1.2.2 Temperature

The optimum temperature for microbial activity depends on the type of microbe; psychrophilic microorganisms have an optimum temperature for growth below about 20 °C. For mesophilic microbial activity it is around 30-40 °C, thermophilic microbes can grow at temperatures above 50°C (Farrell and Rose, 1967). However, in general the optimum temperature for soil microbial activity is 25-30 °C. The increase in microbial activity between 0-30 °C has been well documented in previous studies (Fang and Moncrieff, 2001, Lloyd and Taylor, 1994). Higher temperatures (>30 °C) will be discussed in detail below (Section 1.4).

1.2.3 Organic soil amendments

Substrate availability in soil is an important factor affecting microbial activity. It can be increased by the addition of organic amendments e.g. plant residue, manure. Incorporation of plant residues can provide readily available nutrients particularly C, N and P. It also improves soil physical and chemical properties, such as soil structural stability, water retention, cation exchange, aeration and buffer capacity (Baldock, 2007; Diacono and Montemurro, 2011) which enhances microbial activity.

Moreover composition, particle size and placement of the amendments influence microbial activity. Plant residues are composed of simple and complex organic components (Kögel-Knabner, 2002). Simple organic molecules such as sugars, organic acids and amino acids are rapidly decomposed by microbes. But decomposition of complex organic molecules, e.g., lignin, tannins, cellulose, hemicellulose, is comparatively slow (Vanlauwe et al., 1996). The composition and relative abundance of these components vary depending on maturity and part of the plant (Martens, 2000). For an example the lignin content is higher in mature plants

than young plants. Polysaccharides, hemicellulose and pectin are the major constituent of plant residues and can be decomposed by bacteria and fungi (Romaní et al., 2006) (Sinsabaugh et al., 1991; Kögel-Knabner, 2002). Lignin is the second most abundant macromolecule in plant cell walls. Due to its complex ring structure lignin is comparatively resistant to decomposition. Therefore high lignin content reduces the decomposition rate of organic materials (Kirk, 1975; Kirk and Chang, 1975; Swift et al., 1979). Due to the high polysaccharide content, the C/N and C/P ratios of plant residues are comparatively higher than that of microbes. In order to maintain their growth and metabolic activities microbes contain relatively higher amounts of N and P. Therefore, amendment of plant residues with low C/N and C/P ratio enhances net mineralization increasing nutrient availability, while plant residues with high C/N and C/P ratio can result in at least temporary net immobilization reducing nutrient availability (Paul, 2014). For example, plant residues with C/N < 20 and C/P < 200 enhance net mineralization while C/N > 20 and C/P > 200 enhance net immobilization (Smith, 1993; Hadas et al., 2004).

Particle size influences the decomposition rate by changing the surface/volume ratio. A high surface/volume ratio increases decomposition rate (Angers and Recous, 1997). Therefore smaller residue particles decompose more quickly than larger ones (Amato et al., 1984; Jensen, 1994; Ambus and Jensen, 1997).

Soil-incorporated residues decompose at a faster rate than residues on the soil surface due to the improved interaction between residue and soil microbes (Christensen, 1986). Placing the residue on the soil surface limits soil-residue interactions and slows microbial colonization and water adsorption by the residue, which leads to lowering the rate of decomposition. For example, mass loss after addition of cereal straw was 66% for soil-mixed straw and 13% for straw placed on the soil surface (Curtin et al., 2008).

1.2.4. Rhizosphere

Rhizosphere is the soil surrounding the root that is influenced by the root (Philippot et al., 2013). Due to root exudates, root cell lysates and turnover of a larger microbial biomass organic C availability in rhizosphere soil is higher than the bulk soil where microbes are often limited by organic C availability.

Due to rhizodeposition and plant nutrient uptake, the physical, chemical and biological properties of rhizosphere differ from the bulk soil (Smalla et al., 2001). About 20%-50% of the C assimilated via photosynthesis is translocated to the root in annual plants (Kuzyakov and Domanski, 2000). Rhizodepositions include sloughed-off cells and tissues from roots, mucilage and root exudates. Among them, root exudates have the greatest stimulatory effect on microbial growth and activity (Kraffczyk et al., 1984; Lynch and

Whipps, 1990). Root exudates can be low molecular weight substances (amino acids, organic acids, sugars) or high molecular weight substances polysaccharides and proteins) (Walker et al., 2003). Composition and abundance of different components in root exudates depends on plant species, maturity, soil water content, temperature and plant nutritional status (Rovira, 1959; Reid, 1974; Grayston et al., 1997; Marschner, 1998). Compared to bulk soil, the organic C input via plant roots stimulates higher microbial activity and biomass in the rhizosphere (Ames et al., 1984) and changes microbial community composition (Bowen and Rovira, 1991; Söderberg et al., 2004). By increasing microbial activity, decomposition of native organic matter can be increased in the rhizosphere, i.e. rhizosphere priming (Bottner et al., 1988; Dormaar, 1990). Kuzyakov et al. (2000) reported that microbial biomass N was about two-fold higher in planted than unplanted soil. Moreover, root exudates activate symbiotic microbes by secreting signalling compounds; e.g. flavonoids in root exudates of legumes induce nodulation genes of Rhizobium meliloti (Peters et al., 1986). Nutrient uptake as well as mobilisation of nutrients via root exudates can deplete nutrients in the rhizosphere. For example, available N and P were lower in planted than unplanted soil (Gahoonia and Nielsen, 1992; Gahoonia et al., 1994).

Due to the effect of roots on microbial activity and community composition, the effect of stress may be different in planted compared to unplanted soil. The increased microbial activity may make rhizosphere microbes more susceptible to stress. On the other hand, the greater substrate availability in planted soil may aid recovery after stress (Oren, 2001; Wichern et al., 2006).

1.2.5 Salinity

Salinization is due to the accumulation of water soluble salts in soil which affects soil physical, chemical and biological properties. Salinity can be natural or artificial (Ghassemi et al., 1995). Natural salinity is due to salt-rich parental material and saltwater intrusion. Artificial salinity is human-induced, such as poor irrigation management, insufficient drainage, inorganic fertilizers and amendments such as gypsum (Yan et al., 2015). Saline soils are characterized by an electrical conductivity of the saturation paste EC_e> 4 dSm^{-1} with pH <8.5 (United States Salinity Laboratory Staff (1954). In temperate humid climates salinity affects smaller areas e.g. mainly in salt marshes. However it is very common is arid and semi-arid climates where salt is not leached out of the soil profile by rain (Shannon, 1996). About 6% of total land area in the world is salt affected. According to (Rengasamy, 2006) most of the saline soils in Australia are caused by dryland salinity. Soil salinity becomes a major concern in global agriculture when salts are accumulated above the level which can adversely affects crop production. High salinity affects plants in two ways; higher soil water potential reduces plant water uptake and accumulation of specific ions leads to ion toxicity and oxidative stress

(Shannon, 1996). High soil salinity reduces plant dry biomass, plant height, leaf area, leaf number, chlorophyll content and crop yield (Sultana et al., 1999; Cicek and Cakirlar, 2002).

Salinity also adversely affects soil microbes. High osmotic potential can induce exo-osmosis and cell lysis and thus reduce microbial biomass and respiration (Batra and Manna, 1997; Pathak and Rao, 1998). Due to the high osmotic potential and low organic matter content, microbial biomass and activity are lower in saline soil than in non-saline soil (Sarig et al., 1996; Yuan et al., 2007b; Wong et al., 2009; Rousk et al., 2011). With respect to N mineralization, nitrification is more sensitive to salinity than ammonification (Westerman and Tucker, 1974; McClung and Frankenberger, 1985, 1987). However microbes can adapt to salinity (Paul and Nair, 2008; Sagot et al., 2010; Paul and Lade, 2014), for example by accumulation of compatible osmoregulatory substances such as glycine betaine, amino acids (e.g. proline and glutamine). Osmoregulatory substances increase the intracellular solute concentration and stabilize cellular macromolecules (Le Rudulier and Bouillard, 1983; Parente and Silva, 1984; Csonka, 1989). However osmolyte synthesis is energy-demanding, which is a metabolic burden for microbes. The ability to cope with salinity differs among microbes (Llamas et al., 2008); (Mandeel, 2006). Therefore salinity influences microbial community composition. Increasing salinity induces a shift in microbial community and increases the bacteria–fungi ratio (Pankhurst et al., 2001; Wichern et al., 2006; Yuan et al., 2007a). This can be explained by a greater sensitivity of fungi to salinity compared to bacteria.

1.3 Drying and rewetting

Soil drying and rewetting are common in arid and semi-arid regions. Nearly one third of the earth's soils is in arid, semi-arid, or seasonally arid climates where long dry periods can be interrupted by occasional rainfall events (Schimel et al., 2007). Drying and rewetting (DRW) events influence microbial activity and soil nutrient cycling. The flush of respiration upon rewetting dry soils is well known. This phenomenon has been named "the Birch-Effect" in recognition of its first documentation (Birch, 1958).

1.3.1 Effect of drying and rewetting on soil microbes and nutrient availability

As soil dries, the water film around aggregates becomes thinner and water is held more tightly on soil particles which increases soil matrix potential (Papendick and Camprell, 1981; Skopp et al., 1990; Ilstedt et al., 2000). This limits nutrient diffusion and reduces water availability (Carson et al., 2010). The accumulation of soluble C and ions (Manzoni et al., 2014) increases the solute concentration in the soil solution resulting in a higher

water potential compared to microbial cells. Water can be drawn out of the cells causing desiccation and cell death (Lund and Goksøyr, 1980).

Soil microbes can adapt to overcome drought stress. During unfavourable conditions some microbes can form resting structures, such as endospores, cysts, conidia or other specialized resistant structures and become dormant (Setlow, 1995). During dormancy, microbes can maintain their internal energy for survival mechanisms such as DNA repair (Morita, 1982).

Another drought tolerance mechanism is synthesis or uptake of organic solutes (Bottner, 1985; Wood et al., 2001) to maintain internal water potential. These are low molecular weight compounds termed "osmolytes". Their accumulation results in higher solute concentration in the cytoplasm compared to the surrounding of the cell to avoid exo-osmosis and dehydration (Csonka, 1989). Osmolytes include sugars, sugar alcohols, quaternary ammonium compounds and amines (Imhoff, 1986; Csonka, 1989; Kempf and Bremer, 1998; Kakumanu et al., 2013). According to (Warren, 2014) the osmolyte content was 10-fold higher under water deficit conditions than the moist controls. Production of exo-polysaccharide substances (EPS) is another mechanism to withstand desiccation (Roberson and Firestone, 1992). EPS is a gel-like matrix, and can hold water several times its weight which can be act as a barrier against water loss (Roberson and Firestone, 1992).

Drying can change microbial community structure. Fungi are considered to be more drought-tolerant than bacteria (Schimel et al., 2007; Strickland and Rousk, 2010) because the extensive hyphal network allows them to reach substrates even at very low soil water contents (Griffin, 1981; Allen, 2007; Joergensen and Wichern, 2008). In experimental drought plots, Gram-positive bacteria were more abundant than Gram-negative bacteria (Fuchslueger et al., 2014) likely because of the peptidoglycan layer in the cell wall of Gram-positive bacteria which provides more protection against desiccation than the cell walls of Gram-negative bacteria (Manzoni et al., 2012).

1.3.2 Factors influencing the respiration flush after rewetting of dry soils

Rewetting can be rapid which changes the soil water potential in a short period of time (Kieft, 1987; Fierer and Schimel, 2003). The flush of respiration after rapid rewetting of dry soil is due to increased substrate availability. Substrates may be from either microbes (dead microbes and osmolytes) or soil organic matter (Schimel et al., 2007) which is decomposed by the surviving microbes.

Rapid rewetting of dry soils kills a proportion of the microbial biomass. For example, (Kieft, 1987) found that microbial biomass was reduced by 10-20% due to rewetting dry soils. (Butterly et al., 2009) reported that the reduction of microbial biomass C and P was greater in amended soils (glucose, cellulose and starch) than unamended soil. This may be due to higher microbial activity in amended than unamended soils (Butterly et al., 2009) because active microbes are more susceptible to stress (Van Gestel et al., 1993).Some microbes can survive the rapid reduction in soil water potential upon rewetting by release or polymerization of osmolytes they accumulated during the dry period (Halverson et al., 2000). Physical changes in the soil with rewetting may also contribute to substrate supply to microbes. During rewetting, water enters the aggregates, increasing the internal air pressure which can lead to breakdown of aggregates. This increases the accessibility of previously protected organic matter to microbes (Utomo and Dexter, 1982; Denef et al., 2001).

The magnitude of the respiration flush is governed by many factors, e.g. speed of rewetting, soil water content before and after rewetting, number of DRW cycles and the length of the dry period before rewetting and the length of the moist period prior to drying. Rapid rewetting can cause microbial cell lysis and aggregate breakdown. Slow rewetting allows a more gradual replacement of air with water (Beare et al., 2009; Navarro-García et al., 2012) and a slow change in soil water potential. Therefore slow rewetting results a smaller flush than rapid rewetting (Cosentino et al., 2006).

The water content before and after rewetting also affects the flush size. The flush increases with increasing water potential difference between before and after rewetting (Fischer, 2009; Chowdhury et al., 2011; Dasheng et al., 2018). (Guo et al., 2014) reported a flush of respiration after rewetting soil with 10 and 20% water filled pore spaces (WFPS) compared to constantly moist soil, but there was no flush after rewetting soil with 30 or 45% WFPS. A greater water potential difference between before and after the rewetting increases the stress for microbes and the extent of aggregate breakdown which result in greater substrate availability than a small difference in water content.

The number of DRW cycles is another factor affecting the size of the flush. The size of the respiration flush decreases with increasing number of DRW cycles (Fierer and Schimel, 2002; Mikha et al., 2005; Miller et al., 2005). Several explanations have been proposed. Firstly, increasing stress tolerance of soil microbes. Stress tolerance mechanisms may include, e.g., rapid removal of solutes from cytoplasm and depolymerization of osmolytes (Van Gestel et al., 1993; Lundquist et al., 1999). Secondly, reduction of available substrate with increasing number of DRW cycles; aggregate breakdown is likely to occur mainly in the first DRW cycles, later little additional organic matter will become accessible.

The length of the dry period prior rewetting influences the pulse of respiration. (Shi and Marschner, 2015) reported that the respiration flush increased with increasing number of dry days before rewetting. The effect of the length of the dry period may be due to the relative importance of two processes. Drying causes cell lysis (Borken and Matzner, 2009) due to exo-osmosis which would increase substrate availability upon rewetting. However exo-enzymes released earlier may be still active and can contribute to the higher rate of respiration after rewetting (Potts, 1994; Burns et al., 2013; Maire et al., 2013). An increasing number of moist days prior to drying reduces cumulative respiration of DRW cycles (Shi and Marschner, 2014) likely because decomposition during the moist period decreases susbtrate availability for subsequent DRW events.

1.3.3 Effect of drying and rewetting on nutrient availability

Rewetting of dry soil enhances net N mineralization (Birch, 1958, 1959; Bloem et al., 1992; Cui and Caldwell, 1997). This can be explained by the higher substrate and water availability which enhances microbial activity after rewetting. However, repeated DRW events can reduce nitrification potential (Franzluebbers et al., 2000) because nitrifiers are sensitive to water stress (Stark and Firestone, 1995).

Soil P pools are also affected by DRW events. After rewetting dry soil, MBP and labile organic P increased immediately and labile inorganic P increased gradually (Nguyen and Marschner, 2005). According to (Turner et al., 2003) lysed bacterial cells are the main source for increase in organic P after rewetting. The surviving microbes then mineralise organic P which gradually increases inorganic P. On the other hand rewetting dry soils could reduce P availability because aggregate breakdown may expose more P adsorption sites (Raveh and Avnimelech, 1978).

Mineralization of soil organic matter increases due to rewetting of dry soils (Wu and Brookes, 2005; Xiang et al., 2008). This can be explained by an increase of dissolved organic C after rewetting dry soil (Merckx et al., 2001; Mavi and Marschner, 2012). According to (Mavi and Marschner, 2012) dissolved organic C was about 40% higher after rewetting compared to constantly moist soil. Other sources of dissolved organic C are microbes killed by drying (Stevenson, 1956). Enhanced organic C mineralization after rewetting may also be due to substrate accumulation during the dry period (Kieft, 1987; Prechtel et al., 2000; Denef et al., 2001; Warren, 2014).

1.4 Temperature above 30 °C

In hot summers and fires, soil temperature can be greater than 30 °C. The effect of temperature on microbial activity depends several factors including soil moisture, substrate availability and duration of exposure. Soil

moisture content and substrate availability are important determinants of optimum temperature. The diffusion of substrate increases with increasing temperature, due to an increase in kinetic energy. But high temperature is associated with drying (Rey et al., 2002). Therefore high temperatures reduce substrate and water availability which can be lead to lower microbial activity (Davidson et al., 2006).

1.4.1 Effect of temperature on microbial activity and nutrient availability

There are only few studies on microbial activity at temperatures between 30 and 50 °C (Richardson et al., 2012; Zhou et al., 2014). When water is not limiting, soil respiration rate increases with temperature up to about 50 °C (Anderson and Domsch, 1986; Pietikäinen et al., 2005). (Pietikäinen et al., 2005) studied respiration rate and bacterial and fungal growth in soils after exposure to temperatures between 0 and 45 °C for 5 h-120 h. The respiration rate increased with temperature up to 45 °C, but microbial growth increased only up to 20-30 °C and then decreased. This uncoupling of respiration and microbial growth at higher temperatures is in agreement with previous studies (Anderson and Domsch, 1986). Similarly, (Bárcenas-Moreno and Bååth, 2009b) found that microbial biomass was about 8% lower after incubation at 50 °C for 15 min than at room temperature (~22 °C)

The influence of temperature in the range 50 to 100 °C on microbial activity has been investigated even less (Berard et al. 2011; Barcenas- Moreno et al. 2009b; Shi et al. 2020). Bárcenas-Moreno and Bååth, (2009b) reported that microbial biomass C in soils heated to 100 °C for 15 min was about 80% lower than soils heated to 50 °C. Not only microbial activity but also nutrient availability is influenced by temperatures greater than 30 °C. (Myers, 1975) studied the effect of temperatures between 20 and 60 °C on ammonification and nitrification in tropical soils. He reported that the optimum temperature for nitrification was ~ 35 °C and for ammonification it was 50 °C.

Heating can disrupt cell membranes and cell walls (Welker, 1976) (Hitchener and Egan, 1977),which causes leakage of intracellular substances (Allwood and Russell, 1970; Beuchat, 1978). However, loss of viability is not correlated with membrane damage, which suggests that membrane damage is not the main reason for heat-induced inactivation/death (Russell and Harries, 1968; Allwood and Russell, 1970). Other heat-induced changes include breakage of ribosomes and RNA (Sedgwick and Bridges, 1972; Kadota, 1978) and denaturation of enzymes and other proteins (Harries and Russell, 1967).

During high intensity forest fires, soil temperatures can reach more than 250 °C (Soto 1991;DeLuca and Zouhar, 2000). However in most fires the temperature in the surface soil layer (5 cm) rarely increases above 150 °C and soils layers below 20-30 cm are not influenced by fire (DeBano, 2000). There are extensive studies on the effect of forest fires on soil properties (Choromanska and DeLuca, 2002; González-Pérez et al., 2004; Certini, 2005; Hart et al., 2005). Due to the very high soil temperatures most of the soil microbes in the top layers of the soil die during fires (DeBano, 2000; Certini, 2005).

Fire has considerable effects on soil nutrient cycling, particularly N and P dynamics. Since the majority of soil N is organic, the effect of fire on soil organic matter directly affects soil organic N (Schulten and Schnitzer, 1997). Often inorganic N increases immediately after a fire (Kovacic et al., 1986; Covington and Sackett, 1992; Monleon et al., 1997). In the study by (Covington and Sackett, 1992), ammonium increased about 10 to 20-fold shortly after the fire, while there was no change in nitrate. However one year after the fire, ammonium was low whereas nitrate had increased. The increase in inorganic N after fire can be attributed to deposition of ash on the soil surface and release of NH_4^+ from organo-mineral complexes (Kutiel and Naveh, 1987). Fire reduces labile organic fractions leaving the recalcitrant fractions, and thereby reduces the organic N mineralization (White, 1986). This affects long-term soil nutrient availability. Fire also changes soil P pools. Fire converts organic P into orthophosphate, which is the form of P available for plants and microbes when in soil solution (Cade-Menun et al., 2000; DeBano and Klopatek, 1988; Saa et al., 1993; Romanya et al., 1994; Saa et al., 1998). According to (Cade-Menun et al., 2000) available P in soils immediately after fire was about two-fold higher than unheated soils. However five years after a fire available P was similar as in unheated soil.

Not only the temperature but also its duration is an important factor affecting microbial activity and nutrient availability. Acclimation of microbes to higher temperatures has been reported after long term exposure. Barcenas-Moreno et al., (2009)a) found a shift in optimum temperature for microbial growth after incubation of soil at 50% water holding capacity at 5 to 50 °C for 31 days. Before incubation both bacteria and fungi had optimum growth rates at 30 °C, which decreased rapidly with increasing temperature. After incubation, especially the soils incubated at temperatures above 30 °C, the optimum temperature for growth shifted to high values. i.e. when soils are incubated for one month at 35, 40 and 45 °C, optimum temperature for growth changed from 30 °C to temperatures that they were incubated. This was been explained by thermal acclimation of microbes and may also be due to changes in microbial community composition (Berard et al., 2011).

1.5 Research gaps and aims

The effect of temperature up to approximately 40 °C on microbial activity and nutrient availability has been studied in field and laboratory experiments, and temperatures greater than 100 °C have been studied in relation to fires (Soto et al., 1991). In general microbial activity increases with temperature up to about 30-40 °C and then decreases with further increases in temperature (Richardson et al., 2012). But there are only few studies on the influence of soil exposure to temperatures in the range of 50 to 100 °C on microbial activity and nutrient cycling which can occur during fast moving fires with a low fuel-load (Scotter, 1970; Stoof et al., 2013).

Not only the temperature itself, but also the duration of heat determines the effect of temperature on microbial activity. In previous experiments that investigated the effect of temperature between 50 and 100 °C on microbial activity (e.g., Berard et al., 2011; Barcenas- Moreno et al., 2009b; Riah-Anglet et al., 2015; Shi et al., 2020), heat duration ranged between 18 h and 31 days. However in fast moving forest fires, the soil temperatures will increase and then remain between 50 and 100 °C only for few hours (Zheng et al., 2017). The effect of such short heating events (2-3 h) on soil microbial activity and nutrient availability as well as possible modulating factors should be investigated to better understand the impact of fire on nutrient cycling.

The effect of heating on soil microbes and nutrient availability is likely to be influenced by a number of factors including soil water availability and substrate availability. Soil water availability and substrate availability can be linked because low water availability limits substrate accessibility (Ilstedt et al., 2000) and is particularly relevant in semi-arid regions where fires may occur. Water content before heating could influence the decomposition rate of native soil organic matter and thereby substrate and nutrient availability which may modify the effect of heating. Heating usually results in soil drying (Howard and Howard, 1993; Curiel Yuste et al., 2007) therefore the effect of heating may be, at least partly due to drying and not heat exposure. To better understand the underlying mechanisms, the effect of soil drying should be separated from that of heating and drying. Another factor that can influence water availability is salinity which may exacerbate the effect of heating on microbial activity and nutrient availability because it reduces water availability. Further, soil microbes in saline soils will be exposed to two stressors, salinity and heating. The effect of heating in saline soils has not been specifically studied, but understanding this is important because saline soils are wide-spread in semi-arid regions where they can also be exposed to fire. In previous studies soils were heated once to 50 to 100 °C (Berard et al. 2011; Barcenas- Moreno et al. 2009b; Shi et al. 2020). However, fires may reignite. Thus, for a better understanding of fire effects, the influence of more than one heating event on microbial activity and nutrient availability should be investigated.

Little is known about how substrate and nutrient availability influence microbial activity and nutrients after heating. Substrate and nutrient availability to microbes can be increased by fertiliser or organic matter addition and plant growth. This is relevant for the field because fertiliser addition, litter fall or plant growth may occur before exposure to fire which has not yet been studied in detail. Soil amendment with organic matter, fertilisers or plant growth are likely to have different effects on the impact of heating. Organic matter addition will add both organic C and nutrients (Tian et al., 1992) whereas plant growth would mainly add organic C whereas nutrients may be depleted via plant uptake (Gahoonia et al., 1994 ;Trofymow et al., 1987). The effect of fertiliser amendment is likely to be different from that of organic amendments because no organic C is added and nutrient availability is high.

To address these knowledge gaps, the main aim of this study is to determine the influence of a short heating event to 60 °C followed by rewetting on respiration and nutrient availability.

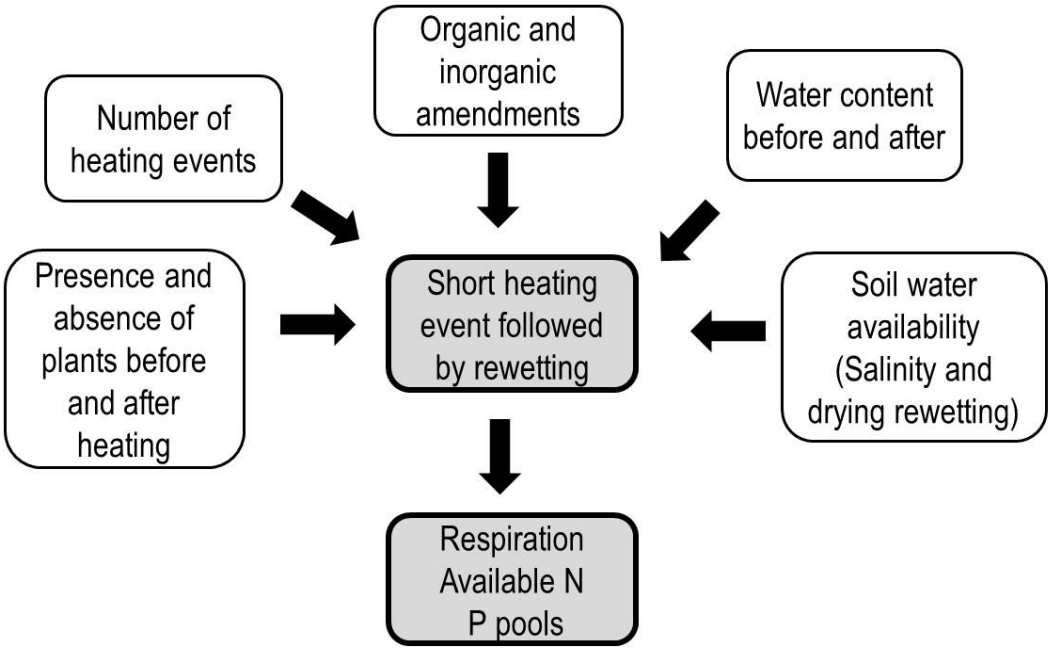

Figure 2. Conceptual diagram of thesis

The detailed aims of the thesis were to study the effect of a number of factors on soil respiration and nutrient availability before and after a short heating event followed by rewetting (Figure 2)

This thesis will examine:

(i) Soil water content before and after heating and rewetting (Chapter 2).

(ii) Amendment form (inorganic nutrients or organic amendment) and time between amendment application and heating (Chapter 3).

(iii) Heating frequency and period between the two heating events (Chapter 4).

(iv) Presence or absence of plants (Chapter 5).

(v) Prior drying and rewetting and salinity (Chapter 6).

During fast moving grass fires soil temperature can range between 50 °C and 100 °C for 15 min- 1 h. Therefore in this project the soils were heated to 60 °C within 1 h and then maintained this temperature for 30 min.

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Chapter 2

Impact of a short heating event followed by rewetting on soil respiration and nutrient availability is not only due to soil drying during heating

Published in Biology and Fertility of Soils
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Statement of Authorship

Title of the Paper	Impact of a short heating event followed by rewetting on soil respiration and nutrient availability is not only due to soil drying during heating
Publication Status	[X] Published [ ] Accepted for Publication [ ] Submitted for Publication [ ] Unpublished and Unsubmitted work written in manuscript style
Publication details	Seneviratne, M., Doolette, A., Marschner, P., 2019. Impact of a short heating event followed by rewetting on soil respiration and nutrient availability is not only due to soil drying during heating. Biology and Fertility of Soils 55, 553-564.

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Contribution to the paper	Performed the experiment, analysed all the samples, data analysis and interpretation data, writing the manuscript. I hereby certify that the contribution is accurate		
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 subjected 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	10/02/2020

Co-Author contributions

By signing the Statement of Authorship, each author certifies that

i. The candidates 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 candidates stated contribution

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Seneviratne, M., Doolette, A., Marschner, P., 2019. Impact of a short heating event followed by rewetting on soil respiration and nutrient availability is not only due to soil drying during heating. Biology and Fertility of Soils 55, 553–56

Biology and Fertility of Soils
https://doi.org/10.1007/s00374-019-01372-9

ORIGINAL PAPER

Impact of a short heating event followed by rewetting on soil respiration and nutrient availability is not only due to soil drying during heating

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Received: 1 March 2019 / Revised: 8 May 2019 / Accepted: 31 May 2019

Abstract

Little is known about the impact of short-term heating to 50–100 °C as it may occur in fast-moving low-intensity fires on microbial activity and nutrient availability. Heating of soils usually induces drying, but it is unclear if the effect of heating is only due to this water loss or if other factors are also important. Two experiments were carried out where soils were heated to 60 °C. The experiments included constantly moist controls and air-dried soils which were dried at 30 °C to the same water content as the heated soils. After heating/air-drying, the soils were rapidly rewet and incubated for 2 weeks at a constant water content optimal for microbial activity. The aim of the first experiment was to assess the effect of water content before heating and heat duration on soil respiration and nutrient availability. Soil was incubated for 14 days at 33, 82, and 165 g water kg^{-1} (referred to as W33, W82, and W165, corresponding to 10, 30, and 50% maximum water-holding capacity) where 165 g water kg^{-1} is optimal for microbial activity in this soil. The soils were then heated to 60 °C and maintained at this temperature for 30 or 90 min. Heat duration had little effect on the measured properties. In heated soils, cumulative respiration after rewetting was about threefold higher than in the constantly moist control. Two days after heating, available N in heated soils was twofold higher than in the constantly moist control and 0.3 to twofold higher than the corresponding air-dried soils. Two weeks after heating, available N differed little between the constantly moist control and heated soils that were at W33 and W82 before heating, but it was about twofold higher in heated soil that was at W165 before heating. Available P 2 days after heating was highest in heated soils, but 2 weeks after heating, available P was lower in heated soils than the constantly moist control. In the air-dried controls which were dried at 30 °C to the same water content as in heated soils prior to rewetting, cumulative respiration, available N and P after rewetting differed little from the constantly moist soil. The aim of the second experiment was to determine the effect of soil nutrient content on soil respiration and nutrient availability after heating. Two soils differing in organic matter, total N, and total P content were used either separately or as mixes with different proportions of the soils. Soils were heated and maintained at 60 °C for 30 min. Before and after heating/air-drying, the soils were maintained at optimal water content (180 g water kg^{-1}). Two and 7 days after heating, available N was 10–30% higher in heated soils than the constantly moist control and air-dried soils. It can be concluded that the effect of short-term heating followed by rewetting on soil respiration and available N and P is not only due to soil drying, but possibly also heating-induced changes in soil organic matter composition and availability as well as soil P sorption capacity.

Keywords Heating · N availability · Rewetting · Respiration · P availability

Introduction

Temperature and soil water content are important environmental factors governing microbial activity and nutrient availability. Between 0 and 50 °C, microbial activity and N availability increase with temperature (Richardson et al. 2012; Zhou et al. 2014; Miller and Geisseler 2018). Exposure of soils to two diurnal heating cycles with maximum temperatures of 50 or 70 °C temporarily increased available N (Zheng et al. 2017). Temperatures greater than about 80 °C reduce microbial activity due to microbial death or protein denaturation (Bárcenas-Moreno and Bååth 2009). The effects of soil temperatures > 100 °C have been studied mainly in fires where, in most studies, the length of time at a given temperature was not known. Fire can increase

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Published online: 18 June 2019

soil-available N and P through chemical mineralization (Saa et al. 1993; Fultz et al. 2016).

In most studies on soil heating, the soil water content before and after heating has not been considered. However, soil water content could influence the impact of heat on soil microbes and nutrient availability. Soil water content is a key driver of microbial survival and functions as a resource, solvent, and transport medium, for example by influencing substrate and gas supply to microbes (Tecon and Or 2017; Schimel 2018). In most soils, soil microbial activity is highest at about 50% of maximum water-holding capacity because both water availability and gas diffusion are not limiting. As a soil dries, the water film around the soil particles becomes thinner and water is held more tightly on soil particles (Ilstedt et al. 2000). The thin water film limits microbial access to dissolved nutrients, restricts water uptake, reduces cellular ATP content (Ciardi et al. 1993), and can cause loss of water from microbial cells (Moyano et al. 2013). Microbial drought tolerance mechanisms include accumulation of osmolytes, production of extracellular polymeric substances which minimize water loss from the cell, and dormancy (Chenu and Roberson 1996; Jones and Lennon 2010; Warren 2016). Low soil water content before heating, compared to optimum water availability, could influence the impact of heating in a number of ways. Firstly, by reducing mineralization before heating leaving more substrate available after heating compared to optimal prior water content. Secondly, it can lead to low microbial biomass and a greater proportion of dormant microbes.

Rapid rewetting of dry soils induces a flush of respiration and nutrient availability which is thought to be due to increased substrate availability for surviving microbes from aggregate breakdown, microbial cell lysis, and release of osmolytes (Birch and Friend 1956; Kieft et al. 1987; Denef et al. 2001; Xiang et al. 2008; Sun et al. 2018). The size of the rewetting flush is influenced by a number of factors, for example, number of drying and rewetting cycles (Wu and Brookes 2005; Yu et al. 2014) and soil water content before and after rewetting. The size of the flush after rewetting to optimal water content increases with decreasing soil water content before rewetting (Kim et al. 2010; Meisner et al. 2017).

In this study, two experiments were conducted. The aim of the first experiment was to assess the effect of water content prior to heating and heat duration on soil respiration and nutrient availability. The aim of the second experiment was to determine the effect of soil nutrient content on soil respiration and nutrient availability after heating. For this, two soils which differed in organic C and available N and P were used. In both experiments, the drying effect of heating was assessed by including air-dried soils (dried at < 30 °C) that had the same water content as the heated soils after heating. The hypotheses were (1) the impact of heating on the measured properties (respiration and N and P availability) will be due to both water loss during heating and heat, thus different from the air-dried controls, (2) the impact of heating will change with time after heating, (3) the impact of heating will be greater after 90 min heating than 30 min, (4) heating will have a greater effect on the measured properties when the soil water content prior to heating was lower than optimal because more mineralizable substrates remain, and (5) the relative impact of heating will be greater in soil with higher nutrient content.

Materials and methods

Two soils (soil 1 and 2) were used in this study and both were collected from 0 to 10 cm depth at Urrbrae, South Australia (Longitude 138°38′3.2″ E, Latitude 34°58′0.2″ S). The area had been under permanent pasture for over 80 years and was recently cropped with oats (*Avena sativa* L.). The climate is Mediterranean with cool, wet winters and hot, dry summers. The soils are Chromosols according to Australian soil classification and Rhodoxeralfs in US Soil Taxonomy. Soil 1 was collected in summer after the area had recently been ploughed and soil 2 in the following autumn. Soil was sampled from several locations, pooled to one composite sample, and air-dried and sieved to < 2 mm. For soil properties, see Table 1.

Experimental design

Experiment 1

The aim of this experiment was to determine the effect of soil water content before heating and heat duration on soil respiration and nutrient availability. Soil 2 was used for this experiment. Before the start of the experiment, the air-dried soil was rewetted and pre-incubated for 14 days at three different water contents; 33, 82, and 165 g water kg^{-1} (corresponding to 10, 30, and 50% of maximum water-holding capacity or approximately − 1.7, − 0.5, and − 0.078 MPa) at 22–25 °C in the dark to reactivate the microbes. The length of pre-incubation period was based on Butterly et al. (2010), who showed that soil

Table 1 Properties of soils 1 and 2

	Soil 1	Soil 2
Clay (%)	40	25
Sand (%)	35	48
Silt (%)	25	27
$pH_{1:5}$	5.8	5.3
Water-holding capacity (g kg^{-1})	400	330
Total organic C (g kg^{-1})	30	22
Total N (g kg^{-1})	2.1	1.6
Total P (g kg^{-1})	0.5	0.4
Available N (mg kg^{-1})	150	53
Available P (mg kg^{-1})	40	13

microbial activity stabilized within 10 days after rewetting of air-dried soil. Thirty grams of air- dried soil was filled into 70-ml polypropylene containers (diameter 4.4 cm, height 5.7 cm), mixed with reverse osmosis water added and adjusted to a bulk density of 1.3 g cm^{-1}. Throughout the pre-incubation period, the desired water content was maintained by adding reverse osmosis water in every second day by weight if necessary.

After the 14-day pre-incubation, the containers with soil for the heated treatments were placed in a fan-forced oven and heated to 60 °C within 1 h and maintained at this temperature for up to 90 min. Soil temperature was monitored with thermocouples. Samples were removed from the oven after being maintained at 60 °C for 30 and 90 min (H30 and H90). These times represent durations of a fast- and slow-moving fire, respectively. The soils were allowed to cool to room temperature and weighed to measure water content after heating. Soil water content was 16, 33, 66, and 100 g kg^{-1}. Irrespective of heating duration, soil that was at 33 and 82 g kg^{-1} dried to 16 and 33 g kg^{-1} (water loss of 17 and 49 g kg^{-1}). For soil that was at 165 g kg^{-1} before heating, heating for 30 and 60 min dried the soil to 100 and 66 g kg^{-1} (water loss of 65 and 99 g kg^{-1}). Then, the soils were rapidly rewetted to 165 g water kg^{-1} by adding reverse osmosis water in a circular motion for uniform rewetting of the soil. The corresponding air-dried controls were pre-incubated under similar conditions as the heated soils. Their water content was reduced by air drying in a fan-forced oven at 30 °C to reach the corresponding water contents after heating; 16, 33, 66, and 100 g kg^{-1} (W16 AD, W33 AD, W66 AD, and W100 AD). Drying duration was 48, 24, 10, and 7 h respectively. After reaching the desired water contents, the soils were rewetted to 165 g kg^{-1} in a similar manner as the heated soils. A constantly moist control was maintained at 165 g kg^{-1} throughout the experiment (W165C). After rewetting on day 15, the soil containers were placed individually in 1 L glass jars sealed with gas-tight lids which had rubber septa for quantification of the CO_2 concentration in the headspace. The jars were incubated in the dark at 22–25 °C, and CO_2 release was measured daily over 14 days. Vials containing about 5 ml of reverse osmosis water were placed inside the jars to minimize water loss from soils. Soil water content was monitored by weight every second day, and if necessary, reverse osmosis water was added to maintain weight. Soils were destructively sampled before heating and on days 17, 22, and 29 (2, 7, and 14 days after rewetting) and analyzed for available N and P. For each sampling time and treatment, there were four replicates.

Experiment 2

The aim of experiment 2 was to assess the impact of soil nutrient concentration on respiration and nutrient availability after heating. Soil 1 had higher available N and P than soil 2. Heat duration had little effect in experiment 1; therefore, only one duration was used in experiment 2 (30 min). To achieve a range of nutrient availabilities, air-dry soils 1 and 2 were used either individually or mixed thoroughly in different ratios (Table 2). The air-dry soil was placed in plastic bags (20 g) and 180 g water kg^{-1} was added. This water content represented approximately 50% of maximum water-holding capacity (− 0.1 MPa) in all soil treatments. After thorough mixing, the soil was placed into 70 ml polypropylene containers (diameter 4.4 cm, height 5.7 cm) and adjusted to a bulk density of 1.3 g cm^{-1}. The containers were pre-incubated for 14 days at 180 g water kg^{-1} at 22–25 °C. Throughout the pre-incubation, the desired water content was maintained by weight. After the 14-day pre-incubation, the containers with soil were placed in a fan-forced oven and heated to 60 °C as described in experiment 1, and then maintained at this temperature for 30 min. Soil temperature was monitored with thermocouples. After 30 min, the soils were removed from the oven, allowed to cool to room temperature, and weighed to measure water content after heating. Water content after heating was 90 g kg^{-1}. Air-dried controls (water content 90 g kg^{-1}) were prepared as described in experiment 1. Heated soils and air-dried controls were rewetted to 180 g kg^{-1}. For all soil treatments, a constantly moist control was maintained at 180 g kg^{-1} throughout the experiment. Soils were incubated for 7 days after rewetting because there was little difference in nutrient availability between 7 and 14 days in experiment 1. Soil respiration was measured daily over 7 days. Soils were destructively sampled before heating, and days 17 and 22 (2 or 7 days after rewetting) and analyzed for available N and P. For each sampling time and treatment, there were four replicates.

Measurements

Soil maximum water-holding capacity was measured at matric potential 10 kPa (Wilke 2005). Soil pH was measured in a 1:5, soil/water suspension ratio after 1 h shaking at 25 °C.

Soil respiration was measured with a Servomex 1450 infrared gas analyzer (Servomex Group, Crowborough, UK) as described in Setia et al. (2011). After each measurement, the jars were opened to remove accumulated CO_2. The calculation of CO_2 concentration was based on a linear regression of

Table 2 Proportion of soils 1 and 2 in mixes of experiment 2

Treatment name	Soil 1 (%)	Soil 2 (%)
100S1	100	0
75S125S2	75	25
50S150S2	50	50
25S175S2	25	75
100S2	0	100

detector reading and injected volumes of CO_2 into empty 1 L glass jars similar to the jars used for the samples.

Available N (exchangeable ammonium and nitrate) concentration was measured after 1 h end-over-end shaking with 2 M KCl at a 1:5 (*w*/*v*) soil to extractant ratio. Ammonium-N was measured as described in Willis et al. (1996) and nitrate-N according to Miranda et al. (2001). Available P was extracted by the anion exchange resin method (Kouno et al. 1995) and determined colorimetrically (Murphy and Riley 1962).

Statistical analysis

There were four replicates for each treatment and sampling time. Data was analyzed by one-way ANOVA for each sampling date separately in Genstat 15th edition (VSN Int. Ltd., UK). Tukey's multiple comparison tests at 95% confidence interval was used to determine significant differences among treatments.

Results

Experiment 1

Heating duration had little effect on respiration rates and cumulative respiration. In all heated soils, the respiration rate was highest 1 day after rewetting, then decreased until about day 7 after which it remained stable (Fig. 1a–c). Respiration rates in the heated soils in the first 2 days after rewetting were two to fivefold higher than in constantly moist control (W165C). Respiration rates were also higher initially in the air-dried soils W16 AD and W33 AD, but remained stable over time in W100 AD and W165C.

In heated soils, cumulative respiration after rewetting was about threefold higher than in W165C, with greater difference in heated soils that were at W33 and W82 before heating than that at W165 before heating (Fig. 1d). Cumulative respiration in heated soils was always higher that in the corresponding air-dried soils. Compared to the corresponding air-dried soils, cumulative respiration in heated soils was 10% higher in W33, 30% higher in W82, and about twofold higher in W165.

Available N and the proportion of nitrate before heating (day 15) increased with soil water content (Fig. 2a). Compared to W33, available N was about 25% higher in W82 and 75% higher in W165. On day 17 (2 days after heating), available N did not differ between air-dried soil and constantly moist control, but it was about twofold higher in heated soils than W165C (Fig. 2b). Heat duration had no effect on available N. Heated soils that were at W33 and W82 before heating had higher available N than the corresponding air-dried soils, but this was not the case in heated soil that was at W165 before heating. In all soils, nitrate was the dominant form of available N. Available N changed little between day

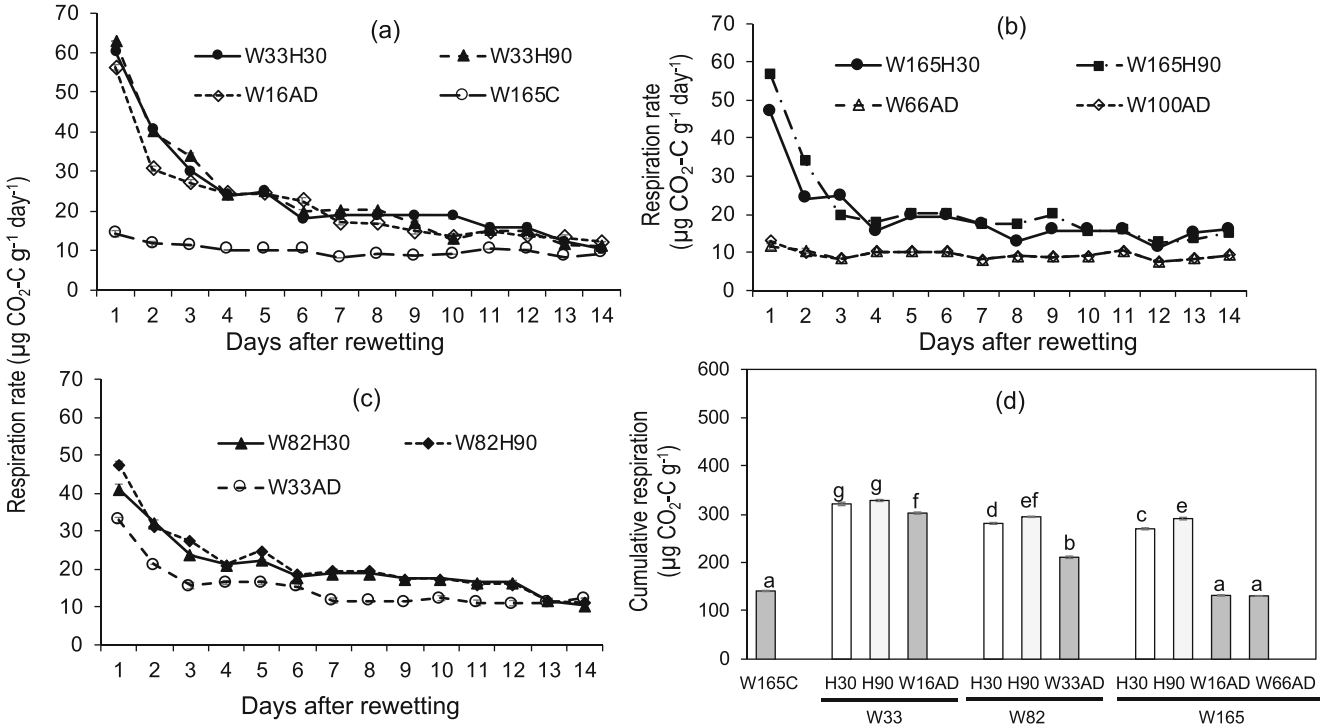

Fig. 1 Respiration rate (**a**, **b**, **c**) and cumulative respiration (**d**) over 14 days after rewetting (day 16–29) in constantly moist soil (W165C) and in soils pre-incubated at different water contents (W33, W82, and W165) which were heated to 60 °C for 30 or 90 min (H30, H90) on day 15 as well as corresponding air-dried soils (W16 AD, W33 AD, W100 AD, W66 AD). In panel **d**, different letters indicate significant differences in cumulative respiration among treatments ($n = 4$, means ± SE). Error bars in panels **a**–**c** are mostly too small to be visible

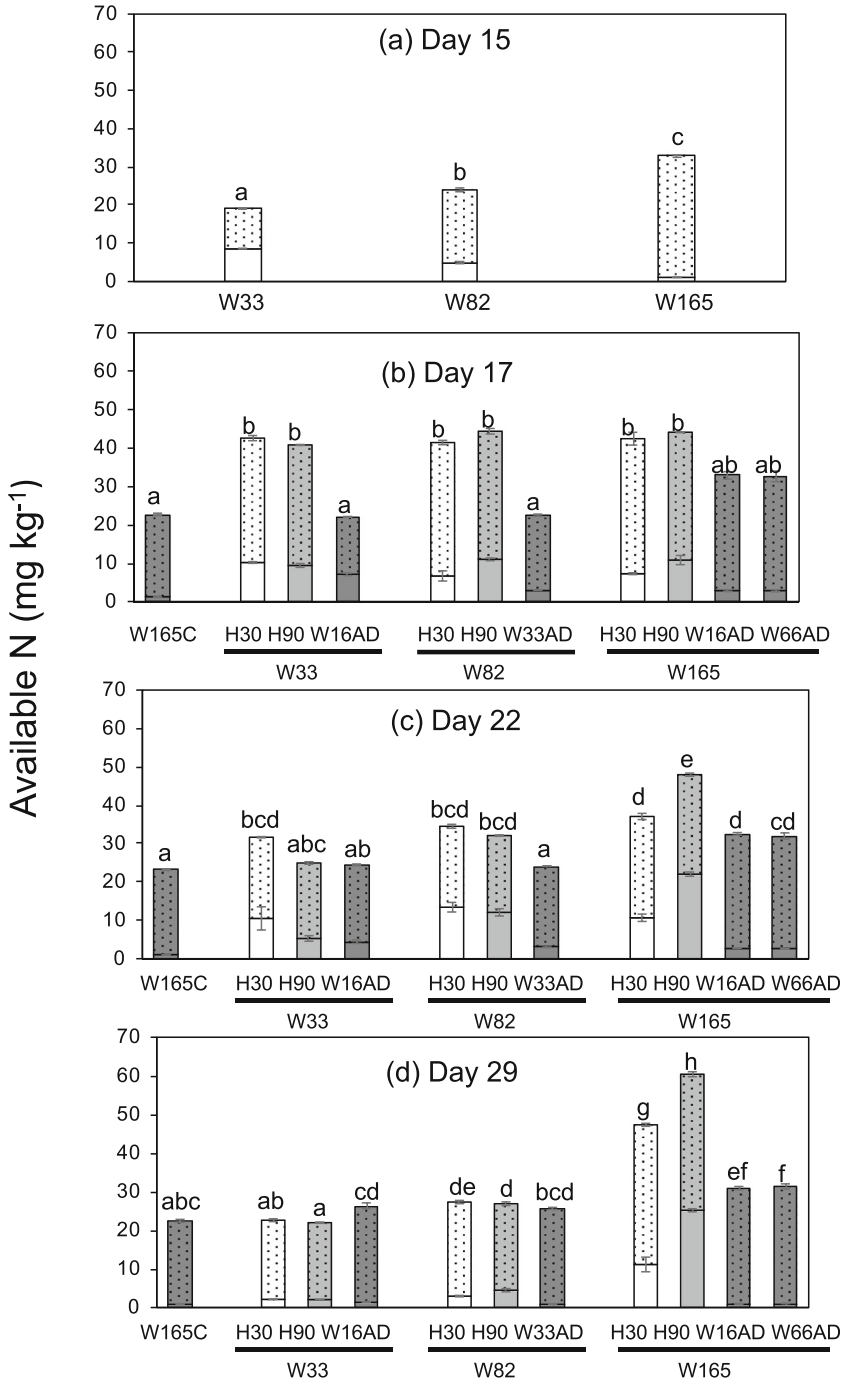

Fig. 2 Available N in constantly moist soil (W165C) and in soils pre-incubated at different water contents (W33, W82, and W165) which were heated to 60 °C for 30 or 90 min (H30, H90) on day 15 as well as corresponding air-dried soils (W16 AD, W33 AD, W100 AD, W66 AD) before heating (**a**) and on days 17, 22, and 29 (2, 7, and 14 days after rewetting, **b**, **c**, **d**) ($n = 4$, means ± SE). Bar segments with no pattern indicate exchangeable ammonium, stippled segments nitrate. At each sampling time, different letters indicate significant differences in total available N among treatments

17 and day 29 in the control and air-dried soils but was lower on day 29 than day 17 in the heated soils that were at W33 and W82 before heating. Available N on days 22 and 29 was similar in W165C, W16C, and W33 AD but about 30% higher in heated soil that was at W66 and W100 before heating (Fig. 2c). Available N on day 22 in soils that were at W33 before heating did not differ between heated soil and W16 AD. But in soils that were at W82 before heating, available N was about 20% higher in heated soils than W33 AD. In heated soils that were at W165 before heating, only the soil kept at 60 °C for 90 min had higher available N on day 22 than the air-dried soil. Compared to W165C, available N on day 29 in heated soils was similar in soil that was at W33 before heating, but about 10% higher in heated soils that were at W82 and twofold higher in heated soils that had been at W165. Compared to the corresponding air-dried soils, available N on day 29 in heated soils was about 15% lower in soil that was at W33 but did not differ in soil that was at W82 before heating (Fig. 2d). In soils that were at W165 before heating, available N was 50%

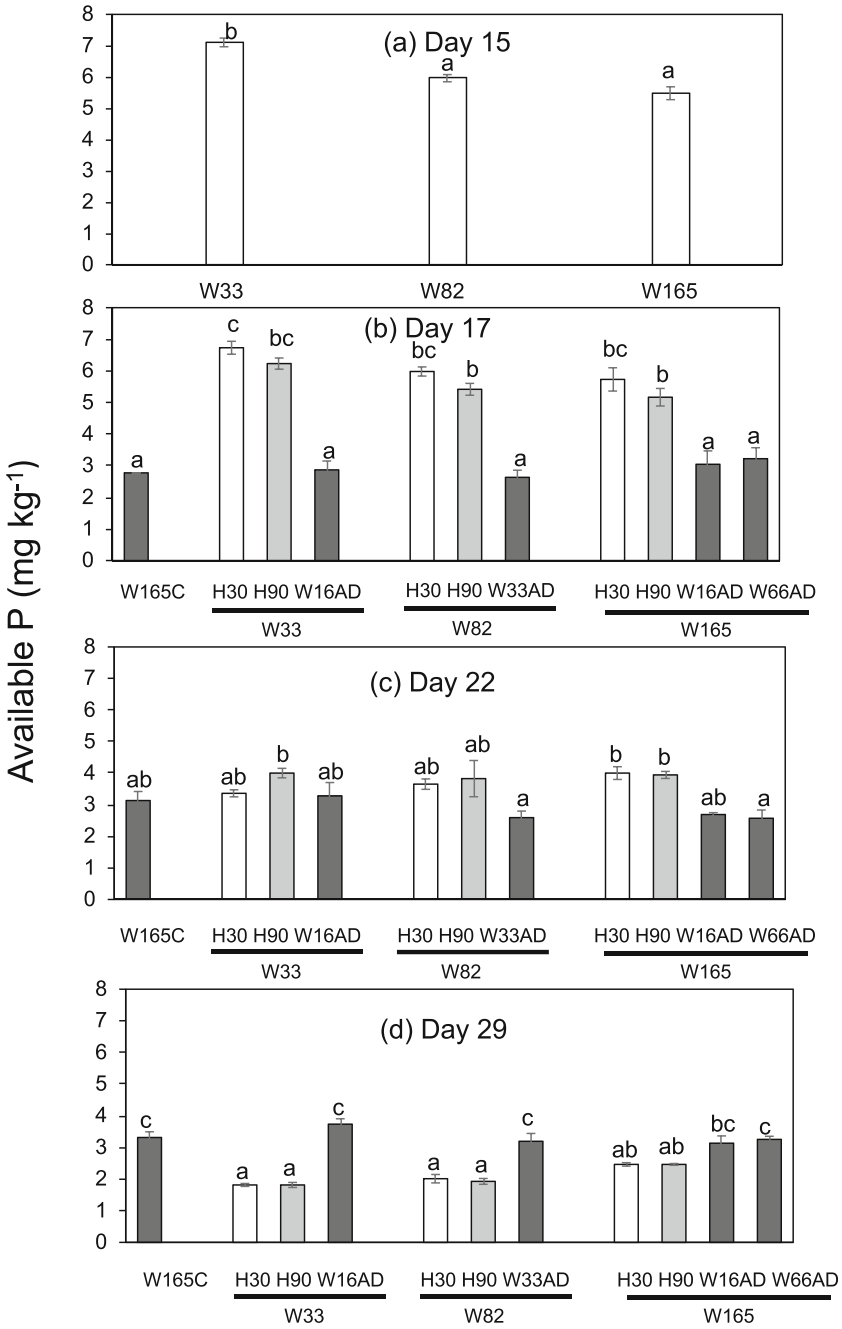

Fig. 3 Available P in constantly moist soil (W165C) and in soils pre-incubated at different water contents (W33, W82, and W165) which were heated to 60 °C for 30 or 90 min (H30, H90) on day 15 as well as corresponding air-dried soils (W16 AD, W33 AD, W100 AD, W66 AD), before heating (**a**) and on days 17, 22, and 29 (2, 7 and 14 days after rewetting, **b**, **c**, **d**) ($n = 4$, means ± SE). At each sampling time, different letters indicate significant differences in available P among treatments

higher than in the corresponding air-dried soils in soil heated for 30 min and twofold higher in soil heated for 90 min.

Available P on day 15 (before heating) was about 20% higher in W33 than at higher water contents (Fig. 3a). Soil water content before heating and heating duration had little effect on available P at all sampling times after heating. Two days after heating (day 17), available P was similar in W165C and the air-dried soils but was 75% to twofold higher in all heated soils (Fig. 3b). In heated soils, available P was higher on day 17 than day 29, but it changed little over time in W165C and the air-dried soils. There was little difference in available P among treatments on day 22 (Fig. 3c). On day 29, available P was about 30% lower in heated soils than W165C and the air-dried soils (Fig. 3d).

Experiment 2

On the first day after rewetting, respiration rates of heated soils were two to threefold higher than in the constantly moist controls and the air-dried soils (Fig. 4a–e). Heating increased respiration rate until about day 5 compared to the controls.

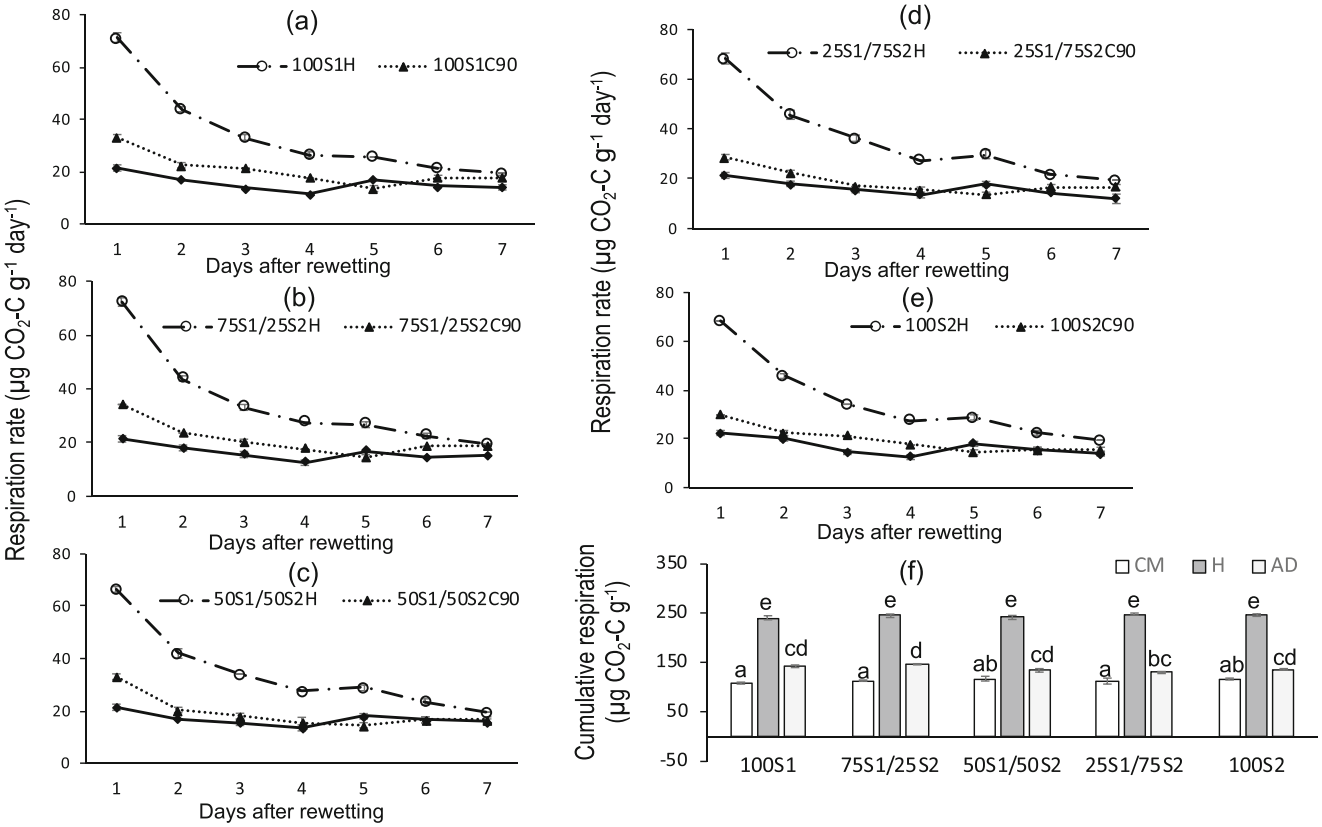

Fig. 4 Respiration rate after rewetting (**a**–**e**) and cumulative respiration (**f**) over 7 days (days 16–22) in soils 1 and 2 (100S1, 100S2) and mixes with different proportions of soil 1 and soil 2 (75S1 + 25S2, 50S1 + 50S2, 25S1 + 75S2) heated to 60 °C for 30 min and rewetted and the corresponding air-dried and constantly moist controls. In panel **f**, bars with different letters indicate significant differences in cumulative respiration among treatments ($n = 4$, means ± SE). Error bars in panels **a**–**e** are mostly too small to be visible

In the air-dried soils, respiration rate was about 20% higher than in the constantly moist controls until about 4 days after rewetting. Compared to the constantly moist soils, cumulative respiration was 25% higher in air-dried soils and twofold higher in heated soils. The proportion of soil 1 and 2 had no effect on respiration.

Available N increased with proportion of soil 1 at all sampling times. On day 15 (before heating), available N was about threefold higher in 100S1 than 100S2 (Fig. 5a). Nitrate was the dominant form of available N, and its proportion was higher in 100S2 (95% of available N) than 100S1 (80%). On day 17 (2 days after rewetting), available N was twofold higher in 100S1 than 100S2 (Fig. 5b). Heating increased available N by about 10% compared to the constantly moist controls and the air-dried soils. This increase was mainly due to an increase in exchangeable ammonium which was two to threefold higher in heated soils, with a greater relative increase in 100S2 than 100S1. Available N in heated soils increased from day 17 to day 22 but remained unchanged in the constantly moist controls and air-dried soils (Fig. 5c). This increase in the heated soils was also due to higher exchangeable ammonium on day 22 than day 17. Available N on day 22 was about 20% higher in heated soils than the controls.

Available P was two to threefold higher in 100S1 than 100S2 at all sampling times (Fig. 6a–c). It increased with the proportion of soil 1 in the soil mixes. Heating had no effect on available P compared to the constantly moist controls and air-dried soils. Available P changed little over time.

Discussion

The results of the two experiments showed that heating induced a flush of respiration and influenced N and P availability after rewetting which can only be partially explained by the low water content after heating. The heating effect was influenced by water content before heating (Experiment 1) and soil properties (Experiment 2).

Based on the results, both the first hypothesis (the impact of heating on the measured properties will be due to both water loss during heating and heat, thus different from the air-dried controls) and the second hypothesis (the impact of heating will change with time after heating) can be confirmed.

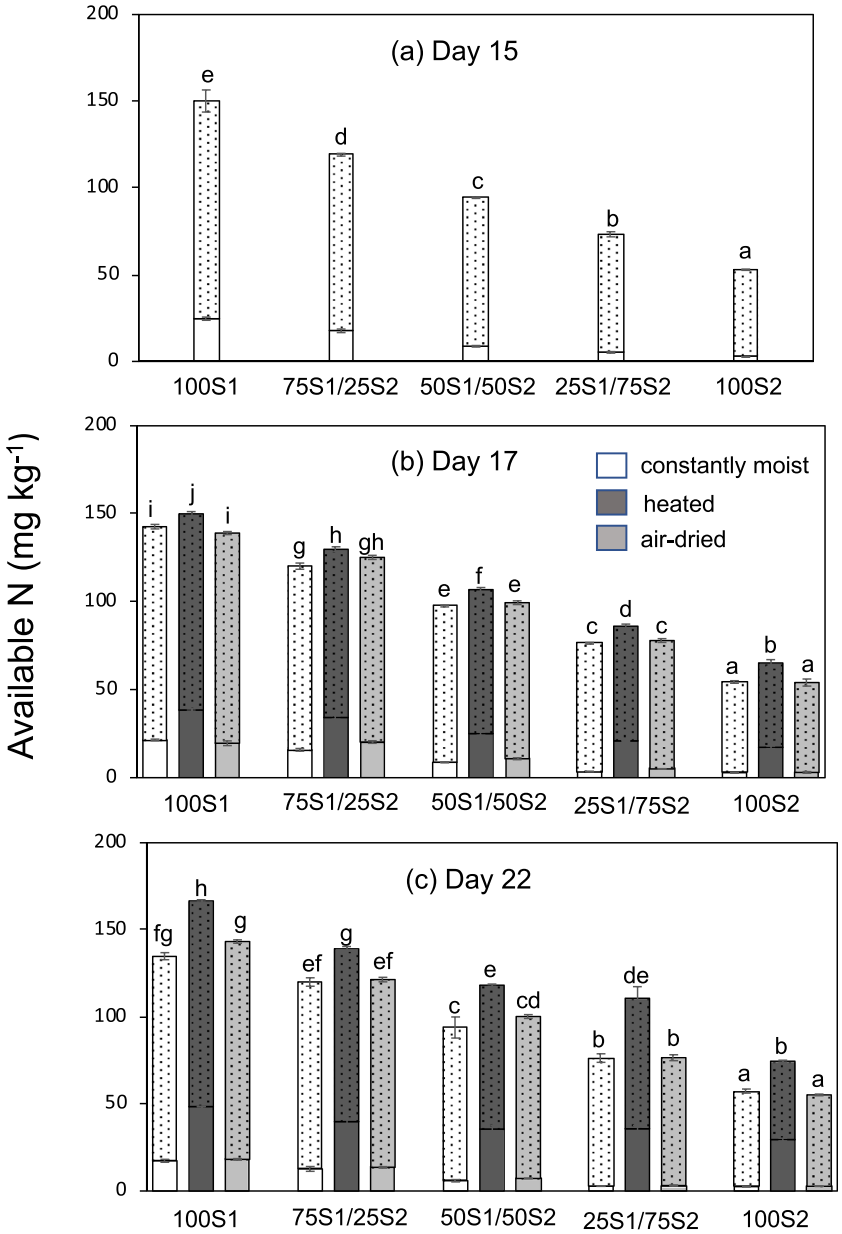

Fig. 5 Available N in soils 1 and 2 (100S1, 100S2) and mixes with different proportions of soil 1 and soil 2 (75S1 + 25S2, 50S1 + 50S2, 25S1 + 75S2) heated to 60 °C for 30 min on day 15 and rewetted, corresponding air-dried soil and constantly moist controls before heating (day 15, **a**) and days 17 and 22 (2 and 7 days after rewetting, **b**, **c** ($n = 4$, means ± SE). Bar segments with no pattern indicate exchangeable ammonium, stippled segments nitrate. At each sampling time, bars with different letters indicate significant differences in available N among treatments

Experiment 1

In the following, heat duration will not be discussed because it had, in general, little effect on the measured parameter. Therefore, the third hypothesis (the impact of heating will be greater after 90 min heating than 30 min) has to be declined. The lack of effect may be because the two durations differed little in water content after heating and thus amount of water added at rewetting. Thus, the impact of heat exposure on soil microbes and nutrient availability was likely quite similar. It is possible that a much longer heat duration, e.g. 300 min would have a different effect on the measured properties than 30 min.

There was a flush of respiration after rewetting in all heated soils which resulted in an about twofold higher cumulative respiration than in the constantly moist control. Rewetting of dry soil has been shown to induce a flush of respiration which can be explained by higher substrate availability from lysed microbes, aggregate breakdown and released osmolytes (Denef et al. 2001; Xiang et al. 2008; Borken and Matzner 2009). A similar flush as in the heated soils occurred in the air-dried control W16 AD and a smaller flush in W33 AD, but there was no flush in W66 AD and W100 AD. The absence of a flush in the air-dried controls W66C and W100C can be explained by the high water content before rewetting. It has been shown that the size of the respiration flush decreases with water content before rewetting (Meisner et al. 2017). The finding that there was a flush also in some air-dried soils suggests that the flush in the heated soils was in part due to the low water content after heating. However, cumulative respiration was always higher in the heated soils than the air-

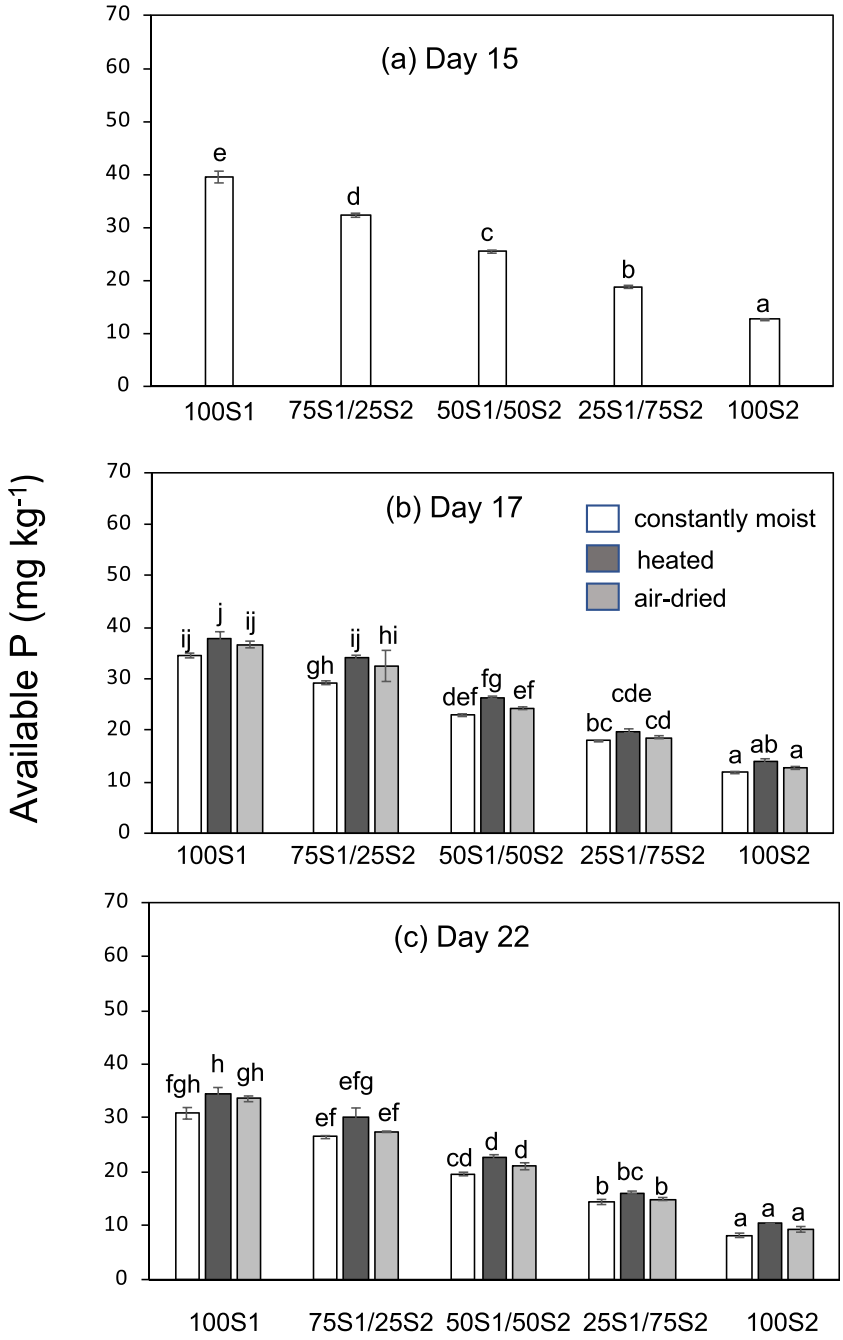

Fig. 6 Available P in soils 1 and 2 (100S1, 100S2) and mixes with different proportions of soil 1 and soil 2 (75S1 + 25S2, 50S1 + 50S2, 25S1 + 75S2) heated to 60 °C for 30 min on day 15 and rewetted, corresponding air-dried soil controls and constantly moist controls before heating (day 15, **a**) and days 17 and 22 (2 and 7 days after rewetting, **b**, **c** ($n = 4$, means ± SE). At each sampling time, bars with different letters indicate significant differences in available P among treatments

dried soils, particularly in heated soils W82 and W165. This indicates that the heating effect was not only due to soil drying. Heating may have increased substrate availability by inducing changes in organic matter chemistry. Prokushkin and Tokareva (2007) reported that experimental heating of forest litter to about 50 or 105 °C for 24 h increased water-soluble organic C compared to unheated soil. Another possible explanation is that a greater proportion of microbes survived heating compared to air-drying because it was shorter (maximal 2.5 h compared to 7–48 h in the air-dried soils). The short duration may have allowed a proportion of microbes to survive by producing small amounts of extracellular polysaccharides to minimize desiccation. The longer air-drying may have required greater polysaccharide production, draining energy cellular energy supply (Roberson and Firestone 1992). Therefore, substrate released after rewetting of heated soils was decomposed quickly by microbes which had greater energy supply than microbes in air-dried soils. This is corroborated by the high N availability after rewetting (see below).

At the end of the pre-incubation (day 15) available N increased with soil water content. This is likely due to stimulation of microbial activity with increasing water content due to higher water availability and greater diffusion of substrates

(Manzoni et al. 2012; Moyano et al. 2013). The proportion of nitrate also increased with water content suggesting rapid nitrification.

The fourth hypothesis (heating will have a greater effect on the measured properties when the soil water content was low prior heating because more mineralizable substrates are left) can only be confirmed for available N and P 2 days after rewetting. Two days after rewetting (day 17), available N was twofold higher in heated soils than the constantly moist control. The increase in available N compared to before heating (day 15) was greatest in heated soils that had been at W33 before heating where it increased more than twofold. The higher available N in heated W33 and W82 cannot be explained by rewetting because available N was about twofold higher than the corresponding air-dried soils (W16 AD and W33 AD) which did not differ from the constantly moist control. The higher N availability in the heated soils is likely due to the high microbial activity after rewetting and possibly increased organic N mineralization as a result of changes in organic matter chemistry. The increase in available N from day 15 to 17 in the treatments where water was limiting during pre-incubation (W33 and W82 prior heating) was likely due to higher microbial activity not an increase in potentially mineralizable N. After day 17, available N remained unchanged in the constantly moist control and air-dried soils. However in heated soils, it decreased in soils that were at W33 and W82 before heating, whereas it increased in heated soil W165. As a result, available N on day 29 was similar in the constantly moist control, heated and air-dried soils W33 and W82, but about twofold higher in heated W165C. This suggests heating induced a rapid increase in N mineralization in heated W33 and W82 where a large proportion of potentially mineralizable organic N had remained after pre-incubation. But in heated soils W165, inorganic N 2 days after heating was similar as prior heating despite higher microbial activity; N mineralization may have increased but was compensated for by high microbial N uptake. The increase in available N over time in heated soils W165 could be due to microbial biomass turnover when respiration was low. In heated W33 and W82 on the other hand, microbial N uptake may have been limited immediately after rewetting because of a smaller microbial biomass at the end of the pre-incubation. Microbial N uptake in these soils likely increased over time. However, we cannot support this explanation because were not able to get reliable microbial biomass N data due to the very high variability among replicates in the heated soils.

Available P on day 15 was higher in W33 than W82 and W165. This is likely due to increased P extractability after rewetting of dry soil (Turner et al. 2002; Butterly et al. 2011), thus does not reflect actual P availability at low water content. Available P did not change from day 15 to day 17 in heated soils but decreased in constantly moist controls and the air-dried soils. The decrease to day 17 may be due to P sorption to soil particles. In the heated soils, sorption apparently occurred later, from day 17 to day 22 and 29. Heating may have initially induced an increase in P mineralization that compensated for P sorption. But after day 17, when microbial activity was low, P sorption dominated. The lower P availability in heated soils than in the constantly moist controls and the air-dried soils on day 29 indicates that heating increased soil P sorption capacity. The higher P sorption capacity may be due to the higher cumulative respiration in the heated soils which could decrease the concentration of soluble organic compounds such as organic acid anions which compete with P for sorption sites on soil particles (Gerke 1994; Iyamuremye et al. 1996).

Experiment 2

The fifth hypothesis (the relative impact of heating will be greater in soil with higher nutrient content) has to be declined because the relative increase in available N after heating was greater in soil 2 which had lower N and P than in soil 1.

Irrespective of the proportion of the two soils, rewetting of heated soils induced a marked flush of respiration but only a very small one in air-dried soils. The small flush in the air-dried soils is likely because their water content before rewetting was quite high (90 g/kg^{-1}). In drying and rewetting experiments (without heating), the flush upon rewetting decreases with increasing water content before rewetting (Meisner et al. 2017). However in the heated soils, where the water content before rewetting was also 90 g kg^{-1}, there was a strong rewetting flush which is similar as in experiment 1. This confirms that the flush of respiration after rewetting of heated soils is only partly due to drying during heat exposure.

Differences in available N and P between soil 1 and 2 and their mixes remained similar at all sampling times. The higher available N and P in soil 1 can be explained by its higher total N and P. As in W165 in experiment 1, available N increased slightly from day 15 to 17 and then further to day 22 in heated soils, but not in the constantly moist controls and the air-dried soils. This increase was mainly due to ammonium with the relative increase greater in soil 2 than soil 1; from day 15 to 22, threefold in soil 2, about tenfold in soil 1. In the soil mixes, the increase in ammonium corresponded to the proportion of soil 1. This suggests that heating induced greater ammonification in the soil with the lower available N concentration, possibly by improving accessibility of organic N. The lack of increase in nitrate suggests inhibition of nitrifiers by heating. Heating may have induced death or dormancy of some microbes which would have a greater effect on nitrification than ammonification because a large proportion of soil microbes can mineralize organic N to ammonium, whereas a limited number of microbes perform nitrification. This interpretation is corroborated by Myers (1975) who reported

that ammonium in soil increased with temperature from 20 to 60 °C whereas nitrate peaked at about 35 °C and then sharply decreased.

Heating had little effect on available P which may be due to the relatively high P availability in all soil treatments, particularly those with a high proportion of soil 1. P availability in 100S2 was higher than in experiment 1 which may be due to the longer storage. Nevertheless, the results confirm that heating has little effect on P availability. P availability did not decrease with time in heated soils as in experiment 1, probably because of the shorter duration of experiment 2.

Conclusion

This study showed that the effect of temperature on soil microbial activity and nutrient availability was due to the effect of both heat and water loss. Short-term heating appeared to increase substrate availability which may be due to changes in organic matter chemistry and accessibility. The immediate effect of heating on respiration and available N was greater in soils that had been dry before heating. This indicates that the amount of decomposable substrate available plays an important role in mineralization after heating. In a field situation, this suggests that a rapidly moving fire followed by rain will have a greater immediate effect on nutrient mineralization if it occurs after a dry period than when the soil is moist. The effects on soil N and P availability after heating changed over time suggesting changes in the ratio of mineralization to immobilization and soil nutrient sorption capacity.

Acknowledgments M. Seneviratne received a Turner Family postgraduate scholarship.

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Chapter 3

Amendment type and time of addition influence the effect of short-term heating on soil respiration and nutrient availability

Published in Journal of Soil Science and Plant Nutrition
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Statement of Authorship

Title of the Paper	Amendment type and Time of Addition Influence the Effect of Short-term Heating on Soil Respiration and Nutrient Availability
Publication Status	[X] Published [ ] Accepted for Publication [ ] Submitted for Publication [ ] Unpublished and Unsubmitted work written in manuscript style
Publication details	Seneviratne, M., Doolette, A., Marschner, P., 2019. Amendment type and time of addition influence the effect of short-term heating on soil respiration and nutrient availability. Journal of Soil Science and Plant Nutrition, 1-8.

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Contribution to the paper	Performed the experiment, analysed all the samples, data analysis and interpretation data, writing the manuscript. I hereby certify that the contribution is accurate		
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 subjected 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	Mihiri Seneviratne	Date	10/02/2020

Co-Author contributions

By signing the Statement of Authorship, each author certifies that

i. The candidates 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 candidates stated contribution

Name of Co-Author	Petra Marschner		
Contribution to the paper	Supervised development of work, data interpretation, manuscript evaluation and correction, acted as the corresponding author		
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Contribution to the paper	Evaluated the manuscript		
Signature		Date	14/02/2020

Seneviratne, M., Doolette, A., Marschner, P., 2020. Amendment type and time of addition influence the effect of short-term heating on soil respiration and nutrient availability. Journal of Soil Science and Plant Nutrition 1-8

Journal of Soil Science and Plant Nutrition
https://doi.org/10.1007/s42729-019-00127-7

ORIGINAL PAPER

Amendment type and Time of Addition Influence the Effect of Short-term Heating on Soil Respiration and Nutrient Availability

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Received: 10 August 2019 / Accepted: 8 November 2019

Abstract
Heating of soil influences soil respiration and nutrient availability. But little is known about how amendment type (plant residue or inorganic N and P fertilisers) or time between amendment application and heating influence the effect of heating. A sandy clay loam was incubated moist (165 g kg^{-1}) for 8 days and amended with the same amount of total N and P as pea residue (C/N 59, C/P 435) or inorganic N and P (0.4 g N kg^{-1} and 0.06 g P kg^{-1}) either 8 or 1 day before heating. On day 8, soils were heated to 60 °C for 30 min followed by rewetting to 165 g water kg^{-1} and moist incubation for 7 days. Soil was sampled before heating (day 8), 2 and 7 days after heating (day 10 and 15). Heating only reduced respiration when residue was added 1 day before heating. Heating had no effect on available N in fertiliser treatments. Heating increased available N on day 10 in the unamended soil by about 20% and in residue treatments about 10-fold, particularly when residue was added 1 day before heating. Heating increased phosphatase P two- to fivefold only on day 10; it increased citrate P on days 10 and 15 up to threefold compared with a greater effect in the residue treatments. Heating of soil had a greater effect of on respiration and available N and P pools after residue addition than unamended soil or soil amended with inorganic fertiliser.

Keywords Available N · Heating · P pools · Pea residues · Respiration

1 Introduction

Temperature is an important driver of soil nutrient cycling. Between 0 and 35 °C, N and P mineralisation increases with temperature, which can be explained by increased microbial activity (Floate 1970; Cookson et al. 2007; Koch et al. 2007). Temperatures > 100 °C have been shown to induce a rapid and short-term increase in nutrient availability (Hernández et al. 1997). This is mainly due to thermal mineralisation of organic matter and incorporation of ash into the soil (Prieto-Fernandez et al. 1993; Fernández et al. 1997; Certini 2005; Alcañiz et al. 2018). However, it is unclear how soil properties are affected by short-term heating to 60 °C, as it may occur in fast-moving fires with low fuel load such as in grassland or shrubland (Scotter 1970; Stoof et al. 2013).

Heating of soils is usually accompanied by drying, thus influencing soil water content which also plays an important role in nutrient cycling (Paul et al. 2003; Xue et al. 2017). Low water content reduces microbial activity because of low substrate diffusion and water availability (Moyano et al. 2013; Schimel 2018). Rapid rewetting of dry soil results in a flush of microbial activity due to increased substrate availability (Birch 1959; Warren 2014). In most previous studies involving heating, soils were rewetted after heating (e.g., Choromanska and DeLuca 2002; Guerrero et al. 2005; Bárcenas-Moreno and Bååth 2009). The higher microbial activity after heating may therefore be due to the combination of heating and rewetting. In a recent study (Seneviratne et al. 2019), we showed that respiration and nutrient availability of heated soils (60 °C for 30 min) after rewetting differed from those of rewetted air-dried soils that had the same water content as the soils after heating. This suggests that the effect of heating on respiration and nutrient availability can not only be explained by rewetting of dry soil.

Soil nutrient availability and microbial activity after heating may also be affected by soil nutrient content. In our recent study (Seneviratne et al. 2019), two soils with similar texture but different organic C and total N content were heated to 60 °C for 30 min and rewetted. Available N was increased by heating only in the soil with low C and N concentration. In

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Published online: 25 November 2019

that study, the reasons for observed differences in heating effect were unclear because the soils differed in a range of properties (e.g., organic matter content).

Not only nutrient content but also nutrient form may influence the heating effect. Nutrients can be added to soil in inorganic form or as organic amendment. Inorganic amendments increase e.g. N and P availability but often have little effect on soil respiration because the microbes are organic C limited (Zhong and Cai 2007; Eberwein et al. 2015). Organic amendments on the other hand add not only N and P but also organic C which increases soil respiration (Ferreras et al. 2006; Flavel and Murphy 2006). With organic amendments, soil nutrient availability is also influenced by the C/N and C/P ratio of the amendment (Hadas et al. 2004). For example, organic amendments with C/N < 20 induce an increase in N availability, whereas addition of organic materials with C/N > 20 can result in lower N availability, at least temporarily. Nutrient availability after amendment application may change over time which can influence the impact of heating on soil properties. After the addition of inorganic N and P, N availability may decrease due to denitrification or leaching and sorption or precipitation may reduce P availability (Gustafsson et al. 2012; Gérard 2016). With organic amendments, depletion of easily decomposable compounds over time reduces soil respiration; N and P availability can change due to lower microbial activity or turnover of the microbial biomass (Ajwa and Tabatabai 1994; Aneja et al. 2006).

Little is known about how amendment form and time of addition influence the impact of soil heating on soil respiration and nutrient availability. Understanding how the effect of short-term heating on soil respiration and nutrient availability is modulated by amendment form and time can improve nutrient management after rapidly moving fires with low fuel load such as grass or shrub fires. In this experiment, soil was heated to 60 °C, maintained at this temperature for 30 min, then allowed to cool and rewet. The aim was to determine how amendment form (inorganic nutrients or organic amendment) and time between amendment application and heating (1 or 7 days) influence the impact of heating on soil respiration, N availability, and P pools. Phosphorus pools were measured to assess the effect on available P and sorbed P. The following hypotheses were tested: (i) fertiliser addition will not influence soil respiration, but increase nutrient availability and change the effect of heating on nutrient availability compared with unamended soil, (ii) with fertiliser, the time between amendment application and heating (1 or 8 days) will have little effect on the measured parameters, (iii) residue addition will increase respiration and nutrient availability compared with unamended soil and increase the impact of heating on the measured parameters, and (iv) the effect of heating will be greater when residue is added 1 day before heating than when added 8 days before. These hypotheses are based on the following assumptions: Hypotheses i and ii, compared with the unamended soil, fertiliser addition will not increase C supply to microbes but will increase nutrient availability to such an extent that binding of N or P to soil particles or heating will have little effect on N and P availability. Hypotheses iii and iv, residue amendment increases C availability to microbes and therefore their activity particularly shortly after residue addition. Active microbes are more susceptible to stress than less active microbes (Van Gestel et al. 1993; Schimel et al. 2007).

2 Materials and Methods

Sandy clay loam was collected on the Waite Campus of the University of Adelaide, South Australia (longitude 138° 38′ 3.2″ E, latitude 34° 58′ 0.2″ S). The climate is Mediterranean with cool, wet winters and hot, dry summers. The soil was a Chromosol based on Australian soil classification (Isbell 2002) and a Rhodoxeralf according to US Soil Taxonomy. Soil was collected at 0–10-cm depth from several randomly selected locations, combined, air-dried, and sieved to < 2 mm. The soil properties were sand 48%, silt 27%, clay 25%, pH (1:5 soil:water) 5.3, total organic carbon 22 g kg^{-1}, total N 1.6 g kg^{-1}, total P 400 mg kg^{-1}, available N 53 mg kg^{-1}, available P 13 mg kg^{-1}, maximum water holding capacity (WHC) 330 g kg^{-1}, and bulk density 1.3 g cm^{-3}.

The pea (*Pisum sativum* L.) residue used in this experiment had the following properties: total organic C 474 g kg^{-1}, total N 7.9 g kg^{-1}, total P 1.1 g kg^{-1} (C/N 59 and C/P 435), water extractable N 0.2 g kg^{-1}, and P 0.5 g kg^{-1}. The residue was dried in a fan-forced oven at 40 °C, finely ground and sieved to particle size 0.25–2 mm.

2.1 Experimental Design

The residue was added at 50 g kg^{-1} which approximately doubled the organic C content of the soil (equivalent to 0.4 g N kg^{-1} and 0.06 g P kg^{-1}), referred to as R treatments. The same amount of N and P was added as inorganic N and P, referred to as F treatments. The inorganic fertiliser solution was prepared by dissolving NH_4Cl and K_2HPO_4 in water. Amendments were added either on day 0, eight days before heating (F7, F7H, R7, R7H, where H indicates heated treatments), or 1 day before heating, on day 7 (F1, F1H, R1, R1H).

On day 0, 30-g air-dried soil was placed in small plastic bags and reverse osmosis (RO) water was added to adjust the soil water content to 165 g kg^{-1} (50% of maximum WHC) which is optimal for microbial activity in this soil. After thorough mixing, the soil was left unamended (UA, UAH) or was amended with residues or fertiliser. The containers were placed in dark at 22–25 °C for 7 days. Reverse osmosis water was added in every second day in order to maintain the soil water content at 165 g kg^{-1}. On day 7, soil from the containers was tipped into small plastic bags to allow thorough mixing.

For treatments amended 1 day before heating (F1, F1H, R1, R1H), residue or fertiliser solution was added and mixed into the soil. For the other treatments (F7, F7H, R7, R7H UA, UAH), soil was mixed in a similar manner. Then the soil was placed back into the 70-ml containers and bulk density was adjusted to 1.3 g cm^{-1}.

On day 8, eight containers of each unamended soil or soil amended with residue or fertiliser were gradually heated to 60 °C in a fan-forced oven within 1 h and maintained at 60 °C for 30 min. Then the oven was turned off and the soil allowed to cool to room temperature which took about 2 h. This heating corresponds to soil temperatures in fast-moving grass fires with low fuel load (Scotter, 1070, Stoof et al. 2013). Temperature was monitored with thermocouples placed in the soil. After reaching room temperature, the soil was rewetted to 165 g kg^{-1}. The other eight containers of each amendment treatment remained at room temperature. The duration (30 min) was chosen because the effect of heating on soil respiration and nutrient availability was similar with heat durations at 60 °C for 30 and 90 min (Seneviratne et al. 2019). Both heated and unheated treatments were placed in the dark at 22–25 °C for a further 7 days (days 9–15) and maintained at 165 g kg^{-1} by adding reverse osmosis water in every second day.

Respiration was measured daily from day 1 to day 15. Soils were destructively sampled before heating (day 8), and on days 10 and 15 (2 and 7 days after rewetting), and analysed for available N and P pools (described below). For each sampling time and treatment, there were four replicates.

2.2 Measurements

Soil texture was measured by the hydrometer method (Gee and Or 2002). Maximum water-holding capacity was measured at matric potential – 10 kPa as described in Wilke (2005). Soil pH was measured in a 1:5 (w/v) soil to RO water ratio after Rayment and Higginson (1992). Wet oxidation and titration (Walkley and Black 1934) was used to measure total organic C in soil and plant residue. The materials were acid digested for the determination of total N (H_2SO_4) and P (HNO_3 and HCl). Total N in the digest was measured by a modified Kjeldahl method (McKenzie and Wallace 1954) and total P by the phosphovanado-molybdate method (Hanson 1950).

Soil P pools were measured as described in DeLuca et al. (2015). Each pool was measured in parallel by shaking 1 g of soil with each extractant (20 ml of 10 mM $CaCl_2$, 10 mM citric acid, 0.2 enzyme units acid phosphomonoesterase, or 1 M HCl) for 3 h on an end-over-end shaker. The respective P pools are referred to as $CaCl_2$ P, phosphatase P, citrate P, and HCl P. Phosphorus in the filtered supernatant was measured by the malachite-green method as described in Ohno and Zibilske (1991). Available N (ammonium and nitrate) was measured after extraction with 2 M KCl at a 1:5 (w/v) soil to extractant ratio. Ammonium-N was measured as described in Willis et al. (1996) and nitrate-N according to Miranda et al. (2001).

Soil respiration was measured with a Servomex 1450 infrared gas analyser (Servomex Group, Crowborough, UK) as described in Setia et al. (2011).

2.3 Statistical Analysis

There were four replicates for each treatment at sampling time. Data was checked for normality using the Shapiro-Wilk test. Cumulative respiration was analysed by one-way ANOVA. The data of available N and P pools was transformed to log10 values to achieve normality and analysed by repeated measures ANOVA with between-subjects factor-treatment and within-subjects factor-time in SPSS statistics version 25. Tukey's multiple comparison test at 95% confidence interval for the treatment × time interaction was used to determine significant differences among treatments and sampling times.

3 Results

In all treatments, respiration rate gradually decreased from day 1 to day 8 (Fig. 1). Fertiliser addition on day 7 (F1, 1 day before heating) had little effect on respiration rate (Fig. 1c). In unamended soil and fertiliser treatments, heating on day 8 (UAH, F1H, F8H) increased respiration rate by about 50% compared with the unheated treatments (UA, F1, F7). After day 9, respiration rate gradually decreased in heated treatments which became similar to the unheated treatments after day 12. In residue treatments (R1 and R7) respiration rates after amendment addition were an order of magnitude higher than the control and fertiliser treatments (Fig. 1b). Residue addition in R1 on day 7 (1 day before heating) resulted in an about 10-fold increase in respiration rate compared with day 6 so that on day 8, respiration rate was about 50% higher in R1 than in R7. Heating had little effect on respiration rate in R8. But in R1H, respiration rate was about 40% lower 1 day after heating (day 9) than before heating (day 8). Respiration rate of R1H remained low until day 12 after which it gradually increased until reaching nearly the same respiration rate on day 15 as after residue addition before heating (day 8). In contrast, respiration rates remained high in R1 until day 10 and then gradually decreased.

Fertiliser addition did not affect cumulative respiration compared with UA and in both UA and F treatments; heating did not influence cumulative respiration (Fig. 1d). Cumulative respiration of the residue treatments was 10- to 12-fold higher than UA (Fig. 1d). It was about 20% higher in R8 than R1. Heating (R1H) reduced cumulative respiration compared with

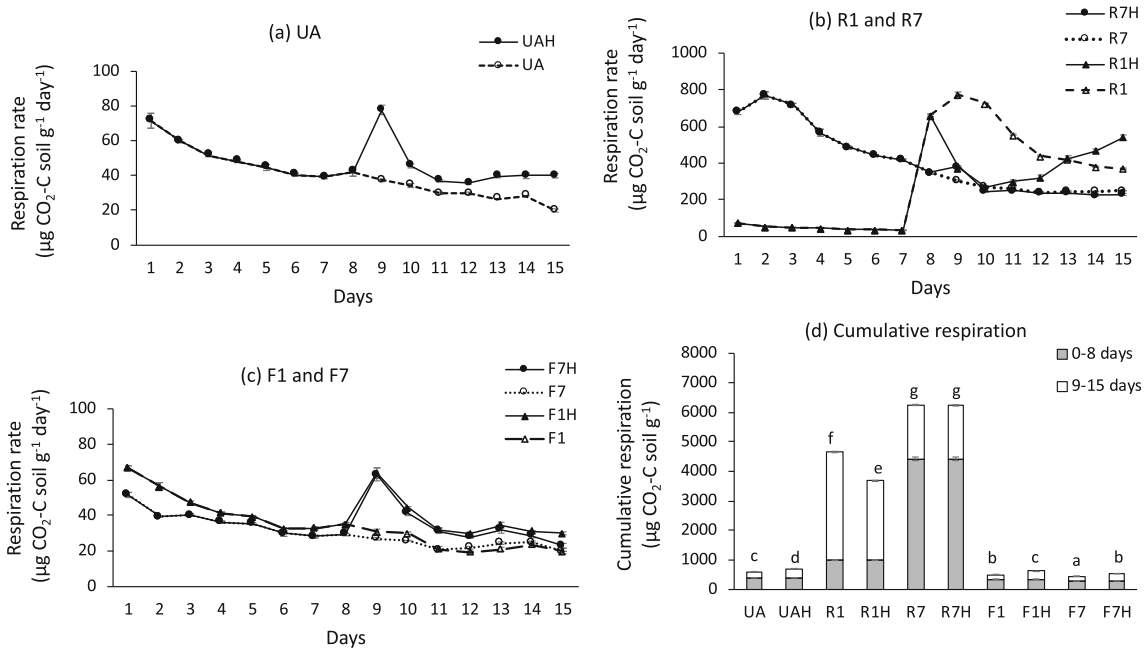

Fig. 1 Respiration rate over 15 days of soils **a** without amendment (UA), or amended with **b** with pea residue (R), or **c** inorganic fertiliser (F) 1 or 7 days before heating. On day 8, soils were heated to 60 °C for 30 min and rewetted (UAH, F1H,F7H, R1H, R7H) and the corresponding constantly moist soils which remained unheated (R1, F1, R7, F7). **d** Cumulative respiration before and after heating. Bars in **d** with different letters have significantly different total cumulative respiration ($P \leq 0.05$, $n = 4$, means ± SE)

R1 by about 15%. On the other hand, cumulative respiration was similar in R7 and R7H.

Before heating (day 8), available N was lowest in the residue treatments (Fig. 2a). Available N was about eightfold higher in UA than in the residue treatments. Compared with UA, available N was fivefold higher in the fertiliser treatments. On day 10 (2 days after heating), available N was highest in the fertiliser treatments where it was not affected by heating (Fig. 2b). In UA and the residue treatments, heating increased available N compared with the unheated soil: in UA by about 20%, in the residue treatments by about 10-fold, with a greater increase in R1H than in R7H. The increase in available N in UA and the residue treatments was due to increase in ammonium whereas nitrate changed little. On day 15 (7 days after heating), available N was highest in the fertiliser treatments and lowest in residue treatments (Fig. 2c). Heating had little effect on available N on day 15. Available N changed a little during the experiment in the fertiliser treatments. In UAH, available N remained high from day 10 to 15, whereas in heated residue treatments (R1H and R7H), available N decreased from day 10 to 15.

On day 8, $CaCl_2$ P was not detectable in the R treatments (Table 1). It was twofold higher in F1 than in UA and F8. Compared with R1, phosphatase P was twofold higher in UA and R7 and two- to threefold higher in the F treatments. Citrate P was lowest in R7 and about twofold higher in the other treatments. HCl P was also lowest in R7 and 10–15% higher in the other treatments. Two days after heating (day 10), $CaCl_2$ P was very low in the unheated soils (Fig. 3a). Heating had no effect on $CaCl_2$ P in the residue treatments. On day 10, $CaCl_2$ P was about fivefold higher in UAH compared with that in UA, threefold higher in F1H than in F1, and more than 10-fold higher in F7H than in F7. On day 15, $CaCl_2$ P was not detectable in the R treatments (Fig. 3b). It was similar in UA, F1, F7, and F7H, but was about twofold higher in UAH than in UA and 50% higher in F1H than in F1. $CaCl_2$ P increased from day 10 to 15 in the unamended treatments, F1, F1H, and F7, but decreased slightly in F7H. Phosphatase P was detectable only on day 10 (Fig. 3c). It was low in most unheated treatments except in F1 where it was about fourfold higher than in UA. Phosphatase P did not differ between R7 and R7H and F1 and F1H, but heating increased phosphatase P in the other treatments. Compared with the unheated treatments, phosphatase P was fivefold higher in UAH and F7H and 10-fold higher in R1H. On day 10, citrate P in the unheated soils was lower in the residue treatments than in the control and the fertiliser treatments (Fig. 3e). Heating increased citrate P in the residue treatments and fertiliser treatments but not in the control. Heating had a greater relative effect in the residue treatments than the fertiliser treatments. Compared with the unheated soils, citrate P was three- and twofold higher in R1H and R7H, but only about 30% higher in F1H and F7H. The relative increase in citrate P in heated compared with unheated soils on day 15 was similar as on day 10 in the residue treatments (Fig. 3f), but heating had no effect in the unamended soil and fertiliser treatments. Citrate P decreased from day 10 to day 15 in most heated soils except F1H. Heating had little effect on HCl P in the unamended soil and in the fertiliser

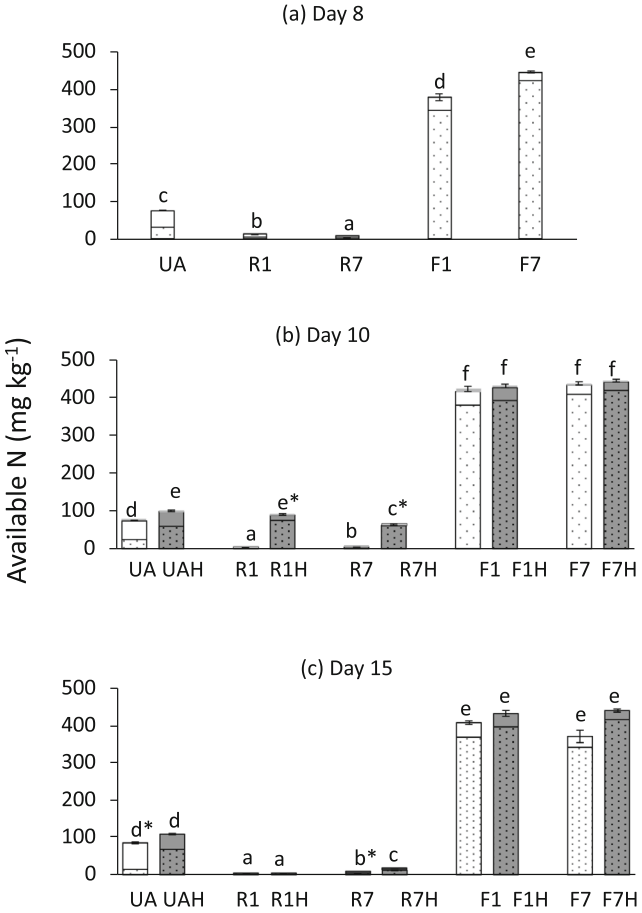

Fig. 2 Available N on days 8, 10, and 15 in soils **a** without amendment (UA), or amended with **b** pea residue (R), or **c** inorganic fertiliser (F) 1 or 7 days before heating. On day 8, soils were heated to 60 °C for 30 min and rewetted (UAH, F1H,F7H, R1H, R7H) and the corresponding constantly moist soils which remained unheated (R1, F1, R7, F7). Bars with different letters at each sampling time are significantly different, asterisk indicates significant increase from previous sampling time ($P \leq 0.05$, $n = 4$, means ± SE). Bar segments with no pattern indicate $NO3^-$ and stippled segments $NH4^+$.

treatments (Fig. 3g, h). In the residue treatments, heating increased HCl P on day 10 by about 20%, but with very little effect on citrate P on day 15. HCl P changed a little over time.

Table 1 $CaCl_2$ P, phosphatase P, citrate P, and HCl P on day 8 in unamended soils (UA), soil with pea residue (R), or inorganic fertiliser before heating (residues and fertiliser was added 1 or 8 days before heating (R1, F1, R7, F7). Values of a given P pool with different letters are significantly different. ($P \leq 0.05$, $n = 4$, means ± SE)

Treatment	$CaCl_2$ P (mg kg^{-1})	Phosphatase P (mg kg^{-1})	Citrate P (mg kg^{-1})	HCl P (mg kg^{-1})
UA	0.2 ± 0.01^{b}	1.7 ± 0.04^{b}	14.2 ± 0.41^{bc}	83.2 ± 1.43^{b}
R1	0.0 ± 0.00^{a}	1.0 ± 0.03^{a}	11.7 ± 0.46^{b}	82.8 ± 1.64^{b}
R8	0.0 ± 0.00^{a}	1.8 ± 0.06^{b}	6.8 ± 0.61^{a}	71.9 ± 4.59^{a}
F1	0.4 ± 0.03^{c}	2.9 ± 0.12^{d}	16.4 ± 0.57^{c}	82.1 ± 0.94^{ab}
F7	0.2 ± 0.02^{b}	2.5 ± 0.03^{c}	15.4 ± 0.31^{c}	78.9 ± 0.86^{ab}

4 Discussion

Based on this study, hypotheses i and ii (fertiliser addition will not influence respiration, but increase nutrient availability and change the effect of heating on nutrient availability compared with unamended soil; and with fertiliser, the time between amendment application and heating will have a little effect on the measured parameters with fertiliser) can be generally confirmed. Hypotheses iii and iv (residue addition will increase respiration and nutrient availability compared with unamended soil and increase the impact of heating on the measured parameters; and the effect of heating will be greater when residue is added 1 day before heating than when added 7 days before) can be confirmed only for some parameters and sampling times.

As expected from other studies (e.g., Fernandez et al. 2003; Baumann et al. 2009), residue addition 8 days before heating (R8) increased respiration rate shortly after amendment compared with unamended soil followed by a gradual decrease in respiration rate. This can be explained by the supply of easily decomposable C compounds with the residues which are depleted over time (Chauvet 1987; Aneja et al. 2006). Residue addition 1 day before heating also increased respiration rate, but heating (R1H) resulted in a rapid decline whereas respiration rate of the unheated soil (R1) remained high for about 3 days. The drastic decline after heating is likely due to death of a proportion of microbes. The small effect of heating on respiration in unamended soil (UA), fertilised treatments, and soil amended with residues 8 days before heating (R8) indicates that heating particularly affected actively growing microbes stimulated by residue addition. This is in agreement with other studies where active microbes were more impacted by stress than less active ones (Van Gestel et al. 1993; Schimel et al. 2007). The gradual increase in respiration rate in R1H after day 12 suggests recovery of the surviving microbes. The higher respiration rate on days 14 and 15 in R1H compared with R1 can be explained by the low respiration rate shortly after heating. Therefore, a greater proportion of easily decomposable compounds remained in R1H compared with R1. Despite the recovery of respiration towards the end of the experiment, cumulative respiration was lower in R1H than in R1.

Fertiliser addition increased N availability compared with the unamended soil irrespective of time since amendment which can be explained by the high solubility of the N added. Compared with the unamended soil, fertiliser addition increased most P pools on day 8 but had little effect on P pools on day 15. This indicates that over time, soluble P added in the fertiliser treatments was converted into stable P forms that were not assessed by the Deluca method. Most P pools in the unamended soil and the fertiliser treatments, except HCl P, were higher in the heated than in the unheated soils. The increase may be due to thermal mineralisation of organic P

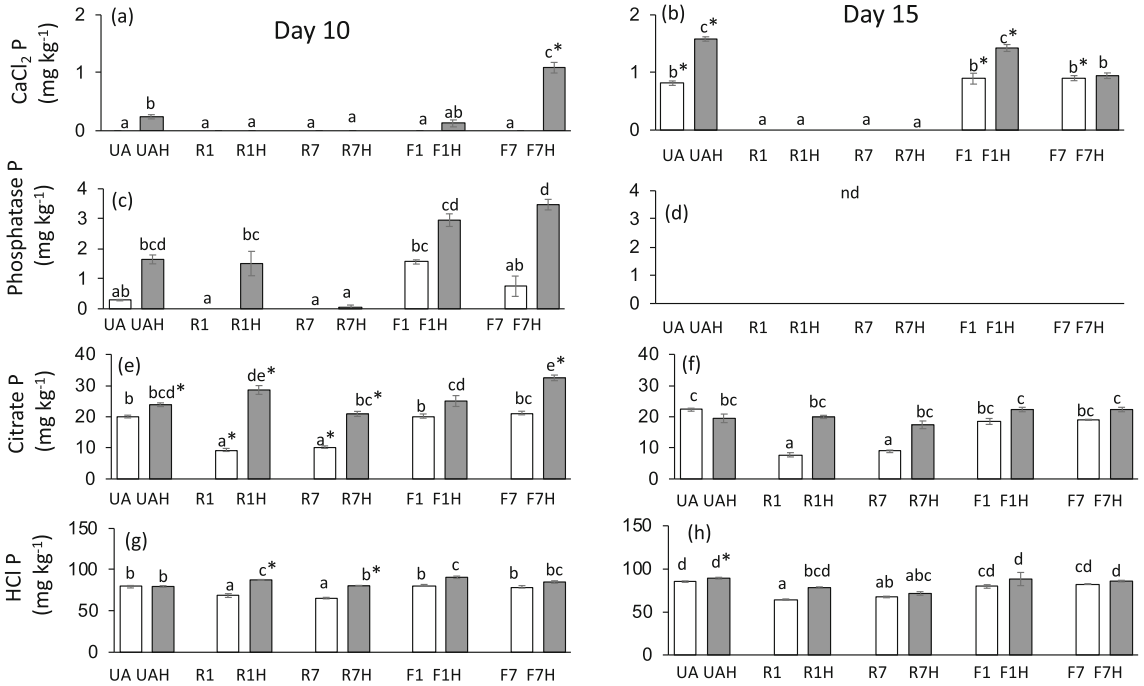

Fig. 3 CaCl2 P (**a**, **b**), phosphatase P (**c**, **d**), citrate P (**e**, **f**), and HCl P (**g**, **h**) on days 10 and 15 in soils without amendment (UA), or amended with pea residue (R) or inorganic fertiliser (F) 1 or 7 days before heating. On day 8, soils were heated to 60 °C for 30 min and rewetted (UAH, F1H,F7H, R1H, R7H) and the corresponding constantly moist soils which remained unheated (R1, F1, R7, F7). Bars with different letters at each sampling time are significantly different; asterisk indicates significant increase from previous sampling time ($P \leq 0.05$, $n = 4$, means ± SE)

(García-Oliva et al. 2018; Merino et al. 2019) and heat-induced microbial death. Another reason for the increase may be the rewetting after heating. Rewetting of dry soil has been shown to increase available P (Turner and Haygarth 2003; Butterly et al. 2011). Within 2 days of rewetting, some of the released P was still available ($CaCl_2$ P), but a proportion had also been converted into phosphatase-labile P, e.g. microbial metabolites, and bound to soil particles (citrate P). From day 10 to 15, phosphatase-labile P may have been mineralised and entered the available P pool ($CaCl_2$ P).

The small effect of residue addition on N availability and $CaCl_2$ P compared with the unamended soil can be explained by the high C/N and C/P ratio (59 and 435) of the pea straw which likely induced N and P immobilisation by the microbial biomass (Tian et al. 1992; Cheshire and Chapman 1996). Growth, and thus immobilisation, was high in the residue treatments as indicated by the higher respiration compared with UA and fertiliser treatments. The lower phosphatase P, citrate P, and HCl P in residue treatments compared with UA may be due to immobilisation, and in the case of citrate P and HCl P by the release of organic acid anions during residue decomposition which compete with P for binding sites on soil particles (Gerke 1993; Iyamuremye et al. 1996; Erich et al. 2002). The lower citrate P and HCl P did not lead to an increase in the other measured P pools which suggests that P was either taken up by microbes or transformed into P pools not assessed by the Deluca method.

In the residue treatments, heating increased available N compared with unheated soil only on day 10, 2 days after heating. The higher available N may be due heat-induced mineralisation of organic N as it has been reported after fire (Certini 2005; Esque et al. 2010). The greater relative increase in available N compared with unheated soil in soil in R1H and R8H than in UAH indicates that residue N is more susceptible to heat-induced mineralisation than N in soil organic matter. The decrease in available N from day 10 to 15 in heated residue-amended soils may be due to immobilisation as indicated by the higher respiration rates compared with the unheated soils.

In the residue treatments, heating increased most P pools compared with unheated soils, at least transiently, except $CaCl_2$ P. The lack of increase in $CaCl_2$ P indicates that P released by heating was rapidly converted into other pools. Heating increased phosphatase P on day 10 in R1H but not in R7H. The transient increase in R1H may be due to release of microbial P by heating-induced death of microbes stimulated by residue addition 1 day before heating. In residue treatments, citrate P was higher in heated compared with unheated soils, particularly in R1H. This is likely due to the lower decomposition rate in R1H than R1 and thus smaller release of organic acid anions competing with P for binding sites. In R7H, the heating-induced increase in citrate P may be due to thermal mineralisation of previously bound organic acid anions and heating-induced increased P binding capacity

(Kwari and Batey 1991). Heating also increased HCl P in the residue treatments on day 10 for the same reasons. The smaller relative increase by heating in HCl P than in citrate P is likely because HCl P is a larger pool (about twofold higher than citrate P); therefore, changes may be less apparent.

The stronger relative increase of available N after heating in the residue-amended soils than fertilised soil is in agreement with our previous study (Seneviratne et al. 2019). In that study, the relative increase in available N induced by heating was greater in the nutrient-poor soil than in the nutrient-rich soil. However, in the present study, the heating effect on available N and P pools was greater with residues than the unamended soil. This indicates that soils with large amounts of decomposable organic matter and thus higher microbial activity are more strongly affected by heat than soils with low organic matter availability.

5 Conclusion

Heating had a greater effect on respiration, available N, and P pools after residue addition than in unamended soil or soil amended with inorganic fertiliser. This is likely because residues activated microbes which were more susceptible to heat stress than the less active microbes in unamended or fertiliser amended soils. N availability was higher in heated residue-amended soils only 2 days after heating, which may be due to the death of a proportion of the microbial biomass or thermal mineralisation of residue N. Regardless of amendment form or time, heating induced only a transient increase in N and P availability. It can be concluded that short-term heating as in grass fires will have negligible effect on fertiliser management post fire. Studies with ^{15}N- and ^{33}P-labelled residues could be used to provide more detailed information about the impact of heating on the fate of residue N and P.

Funding Information M. Seneviratne received a Turner Family postgraduate scholarship. This study was funded by a postgraduate scholarship from the Turner Family Trust.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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Chapter 4

Soil respiration and nutrient availability after short heating followed by rewetting differ between first and second heating and are influenced by the interval between heating events

Published in Soil Biology and Biochemistry

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Statement of Authorship

Title of the Paper	Soil respiration and nutrient availability after short heating followed by rewetting differ between first and second heating and are influenced by the interval between heating events
Publication Status	[X] Published [ ] Accepted for Publication [ ] Submitted for Publication [ ] Unpublished and Unsubmitted work written in manuscript style
Publication details	Seneviratne, M., Marschner, P., 2019. Soil respiration and nutrient availability after short heating followed by rewetting differ between first and second heating and are influenced by the interval between heating events. Soil Biology and Biochemistry 136, 107537.

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Contribution to the paper	Performed the experiment, analysed all the samples, data analysis and interpretation data, writing the manuscript. I hereby certify that the contribution is accurate		
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 subjected 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	Mihiri Seneviratne	Date	10/02/2020

Co-Author contributions

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iii. The sum of all co-author contributions is equal to 100% less the candidates stated contribution

Name of Co-Author	Petra Marschner		
Contribution to the paper	Supervised development of work, data interpretation, manuscript evaluation and correction, acted as the corresponding author		
Signature		Date	10/02/2020

Seneviratne, M., Marschner, P., 2019. Soil respiration and nutrient availability after short heating followed by rewetting differ between first and second heating and are influenced by the interval between heating events. Soil Biology and Biochemistry107537

Soil Biology and Biochemistry 136 (2019) 107537

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

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

Soil respiration and nutrient availability after short heating followed by rewetting differ between first and second heating and are influenced by the interval between heating events

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ARTICLE INFO

Keywords:
Available N
Heating
Microbial biomass P
P pools
Rewetting

ABSTRACT

Soils may be exposed to short heating events for example in fast-moving grass fires which can reoccur if not all fuel was burnt in the first heating. Little is known about the effect of a second heating on microbial activity and nutrient availability. The aim of this study was to determine the effect of one and two heating events on soil respiration and nutrient availability. The soil was incubated moist (165 g kg^{-1}) for 7 days. On day 8, soils were left unheated (CM) or heated and then maintained for 30 min at 60 °C followed by rapid rewetting to 165 g kg^{-1}. Heated treatments included heated once (H8) or heated a second time 4, 8 and 16 days after the first heating event (H8-12, H8-16 and H8-24). Soil respiration was measured from day 8–26, soils were sampled on day 10, 14, 18 and 26 for available N and P pools. Heating and rewetting induced a flush of respiration. Compared to unheated soil cumulative respiration was about 10 % higher in H8 and 20% higher in - H8-12 and H8-16, but 30% higher in H8-24. From two days after heating, available N and P were about 25% higher in heated soils than the unheated control. The second heating induced a further increase in available N and P compared to the first heating. Microbial biomass P was generally 25–50% lower in heated soils than the control and was lower after the second than the first heating. Citrate P was 30–50% higher in heated soils than the control, irrespective of number of heating events. HCl P which was the largest P pool was not affected by heating. It can be concluded that a short heating event followed by rewetting increases N and P availability with a further increase after the second heating, particularly when the interval between heating events is long. The increase in nutrient availability may aid plant recovery after heating, but could also increase nutrient loss by leaching.

1. Introduction

Soil microbial activity is influenced by environmental factors, particularly soil water content and temperature. High soil temperatures (> 80 °C) such as those reached in fires, can kill a proportion of soil microbes and change nutrient availability (Bárcenas-Moreno and Bååth, 2009). Soil microbial activity is often highest at 25–30 °C (Pietikainen et al., 2005). However, the response of microbes to temperature is influenced by prior temperature. For example, Ranneklev and Bååth (2001) incubated soils at 25–50 °C for several days or weeks and then measured bacterial growth. They found that the optimum temperature for growth was shifted to higher temperature in soils incubated at 45 or 55 °C compared to soil that had been at 25 °C. They explained this with a change in the microbial community towards a dominance of heat-tolerant genotypes. The effect of a short exposure to high temperature, such as in a fast-moving grass fire could have a different effect than incubation for days or weeks at a higher temperature because a short heat exposure may not allow the microbial community to adapt.

The effect of rapid rewetting of air-dry soil has been studied extensively (for example, Franzluebbers et al. (2000); Xiang et al. (2008)). The flush of respiration has been explained by increased substrate availability upon rewetting (Fierer and Schimel, 2003; Borken and Matzner, 2009). Rewetting can also temporarily increase N and P availability (Fierer and Schimel, 2002; Butterly et al., 2011). Substrate after rewetting include microbes killed by rewetting, release of osmolytes accumulated during the dry period and exposure of previously occluded organic matter through aggregate breakdown (Franzluebbers et al., 2000; Gordon et al., 2008; Warren, 2014). The flush of respiration is highest at the first rewetting and decreases with number of drying and rewetting (DRW) cycles (Wu and Brookes, 2005; Shi and Marschner, 2014). This decrease has been explained by microbial death, change in microbial community composition and reduced

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https://doi.org/10.1016/j.soilbio.2019.107537
Received 29 April 2019; Received in revised form 1 July 2019; Accepted 10 July 2019
Available online 10 July 2019
0038-0717/

Table 1
Soil analyses as described in Marschner et al. (2015).

Parameter	Details	Reference
Soil texture	Hydrometer method	Gee and Or (2002)
Soil pH	1:5 soil:water ratio, 1 h shaking	Rayment and Higginson (1992)
Soil maximum water holding capacity	At matric potential −10 kPa	Wilke (2005)
Total organic C	Wet oxidation and titration	Walkley and Black (1934)
Total N	Digestion with H_2SO_4, measurement by modified Kjeldahl method	Bremner and Mulvaney, 1982
Total P	Digestion with 1:3 HNO_3 and HCl, measurement by phosphovanado-molybdate	Hanson (1950)
Available N extraction	2 M KCl at a 1:10 soil extractant ratio, 1 h shaking	Bremner and Mulvaney (1982)
Ammonium N measurement		Willis et al. (1996)
Nitrate N measurement		Miranda et al. (2001)
Available P extraction	Anion exchange resin	Kouno et al. (1995)
Available P measurement		Ohno and Zibilske (1991)
Microbial biomass P	Anion exchange resin with hexanol, biomass P = fumigated-unfumigated	Kouno et al. (1995)
Soil respiration	CO_2 concentration in headspace of jars	Setia et al. (2011)

substrate availability (Cosentino et al., 2006; Borken and Matzner, 2009).

Zheng et al. (2017) exposed air-dry soil to one or two diurnal heating events on consecutive days where the soil temperature was gradually increased over 3 h, maintained at 50 °C for 2 h and then allowed to cool over 3 h after which the soil was rewet. They found that compared to soil maintained at ambient temperature, one heating event increased respiration rate one day after rewetting, but the flush of respiration was reduced after the second heating event. In a previous study on short-term heating, we showed that 30 min at 60 °C followed by rewetting induced a flush of respiration and increased N and P availability (Seneviratne et al., 2019). Soils that were air-dried to the same water content as after heating and were then rewetted also had a flush of respiration and higher nutrient availability than constantly moist soil, but the effect was smaller than in heated soil. This indicates that the heating effect is not only due to rewetting of dry soil. Nevertheless, rewetting of soil dried during heating can increase nutrient availability.

Little is known about the effect of two short heating events as they may occur when a grass or stubble fire is temporarily stopped by rain, but then re-ignites in the remaining stubble. The aims of this study were to determine the effect (i) of two short-term heating events (30 min at 60 °C) followed by rewetting on soil respiration, N availability and P pools, and (ii) of the period between the two heating events (4–14 days). The hypotheses were (i) heating will induce a flush of respiration and increase available N and P, (ii) the flush will be smaller after the second heating than the first, and (iii) the flush after the second heating will increase with time between heating events. The second hypothesis is based on findings in DRW experiments with more than one DRW cycle. The third hypothesis assumes that a longer period between the heating events will allow recovery of microbes.

2. Materials and methods

A loamy soil was collected in early autumn from 0 to 10 cm depth in Urrbrae (Longitude 138°38′3.2″ E, Latitude 34°58′0.2″ S) South Australia. The climate in this region is Mediterranean. The soil is classified as Chromosol in Australian soil classification and as Rhodoxeralf in US Soil Taxonomy. After collection, the soil was air-dried and sieved to < 2 mm. It has the following properties: sand 48%, silt 27% and clay 25%, pH (1:5 soil:water) 5.3, total organic C 22 g kg^{-1}, total N 1.6 g kg^{-1}, total P 400 mg kg^{-1}, available N 53 mg kg^{-1}, available P 13 mg kg^{-1}, maximum water holding capacity (WHC) 330 g kg^{-1} and bulk density 1.3 g cm^{-3}.

2.1. Experimental design

On day 0, 30 g of air-dried soil was mixed thoroughly with 165 g kg^{-1} (50% maximum water holding capacity) reverse osmosis (RO) water in a plastic bag, placed in 70 ml plastic containers and adjusted to bulk density 1.3 kg cm^{-3}. Eighty containers were prepared and kept in the dark at 20–22 °C for 7 days and soil water content was maintained by weight and adding RO water if necessary. Throughout the experiment, the containers were left open to allow gas exchange.

On day 8, 64 of the soil containers were heated to 60 °C in a fan forced oven within 1 h and maintained at 60 °C for 30 min. Thermocouples were inserted in heated soils to monitor the soil temperature. After 30 min at 60 °C the containers were removed from the oven. After cooling, the soil water content was 100 g kg^{-1}. Then the soil was rewetted to 165 g kg^{-1}. One treatment (H8) was heated only once, on day 8. In the other three treatments, soils were heated and rewet twice, with an interval of 4, 8 or 16 days (H8-12, H8-16, H8-24) between the first and the second heating event. Temperature, duration of heat, water content before and after rewetting were same as in the first heating event. Controls were kept at room temperature. Heated soils after rewetting and unheated control were maintained at 165 g kg^{-1}. Respiration was measured from day 9 to day 26. Soil was sampled destructively on day 10, 14, 18 and 26 for available N and P pools.

2.2. Analyses

Soil analyses were carried out as described in Marschner et al. (2015). For details see Table 1.

Soil P pools were measured after DeLuca et al. (2015). For each extractant (20 ml of 10 mM $CaCl_2$, 10 mM citric acid, 0.2 enzyme units acid phosphomonoesterase, or 1 M HCl), 1 g of soil was shaken for 3 h on an end-over-end shaker. These P pools are referred to $CaCl_2$ P, citrate P, phosphatase P and HCl P. $CaCl_2$ P was not detectable. Therefore, available P was extracted using anion exchange resin (Kouno et al., 1995). This method was also used for microbial biomass P (MBP, Table 1). No correction factor was used for P because recovery of a P spike in soil from the same location was 98% (Butterly et al., 2010). The P concentration in all extracts was measured by the malachite-green method (Ohno and Zibilske, 1991).

2.3. Statistical analysis

There were four replicates for each treatment and sampling time. Normal distribution of residuals was tested by Shapiro-Wilk test. Cumulative respiration was analysed by one-way ANOVA. The data of P pools and available N was log10 transformed to achieve normal distribution. Then the data was analysed by two-way repeated measures ANOVA with between subjects factor-treatment, within subjects factor-time in SPSS statistics version 25. Tukey's multiple comparison test at 95% confidence interval for the treatment × time interaction was used to determine significant differences among treatments and sampling times.

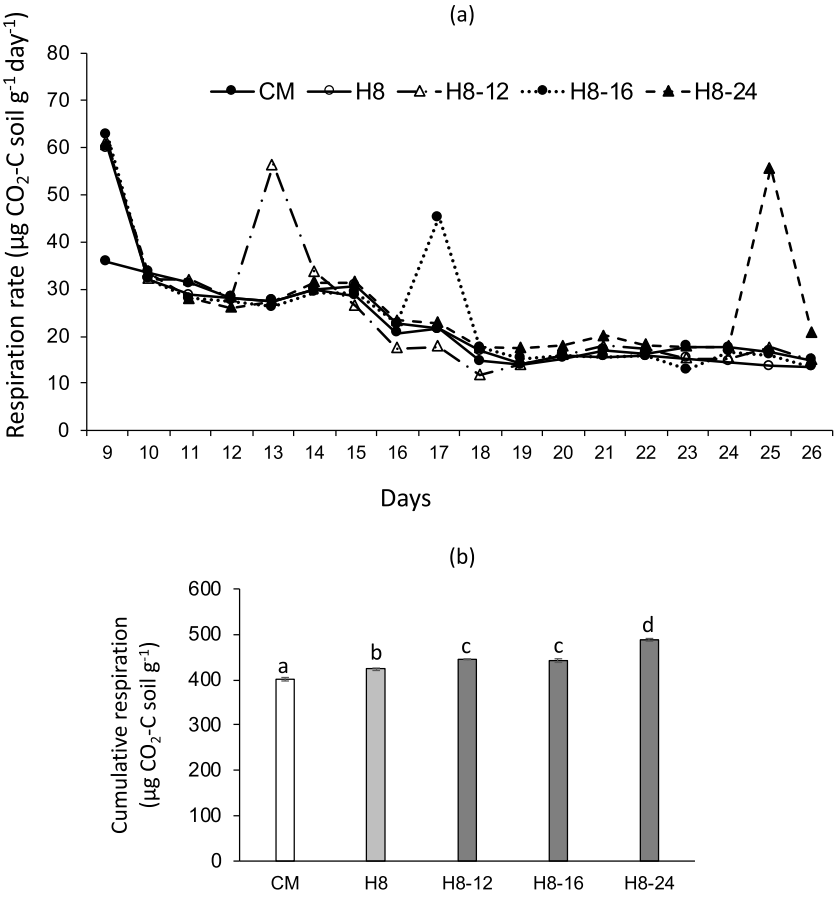

Fig. 1. Respiration rate over 18 days (a) of soils heated to 60 °C for 30 min on day 8 followed by rewetting once (H8) or a second time 4, 8 and 16 days after the first heating event (H8-12, H8-16, H8-24) and the unheated, constantly moist control (CM); cumulative respiration over 18 days (b). Bars in (b) with different letters are significantly different ($P \leq 0.05$, (n = 4, means ± SE).

3. Results

Heating induced a flush of respiration one day after rewetting (Fig. 1 a). This flush was two to three-fold higher than unheated soil. The flush after the second heating in H8-12 and H8-16 was similar to that after the first heating (Table 2). However the flush after the second heating in H8-24 was about 50% higher than after the first heating. Cumulative respiration was higher in heated than unheated soil (Fig. 1b). Compared to unheated soil it was about 10% higher in H8, 20% higher in H8-12 and H8-16, but 30% higher in H8-24.

At all sampling times, available N was higher in heated treatments than the unheated control (Fig. 2). This increase was due to a two to eight-fold increase in ammonium in heated soils compared to the control whereas the proportion of nitrate was lower than the control. Available N in the control was higher on days 14 and 26 than day 10 and the proportion of ammonium decreased while the nitrate proportion increased. On day 10, two days after the first heating, available N was about 20% higher in heated soils than the control (Fig. 2a). On day 14, two days after the second heating in H8-12, available N was about 10% higher in H8-12 than H8. It increased significantly from day 10 to day 14 in the control and in H8-12. On day 18, two days after the second heating in H8-16, available N was about 20% higher in the soils heated twice (H8-12 and H8-16) compared to the soils that had been heated once H8 and H8-24 and the control (Fig. 2c). On day 26, two days after the second heating in H8-24, available N was highest in H8-24, where it was about 25% higher than the other heated treatments and about 30% higher than the control. Only in H8-24, available N increased from day 18 to day 26.

HCl P was the largest P pool, it was about three-fold higher than the other pools (Fig. 3). MBP was generally lower in heated soils than the unheated control except on day 26 where it was similar in H8 and the control (Fig. 3 a-d). In the control, MBP increased by about 30% from day 12 to day 14, but then decreased so that it was similar on day 26 as on day 12. On day 12, two days after the first heating, MBP was about 25% lower in heated soils than the control (Fig. 3a). Two days after the second heating in H8-12 (day 14), MBP was about 15% lower in H8-12 than the soils heated once (H8, H8-16, H8-24) and three-fold lower than the control (Fig. 3b). On day 18, MBP was about 30% lower than H8 in the two treatments that had been heated twice, H8-12 and H8-16 (Fig. 3c). MBP only decreased from day 14 to day 18 in H8-16 where it was about 50% lower on day 18 than day 14. Two days after the second heating in H8-24 (day 26), MBP was 20–50% lower in H8-16 and H8-24 than the other treatments. From day 18–26 MBP in heated soils increased in H8 and H8-12, remained unchanged in H8-14, but halved in H8-24.

Resin P differed little among treatments on day 12, but was higher in heated soils than the control at the other sampling times (Fig. 3e–h).

Table 2
Increase in respiration rate compared to the constantly moist soil one day after heating in soils heated to 60 °C for 30 min followed by rewetting once (H8) or a second time 4, 8 and 16 days after the first heating event (H8-12, H8-16, H8-24). Values in each column with different letters are significantly different ($P \leq 0.05$, n = 4, means ± SE). Asterisks indicate significantly higher respiration rate after the second compared to the first rewetting.

Treatment	First heating event	Second heating event
	µg CO_2–C soil g^{-1} day^{-1}	
H8	24.6 ± 1.2[a]	
H8-12	25.5 ± 1.5[a]	28.9 ± 0.7[b]*
H8-16	26.8 ± 0.7[a]	25.7 ± 0.0[a]
H8-24	24.8 ± 1.3[a]	40.0 ± 0.9[c]*

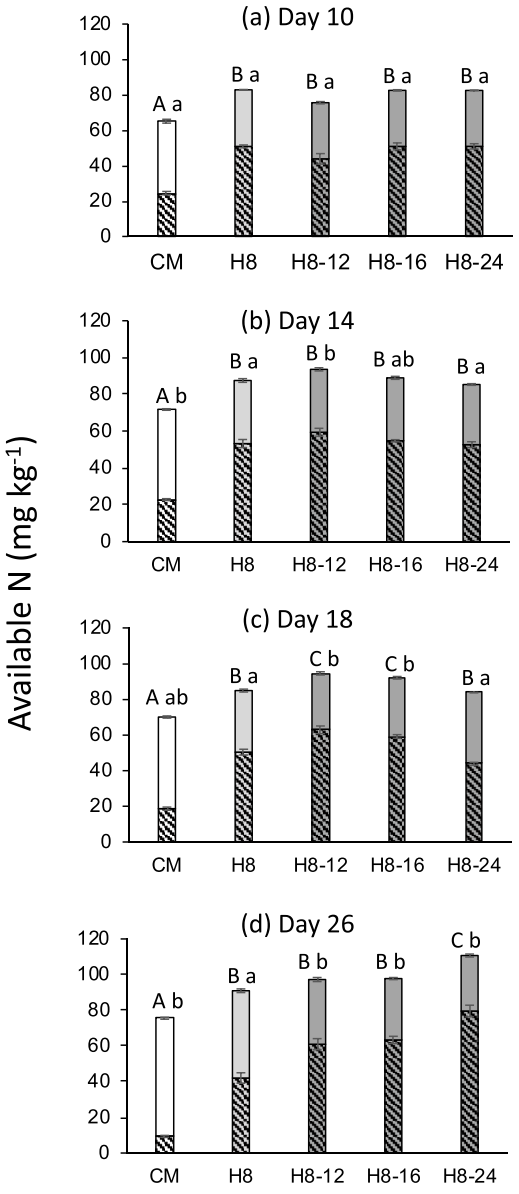

Fig. 2. Available N on day 10 (a), 14 (b), 18 (c) and 26 (d) in soils heated to 60 °C for 30 min followed by rewetting once (H8) or a second time 4, 8 and 16 days after the first heating event (H8-12,H8-16, H8-24) and the unheated, constantly moist control (CM). Bar segments with no pattern indicate nitrate - and stippled segments ammonium. Bars in with different letters are significantly different ($P \leq 0.05$, ($n = 4$, means ± SE), upper case letters indicate differences between treatments at a given sampling time, lower case letters indicate differences between sampling times of a given treatment.

The second heating induced an increase in resin P of about 25% compared to the sampling before heating. On day 14, resin P was about 10% higher than the control in soils heated once (H8, H8-16, H8-24), but 25% higher in H8-12 which had been heated the second time two days before. Only in H8-12, resin P increased from day 10 to day 14. Two days after the second heating in H8-16 (day 18), resin P was higher than on day 14 in that treatment, but it did not change in the other treatments. On day 26, two days after the second heating in H8-24, resin P was about 20% higher in H8-16 and H8-24 than the other heated treatments. Resin P increased from day 18 to day 26 in H8-24, but remained unchanged in H8-16.

Citrate P did not differ among treatments on day 12, two days after the first heating (Fig. 3 i). But at all other sampling times, citrate P was 30–50% higher in heated soils than the control (Fig. 3j-l). Citrate P did not differ between H8 and the treatments heated twice. HCl P was lowest on day 10 (Fig. 3m–p). Heating had no consistent effect on HCl P except on day 26 where it was about 10% higher in soils heated twice than the control.

4. Discussion

The study showed that heating followed by rewetting influence soil respiration, available N and P. In our previous study, the short-term increase in soil respiration and available N after one heating and rewetting was greater than in soils that were air-dried to the same water content as after heating and then rewet (Seneviratne et al., 2019). Therefore, the effect of heating and rewetting is not only due to the sudden increase in soil water content upon rewetting.

4.1. First heating

The first hypothesis (heating will induce a flush of respiration and increase available N and P) can be confirmed. The flush of respiration after the first heating lasted only one day after which the respiration rates were similar to CM. This is different from the respiration flush after rewetting of dry soil where the respiration rate usually remains above that of the constantly moist soil for several days after the flush (e.g., Shi and Marschner (2014)). The short duration of the flush may be due to the fact that the soil water content after heating (100 g kg^{-1}) was not as low as in air-dry soil (< 20 g kg^{-1}). The water content before rewetting has been shown to influence respiration after rewetting; the flush decreases with increasing water content before rewetting (Chowdhury et al., 2011; Meisner et al., 2017). A further reason may be that the water content and thus microbial activity, were similar to CM until the day when the soils were heated. In DRW experiments, soils remain air-dry for days or weeks before rewetting. Compared to the constantly moist control the low microbial activity during the dry period leads to an accumulation of decomposable organic matter which can be decomposed upon rewetting.

Heating induced a 25% increase in available N within two days which remained higher than CM throughout the experiment. The increase was due to ammonium which was two to four-fold higher than in CM, whereas the proportion of nitrate was lower than in CM. This is in agreement with our previous study (Seneviratne et al., 2019) and suggests that heating stimulated ammonification, but inhibited nitrification. The initial increase in ammonium may be due to heat-induced mineralisation of organic N (Certini, 2005; Esque et al., 2010). The reduction of proportion of nitrate indicates that nitrifiers were susceptible to heating. Myers (1975) showed that ammonium in soil increased with temperature from 20 to 60 °C whereas nitrate peaked at about 35 °C and then sharply decreased. The sustained increase in available N after heating occurred although respiration rates were increased only for the first day after rewetting. This suggests that microbial nutrient uptake was limited after respiration rates had dropped which is corroborated by the reduction of MBP after heating.

The increase in resin P six days after the first heating may be due to heat-induced P mineralisation (Merino et al., 2019). An increase in available P has also been observed after rewetting of air-dry soil (Butterly et al., 2011; Buenemann et al., 2013) which was explained by mineralisation of microbial P. However, this flush lasts only a few hours after rewetting. In contrast, resin P in this study remained higher than CM until the end of the experiment. This and the higher citrate P in heated soils than CM suggest sustained increase in P mineralisation. On the other hand, MBP was reduced by heating and remained low until day 18 (10 days after heating) when respiration rates were low. This indicates that a proportion of microbes was killed by heating and rewetting. Since the rewetting stress was moderate, the loss of MBP is likely due to the heating. However, the flush of respiration after heating, high available N and resin P indicate that the remaining

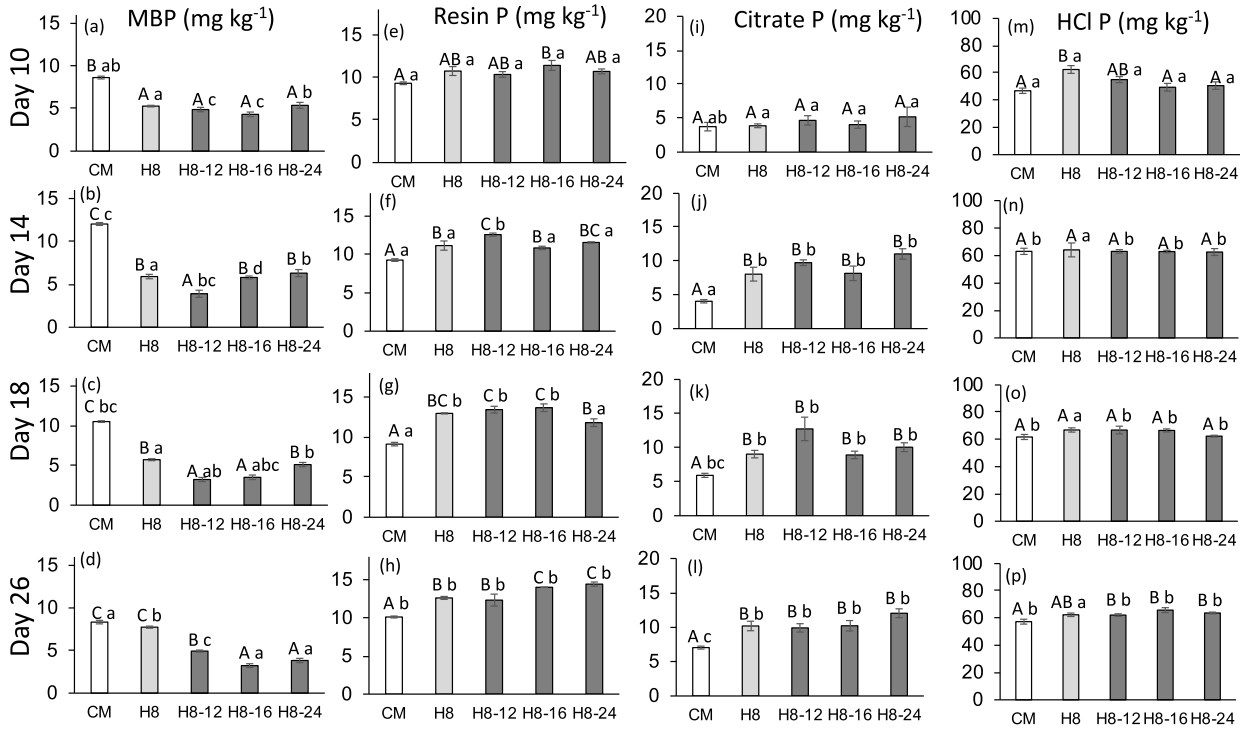

Fig. 3. Microbial biomass P (a–d), Resin P (e–h), Citrate P (i–l) and HCl P (m–p) on day 10 (a, e, i, m), 14 (b, f, j, n), 18 (c, g, k, o) and 26 (d, h, l, p) in soils heated to 60 °C for 30 min followed by rewetting once (H8) or a second time 4, 8 and 16 days after the first heating event (H8-12, H8-16, H8-24) and the unheated, constantly moist control (CM). Bars in with different letters are significantly different ($P \leq 0.05$, (n = 4, means ± SE), upper case letters indicate differences between treatments at a given sampling time, lower case letters indicate differences between sampling times of a given treatment.

microbes were very active and rapidly decomposed substrates released from dead microbes and organic matter. Thus, P was apparently available, but not taken up by microbes until their activity was low. P content of cells can differ among microbial genotypes and communities (Fagerbakke et al., 1996; Ehlers et al., 2010). It is possible that heating killed P accumulating microbes (Buenemann et al., 2008) which took longer to recover than microbes which don't accumulate P or that the surviving microbes had lower P requirement.

4.2. Second heating

The second hypothesis (the flush will be smaller after the second heating than the first) has to be declined because the respiration rate after the second heating was similar or higher than the rate after the first heating. The hypothesis was based on studies with multiple DRW cycles where the size of the rewetting flush was highest after the first rewetting (Wu and Brookes, 2005; Shi and Marschner, 2014). This has been explained by microbial death, changes in microbial community composition, decomposition of available substrate during the moist period and lack of additional release of soil organic matter in subsequent DRW cycles (Cosentino et al., 2006a; Borken and Matzner, 2009). The third hypothesis (the flush after the second heating will increase with time between heating events) can be confirmed for respiration rate and available N in H8-24 compared to shorter intervals, but not for the P pools. There are several possible explanations for the similar flush after the first and second rewetting. Respiration rate before the second heating was lower than before the first. Before the first heating it was 36 ± 1 μg CO_2–C soil g^{-1} day^{-1} (assuming it was similar to CM on day 9) compared to 18–27 ± 1 μg CO_2–C soil g^{-1} day^{-1} before the second heating. This suggests that microbes were less active before the second heating. Generally, it is assumed that active microbes are more susceptible to stress than less active microbes (Van Gestel et al., 1993; Schimel et al., 2007). The size of the rewetting flush in DRW experiments has been used to indicate stress with a larger flush suggesting greater stress (Fierer and Schimel, 2002). However, the low respiration rate before the second heating in this study, particularly before the heating on day 24 in H8-24, could also mean that the microbes were starved. The flush of substrate availability upon heating and rewetting may trigger very high respiration rates similar to those observed after addition of small amounts of available C to soils with low basal respiration rates (De Nobili et al., 2001). Another possible explanation for the similar flush size after the first and second heating could be that rewetting of heated soil induced a greater release of organic matter from soil aggregates than in DRW cycles. Heating has been shown to increase extractable organic C (Prokushkin and Tokareva, 2007) which may be further increased by rapid rewetting. Lopez-Sangil et al. (2018) suggested that the disruption of the organo-mineral matrix is an important driver for the flush of respiration in DRW. In contrast, Zheng et al. (2017) reported that respiration rate was much lower after a second diurnal heating to 50 °C than the first. The difference between the current study and Zheng et al. (2017) may be because of the longer duration of the heating in Zheng et al. (2017) which included 3 h gradual heating, 2 h at 50 °C, 3 h cooling and because the soils were heated on consecutive days.

The flush of respiration also induced increased N and P mineralisation. The increase in ammonium was strongest in H8-24 which is in agreement with the high respiration flush. In contrast, the increase in resin P was not affected by the length of time between the two heating events. The second heating caused a further decrease in MBP. Although MBP in H8-12 was higher on day 26 than day 14, it remained lower than in H8. This indicates a sustained reduction of microbial P, particularly in soils heated twice. The second heating did not induce a further increase in citrate or HCl P compared to the first heating although resin P was higher. Binding of organic acid anions produced during decomposition of native organic matter to anion exchange sites between heating events (Gerke, 1994; Iyamuremye et al., 1996) may have

prevented a further increase in these pools. The limited P binding to soil particles then led to the increase in resin P after the second heating.

5. Conclusion

Short-term heating and rewetting induced a flush of respiration, loss of MBP and increased available N, resin P and citrate P. The second heating caused another similar respiration flush, further loss of MBP and increased available N and resin P compared to the single heating. This suggests that multiple heating events separated by periods with sufficient soil water content could increase C loss from soil, but also temporarily increase nutrient availability which may aid recovery of plants after heating. On the other hand, the higher N availability after the second heating may increase N leaching if the heating is followed by heavy rain. However, the predominance of ammonium N may limit leaching because ammonium is sorbed to cation exchanged sites on soil particles.

Acknowledgement

Mihiri Seneviratne thanks the Turner family and the University of Adelaide for providing the postgraduate scholarship.

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Chapter 5

Impact of heating and rewetting on soil respiration and nutrient availability is enhanced by prior growth of plants

Published in Journal of Soil Science and Plant Nutrition
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Statement of Authorship

Title of the Paper	Impact of Heating and Rewetting on Soil Respiration and Nutrient Availability Is Enhanced by Prior Growth of Plants
Publication Status	[X] Published [ ] Accepted for Publication [ ] Submitted for Publication [ ] Unpublished and Unsubmitted work written in manuscript style
Publication details	Seneviratne, M., Alamgir, M., Marschner, P., Impact of heating and rewetting on soil respiration and nutrient availability is enhanced by prior growth of plants. Journal of Soil Science and Plant Nutrition, 1-8.

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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 subjected 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		
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Co-Author contributions

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Seneviratne, M., Marschner, P., 2020. Impact of heating and rewetting on soil respiration and nutrient availability is enhanced by prior growth of plants. Journal of Soil Science and Plant Nutrition 1-8

Journal of Soil Science and Plant Nutrition
https://doi.org/10.1007/s42729-020-00179-0

ORIGINAL PAPER

Impact of Heating and Rewetting on Soil Respiration and Nutrient Availability Is Enhanced by Prior Growth of Plants

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Received: 14 October 2019 / Accepted: 19 January 2020

Abstract

The effects of heating and rewetting on nutrient availability and microbial activity have been studied extensively. But little is known about the effect of prior presence of plants on the impact of heating on microbial activity and nutrient availability. Soil was planted with wheat or left unplanted. After 4 weeks, roots were removed from the planted soil, washed and then added to half of the previously planted and unplanted soil at 1 g dw kg^{-1}. Half of the replicates from each treatment were heated and then maintained at 60 °C for 30 min followed by rapid rewetting, while others remained unheated. Soil respiration was measured continuously for 14 days. Soils were sampled 2, 7 and 14 days after rewetting for available N and P pools. Heating and rewetting induced a flush of respiration and increased available N and P. The heating-induced increase in initial respiration rate, cumulative respiration and available N and P was greater in previously planted soil than unplanted soil. Heating increased available N and P three- to fivefold in planted soil, but only twofold in unplanted soil. Plant-induced changes in nutrient availability increase the impact of heating on soil respiration and nutrient availability.

Keywords Presence of plants · Soil heating · Respiration flush · Available N and P

1 Introduction

Shrub or grassland fires can induce short heating events (Scotter 1970, Stoof et al. 2013), but the effect of prior growth of plants on the heating impact is not clear. Growing plants influence soil properties through root exudation and nutrient uptake. Root exudates include a variety of organic compounds that can be rapidly decomposed by microbes increasing their growth and activity and changing microbial community composition (Jones et al. 2009; Chaparro et al. 2014; Semenov et al. 1999; van Hees et al. 2005). The greater activity of microbes in the rhizosphere may affect the response of soil microbes to stress such as heating and rewetting of dry soil because active microbes are more susceptible to stress than inactive ones (Schimel et al. 2007; Van Gestel et al. 1993). Further, microbial communities differ in susceptibility to heating and rewetting (Fierer and Schimel 2002; Oliverio et al. 2017). On the other hand, nutrient uptake by roots, can, deplete nutrients such as available N and P (Xue et al. 2017). These plant-induced changes may influence the effect of heating on nutrient availability.

Stressors such as heating or rewetting of dry soil can kill a proportion of the microbial biomass, but also increase substrate availability of the surviving microbes and change microbial community composition (Bárcenas-Moreno and Bååth 2009; Bérard et al. 2011; Fierer and Schimel 2003; West et al. 1992). Heating or rewetting may influence nutrient availability by exposing previously occluded organic matter or sorption sites (Denef et al. 2001; Romanya et al. 1994; Serrasolses et al. 2008). Heated soils can be exposed to both stressors as heating often dries soil and may be followed by rewetting.

In previous studies, we showed that a short heating event (gradual heating over 1 h, maintained at 60 °C for 30 min, then allowed to cool) followed by rewetting induced a flush of respiration and increased N and P availability (Seneviratne et al. 2019, Seneviratne and Marschner 2019). These experiments were carried out in a relatively nutrient- and organic

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s42729-020-00179-0) contains supplementary material, which is available to authorized users.

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Published online: 27 January 2020

matter–rich soil. Many agriculturally used soils are low in organic matter where organic substrate input by plants may be particularly relevant. It is unclear how soil respiration and nutrient availability are affected by heating and rewetting in a nutrient-poorer soil and if the response is modulated by the presence of plants.

The aim of the experiment was to determine the impact of heating and rewetting on soil respiration and nutrient availability in previously planted or unplanted soil. The effect of roots was studied by removing them from planted soil and then adding them to half of each planted and unplanted soil. We assumed that the effect of heating will differ between planted and unplanted soil because of differences in nutrient availability and microbial activity. The hypotheses were (i) the increase in respiration, available N and P by heating and rewetting will be greater in planted than unplanted soil, and (ii) presence of roots will increase the impact of heating on the measured parameters.

2 Materials and Methods

2.1 Soil

A loamy sand soil was collected from 0 to 10 cm depth at Monarto, South Australia (35°04′ S 139° 07′ E). The soil was air-dried and sieved to < 2 mm. The soil had the following properties: sand 74%, silt 17% and clay 9%, $pH_{1:5}$ 7.5; $EC_{1:5}$ 0.02, total organic carbon 6.3 g kg^{-1}, total N 0.5 g kg^{-1}, available N 20 mg kg^{-1}, available P 2 mg kg^{-1} and maximum water holding capacity (WHC) 180 g kg^{-1}.

2.2 Experimental Design

About 500 g (dry weight equivalent) of soil was filled into pots lined with plastic bags. Soil water content was adjusted to 150 g kg^{-1} (75% of maximum WHC) by mixing with reverse osmosis (RO) water. This water content was chosen, because in previous experiments, it was optimal for plant growth and microbial activity in this soil (Alamgir et al. 2012). Six pots remained unplanted; the other six pots were planted with 18 germinated wheat seeds (*Triticum aestivum* L., variety Mace) per pot. This high planting density was to ensure that most soil in the pot was affected by roots. The planted and unplanted pots were placed in a glasshouse with natural light and temperature 20–25 °C. After 1 week, the plants were thinned to 15 plants per pot. The soil water content was maintained at 150 g kg^{-1} by adding reverse osmosis water if necessary. Weeds growing in unplanted pots were removed. Wheat plants were removed 4 weeks after planting when a dense plant cover was established. The shoots were cut at the soil surface, and all visible roots were removed manually from the soil. The soil in the planted pots is referred to as planted soil and soil from unplanted pots as unplanted soil.

The collected soil was immediately used for the heating experiment. Unplanted and planted soils were weighed into plastic bags, 30 g each (U, P). The collected roots were washed carefully to remove any adhering soil particles and patted dry with paper towels. Root nutrient concentrations were 505 g organic C kg^{-1} and total N 7.0 g kg^{-1} (dry weight basis). The fresh root mass was cut into 0.5-cm pieces and added at 1 g dw equivalent kg^{-1} with unplanted and planted soil (U + R, P + R). This represents the root density in the planted pots. Soil and roots were mixed in plastic bags and filled into 70-ml containers. Unamended soil was mixed in a similar manner in plastic bags before filling into the containers. The bulk density of soil in the containers was adjusted to 1.3 g cm^{-3} by compacting the soil to a certain height.

Twelve containers from each planted or unplanted soil without or with roots were gradually heated to 60 °C in a fan-forced oven within 1 h and maintained at 60 °C for 30 min (UH, PH, U + RH, P + RH). Such short heating to moderate temperature may occur in fast-moving fires with low fuel load (Scotter 1970, Stoof et al. 2013). Soil temperature was monitored with thermocouples placed in the soil. The heated soils were allowed to cool to room temperature and then rewetted within a few minutes to 150 g kg^{-1} by adding water dropwise in a circular motion. The 12 unheated containers remained at room temperature, i.e. at 18–20 °C (U, P, U + R, P + R). Both heated and unheated treatments were incubated in the dark at 18–20 °C for 14 days and maintained at 150 g kg^{-1} by adding reverse osmosis water in every second day. Four containers of each treatment were placed in glass jars to measure soil respiration.

Respiration was measured daily from day 1 to day 14. Four containers from each treatment were destructively sampled on 2, 7 and 14 days after rewetting for available N and P pools. The P pools citrate P and HCl P were measured because they can be influenced by microbial and root exudates (Deluca et al. 2015). Between sampling times, only the containers to be sampled at the end of the period were placed in the jars. The remaining cores were incubated under the same conditions in large plastic trays covered with aluminium foil.

2.3 Analyses

Soil maximum water holding capacity was measured matric potential = − 10 kPa (Wilke 2005). Soil texture was determined according to Gee and Bauder (1986). Soil pH was determined in a 1:5 (w/v) soil to RO water ratio (Rayment and Higginson 1992). Total organic C of soil and roots was determined by wet oxidation (Walkley and Black 1934). Total N in soil and roots was determined using the Kjeldahl method (Bremner and Mulvaney 1982).

Soil respiration was measured as described in Setia et al. (2011) using a Servomex 1450 infra-red analyser (Servomex Group, Crowborough, UK). Linear regression based on injection of known amounts of CO_2 in similar jars was used to define the relationship between CO_2 concentration and analyser reading.

Citric acid– and HCl-extractable P were measured after DeLuca et al. (2015). P pools extracted are potentially bioavailable inorganic P pools sorbed to clay particles or weakly bound in inorganic precipitates, respectively. To determine citric acid– and HCl-extractable P, 0.5 g of soil was extracted in parallel by shaking with 10 ml of each extractant (10 mM citric acid or 1 M HCl, respectively) on an end-over-end shaker in separate 50-ml tubes for 3 h. Resin (available) P and microbial biomass P (MBP) were determined using the anion exchange resin method (Kouno et al. 1995) with hexanol fumigation. The P concentration in the extracts was determined by the malachite-green method as described in Ohno and Zibilske (1991). MBP is the difference in P concentration between fumigated and unfumigated soil (Kouno et al. 1995). Available N (ammonium and nitrate) concentration was measured after 1-h shaking with 2 M KCl in a 1:5 soil to extractant ratio. Ammonium-N and nitrate-N were determined after Willis et al. (1996) and Miranda et al. (2001), respectively.

2.4 Statistical Analysis

There were four replicates per treatment and sampling time. Data variance was checked for normality using the Shapiro-Wilk test. Cumulative respiration was analysed by one-way ANOVA. The data of available N and P pools was transformed to log10 values to achieve normality and analysed by repeated measures ANOVA with between-subject factor (treatment), within-subject factor (time) in SPSS statistics version 25. Tukey's multiple comparison test at 95% confidence interval for the treatment × time interaction was used to determine significant differences among treatments and sampling times. The data was also analysed by three-way ANOVA with factors plant × heating × root for each sampling time separately (supplementary Table S1).

3 Results

Heating and root addition increased soil respiration about twofold in the first 2 days (Fig. 1a, b). One day after rewetting, the respiration rate was higher in heated than unheated soil, about twofold higher in unplanted soil, but more than twofold higher in planted soil. In heated soils, respiration rate in the first 2 days was higher in planted than unplanted soil. After day 2, heating, planting or root addition had little effect on respiration rate. Cumulative respiration was about 30% higher in heated than unheated soil (Fig. 1c, Table 1, Table S1). It was about 20% higher in planted than unplanted soil.

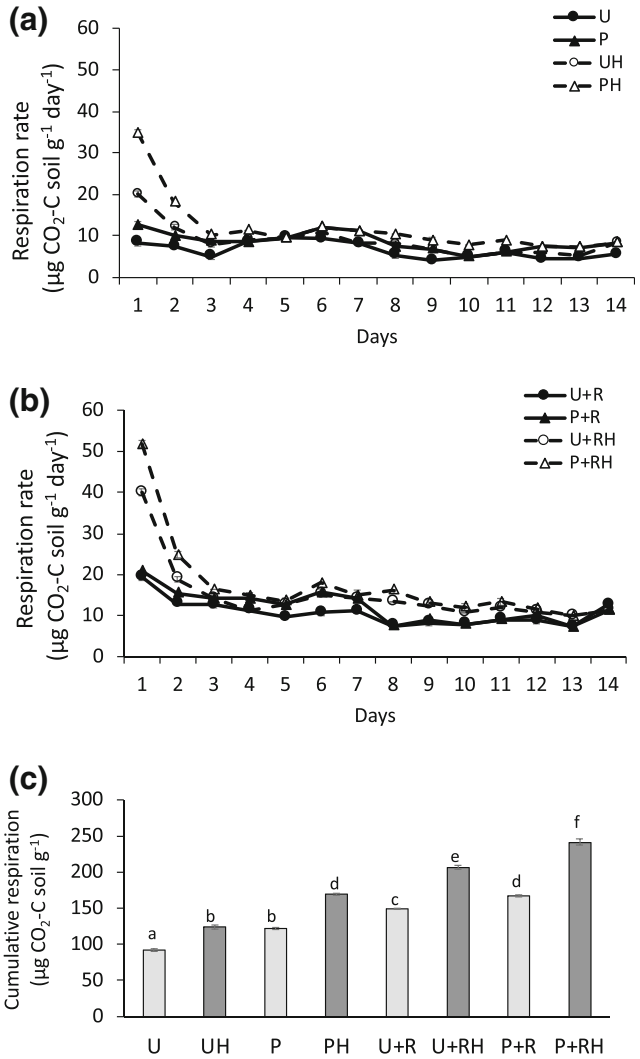

Fig. 1 Soil respiration rate (**a**, **b**) and cumulative respiration (**c**) over 14 days in previously unplanted and planted soil without roots which remained unheated (U and P) or were heated and rewet (UH and PH) or with roots (U + R, P+R, UH + R, PH + R). In panel **c**, bars with different letters are significantly different ($P \leq 0.05$, $n = 4$, means ± SE)

Available N was lower in planted than unplanted soil which was due to the about tenfold higher nitrate concentration in unplanted soil (Fig. 2, Table S1). Heating increased available N in planted soil three- to fivefold at all sampling dates due to an increase in ammonium N whereas nitrate was little influenced by heating (Table 1). In unplanted soil, heating had no effect on available N on day 2 (Fig. 2a). Available N in unplanted soil on day 7 was about twofold higher in heated soil without roots and about 0.2-fold higher with roots (Fig. 2b). On day 14, heating increased available N in heated unplanted soil only without roots (Fig. 2c). Root addition generally had little effect on available N. Available N changed little over time.

Table 1 Difference between heated and unheated soils in percentage of unheated soil for planted and unplanted treatments without (UP, P) or with roots (UP + R, P + R) for cumulative respiration, available N and resin P. Different letters indicate significant differences among treatments ($n = 4$, $P \leq 0.05$)

	Treatment	Difference in percentage of unheated soil
Cumulative respiration ($\mu g\ CO_2$-C soil g^{-1})		
	UP	35 a
	P	37 ab
	UP + R	39 ab
	P + R	43 b
Available N (mg kg^{-1})		
Day 2	UP	13 a
	P	189 b
	UP + R	14 a
	P + R	152 b
Day 7	UP	64 b
	P	260 c
	UP + R	30 a
	P + R	196 c
Day 14	UP	95 b
	P	192 c
	UP + R	14 a
	P + R	151 c
Resin P (mg kg^{-1})		
Day 2	UP	108 a
	P	499 b
	UP + R	116 a
	P + R	885 c
Day 7	UP	89 a
	P	678 c
	UP + R	123 b
	P + R	712 c
Day 14	UP	64 a
	P	296 b
	UP + R	76 a
	P + R	614 b

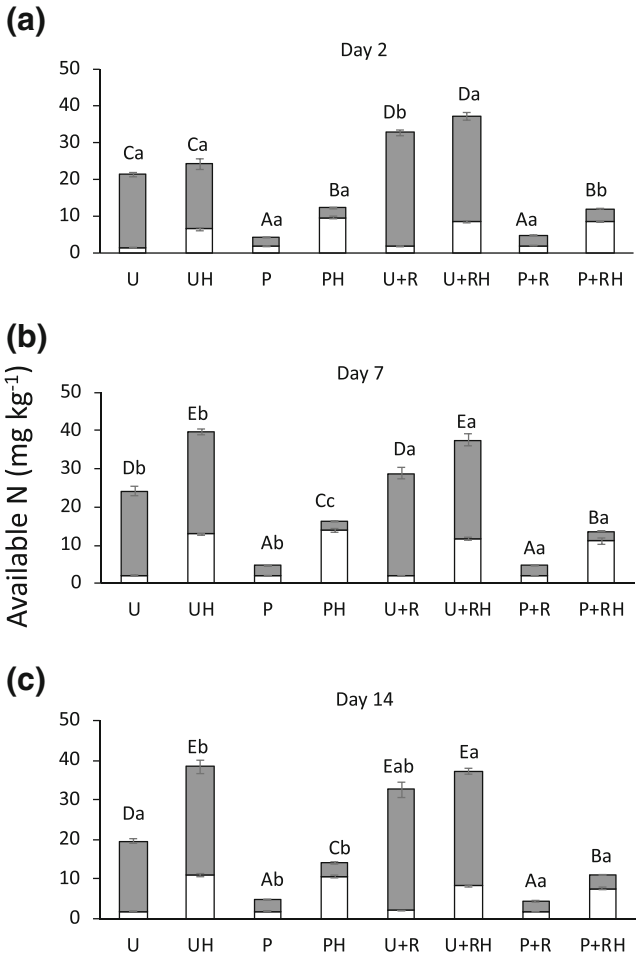

Fig. 2 Available N on days 2 (**a**), 7 (**b**) and 14 (**c**) in previously unplanted and planted soil without roots which remained unheated (U and P) or were heated and rewet (UH and PH) or with roots (U + R, P +R , UH + R, PH + R). Different uppercase letters indicate significant differences among treatments at a given sampling time, and different lowercase letters indicate differences over time in a given treatment ($P \leq 0.05$, $n = 4$, means ± SE). In each bar, ammonium is shown in white columns, nitrate in grey

Resin P was two- to threefold higher in unplanted than planted soil, but root addition had little effect on resin P (Fig. 3a–c, Table S1). Heating increased resin P about twofold in unplanted soil and three- to fourfold in planted soil (Table 1). Resin P changed little over time. MBP differed little among treatments on day 2 (Fig. 3d). But on day 7 and 14, heating reduced MBP by 30–50% except in unplanted soil without roots on day 14 where heating had no effect on MBP (Fig. 3e, f). The effect of heating on MBP was similar in planted and unplanted soil. MBP changed little over time in unheated soils, but was lower on day 7 than day 2 in heated soils, except in planted soil with roots.

Citrate P was not affected by root amendment (Fig. 4a–c, Table S1). On day 2, citrate P was lower in planted than unplanted soil only in unheated treatments. Heating increased citrate P on day 2 fourfold in planted soil without roots and nearly threefold in unplanted soil with roots, but had no effect in unplanted soil without roots and planted soil with roots (Fig. 4a, Table S2). Citrate P on day 7 did not differ between planted and unplanted soil, and heating affected citrate P only in planted soil with roots where it was about twofold higher than in unheated soil (Fig. 4b). Citrate P on day 14 differed between planted and unplanted soil only in heated treatments, where it was about 50% lower in planted soil (Fig. 4c). Heating increased citrate P on day 14. In unplanted soil, the heating-induced increase in citrate P was twofold without roots, but only 0.5-fold with roots. In planted soil without

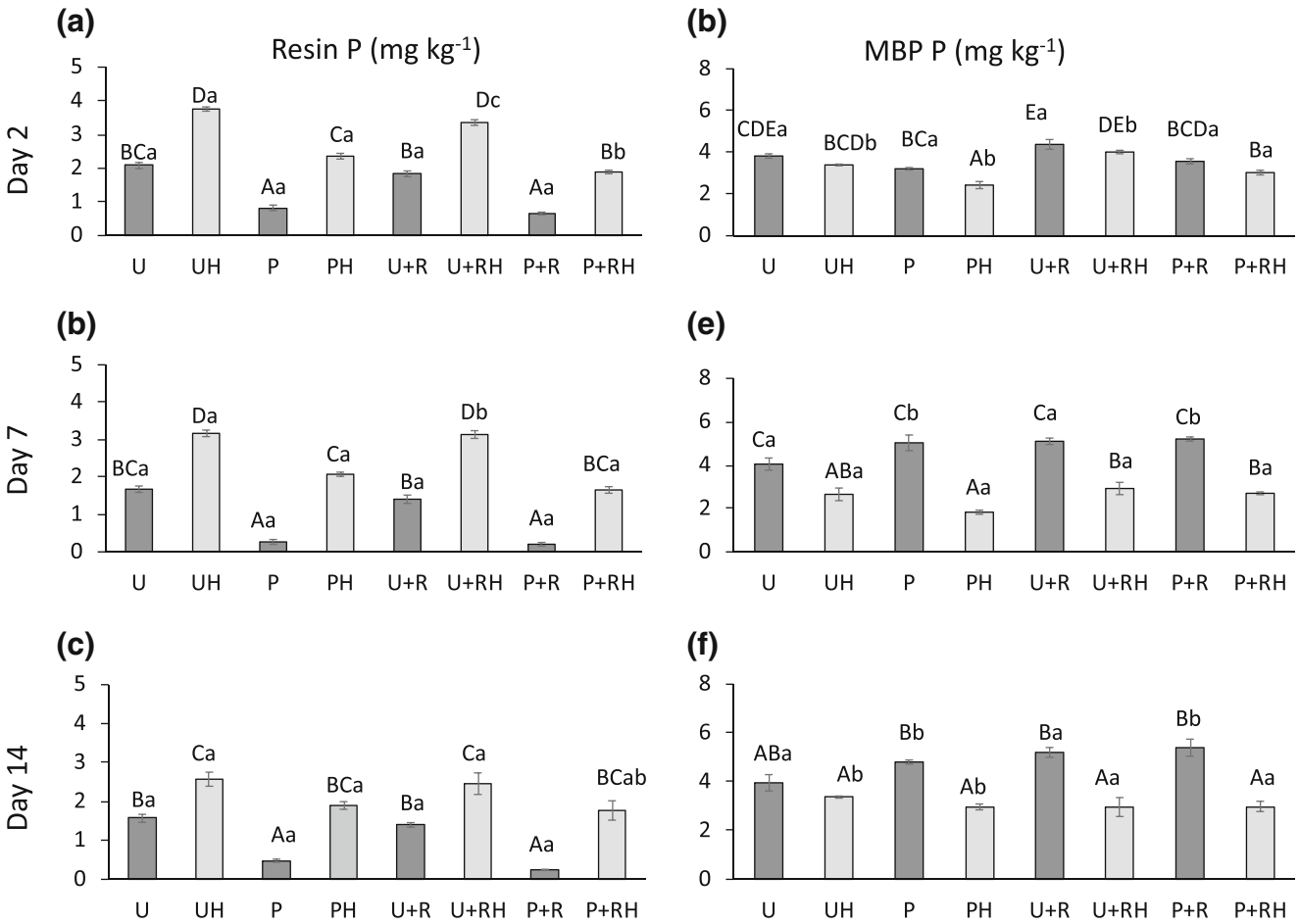

Fig. 3 Resin P (**a**–**c**) and microbial biomass P (**d**–**f**) on days 2 (**a**, **d**), 7 (**b**, **e**) and 14 (**c**, **f**) in previously unplanted and planted soil without roots which remained unheated (U and P) or were heated and rewet (UH and PH) or with roots (U + R, P + R, UH + R, PH + R). Different uppercase letters indicate significant differences among treatments at a given sampling time, and different lowercase letters indicate differences over time in a given treatment ($P \leq 0.05$, $n = 4$, means ± SE)

or with roots, heating increased citrate P more than two-fold. Citrate P changed little over time.

HCl P did not differ between planted and unplanted soil and was not affected by root amendment (Fig. 4d–f, Table S1). Heating influenced HCl P on day 2 only in unplanted soil without roots where it was about 50% higher in heated than unheated soil (Fig. 4d, Table S2). On day 7, HCl P was not influenced by heating (Fig. 4e). Heating increased HCl P on day 14 by about 50% without roots, but had no effect with roots (Fig. 4f). HCl P changed little over time. There was no consistent difference in the effect of heating on citrate P and HCl P between planted and unplanted soil.

4 Discussion

The results of this experiment showed that a short heating event had a greater relative effect on N and P availability in planted than unplanted soil. However, root addition had little effect on the measured parameters (Table S1). This suggests that the amount of organic matter added with the roots (1 g dw kg^{-1}) was too small to influence microbial activity and nutrient availability. Therefore, the second hypothesis (presence of roots will increase the impact of heating on the measured parameters) is declined.

The higher respiration in planted than unplanted soil can be explained by the higher substrate availability from root and root exudates (Farrar et al. 2003; Jones et al. 2009). Previous studies found higher microbial biomass in the rhizosphere (Haynes and Francis 1993; Joergensen 2000). However, in this study, presence of plants had little effect on MBP. This may be due the low P availability in planted soil which likely limited microbial P uptake. Nevertheless, respiration was higher in planted soil which indicates a greater activity per unit biomass.

Presence of plants reduced available N and resin P due to plant N and P uptake (Richardson et al. 2009). Plants seem to have taken up mainly nitrate because ammonium differed little between planted and unplanted soil. The lower citrate P in planted soil is likely due to organic acid anions released by

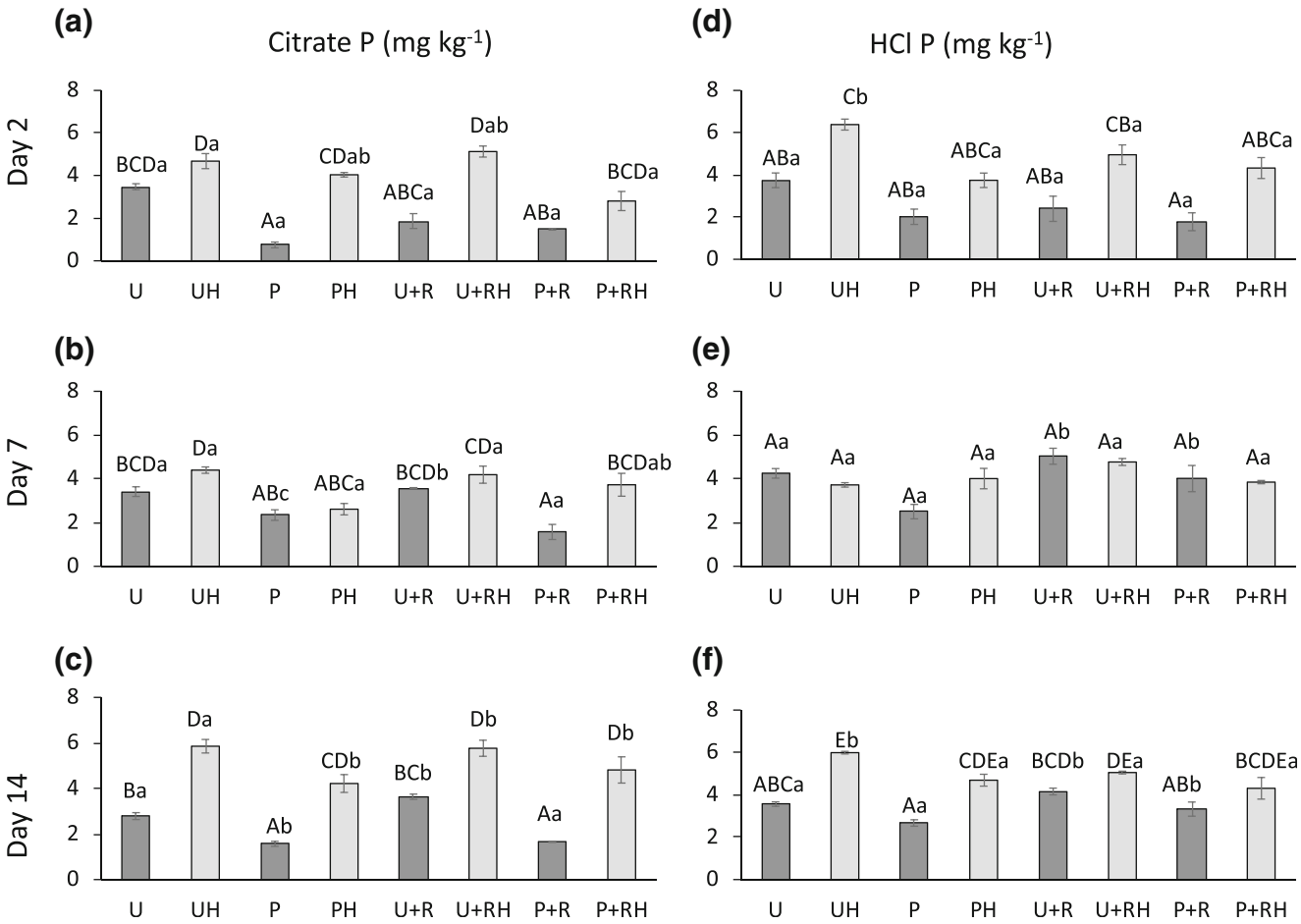

Fig. 4 Citrate P (**a**–**c**) and HCl P (**d**–**f**) on days 2 (**a**, **d**), 7 (**b**, **e**) and 14 (**c**, **f**) in previously unplanted and planted soil without roots which remained unheated (U and P) or were heated and rewet (UH and PH) or with roots (U + R, P + R, UH + R, PH + R). Different uppercase letters indicate significant differences among treatments at a given sampling time, and different lowercase letters indicate differences over time in a given treatment ($P \leq 0.05$, $n = 4$, means ± SE)

roots which would deplete citrate-extractable P (Bolan et al. 1994; Jones et al. 2003).

In agreement with our previous studies (Seneviratne et al. 2019; Seneviratne and Marschner 2019), heating followed by rewetting increased respiration and available N and P. This increase can be explained by higher substrate availability after heating which stimulated microbial activity. Heating as well as rewetting of dry soil has been shown to increase available substrate by release of organic matter from aggregates and death of a proportion of the microbial biomass (Kaiser et al. 2015; Prokushkin and Tokareva 2007).

The hypothesis that the increase in respiration and available N and P by heating and rewetting will be greater in planted than unplanted soil can be confirmed for available N and P. The greater heating-induced increase in available N and P in planted soil could be due to the low nutrient availability before heating. Microbes may have been starved for N and P and rapidly mineralised organic matter released by heating. However, available N and P after heating were similar or lower in planted than in unplanted soil likely because of the relatively low OM content in this soil (6.3 g kg^{-1}) and the short duration of the heating-induced increase in respiration.

The increase in available N by heating was due to ammonium whereas nitrate was not affected. The higher N availability after heating can be explained by heat-induced mineralisation (Certini 2005; Esque et al. 2010). The increase in ammonium indicates that heating and rewetting stimulated particularly ammonification without a corresponding increase in nitrification. Similarly, in the study by Myers (1975), ammonium increased as soil was heated from 20 to 60 °C, but nitrate was maximal at 35 °C and then decreased at higher temperatures.

Available P was higher in heated soils. Stimulation of P mineralisation has been observed after heating (Merino et al. 2019) as well as after rewetting of dry soil (Buenemann et al. 2013; Butterly et al. 2011). The increase in available P after rewetting was explained by mineralisation of microbial P. However, in the present study, heating and rewetting had no effect on MBP on day 2. This suggests that shortly after heating and rewetting, the higher P availability was not due

to P release from the microbial biomass. More likely, it was due to increased net mineralisation which is in agreement with the high respiration rate in the first 2 days after rewetting. The decline in MBP in heated soils from days 2 to 7 suggests that shortly after heating, microbial P uptake may have compensated for microbial death due to high microbial activity. But later, when respiration rates were low, microbial death dominated. This is in contrast to our earlier study (Seneviratne and Marschner 2019) where MBP was 50% lower in heated than unheated soils 2 days after heating. However, the soil used in that experiment was richer in nutrients than the soil used here, and MBP in unheated soil was twofold higher. The low MBP in the soil used in the present study may have masked the initial heat-induced reduction. The lower MBP in heated soils on days 7 and 14 indicates that heating reduced microbial biomass P uptake despite the higher available P than in unheated soil. Butterly et al. (2011) showed that after rewetting of dry soil, P availability was increased only for about 2 days. In contrast, P availability remained high in the present study where heating and rewetting were combined. This suggests that a short heating event induces in sustained increase in P mineralisation. Another reason could be a reduction in P sorption capacity induced by heating (Serrasolses et al. 2008).

In our previous study (Seneviratne and Marschner 2019), citrate P was up to twofold higher in heated than unheated soils 6 to 16 days after heating. This can be explained by increased P mineralisation and binding of proportion of mineralised P to soil particles. A further explanation could be exposure of previously occluded P binding sites after rewetting due to aggregate breakdown (Kaiser et al. 2015). In the current study, however, the effect of citrate P was variable with an increase in heated soils only on day 14. Citrate P in the current study was lower than in the soil used in Seneviratne and Marschner (2019). This low citrate P together with the lower soil organic C may have slowed the response of citrate P to heating.

5 Conclusion

This study showed that a short heating event followed by rewetting increased nutrient availability more strongly in planted than unplanted soil. This increase in nutrient availability would be beneficial for plants that survive the heating event. However, nutrient availability after heating in planted soil was either lower or similar to that of unplanted soil. Thus prior plant growth may have little effect on establishment of seeds blown in or sown after heating. To test this, future experiments could investigate establishment of seedlings after heating of previously planted or unplanted soil.

Acknowledgements Mihiri Seneviratne thanks the Turner family and the University of Adelaide for providing the postgraduate scholarship.

Funding Information This study was funded by a postgraduate scholarship from the Turner Family Trust.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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Chapter 6

Soil respiration and nutrient availability after heating are influenced by salinity but not by prior drying and rewetting.

Published in Biology and Fertility of Soils
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Statement of Authorship

Title of the Paper	Soil respiration and nutrient availability after heating are influenced by salinity but not by prior drying and rewetting
Publication Status	[X] Published [ ] Accepted for Publication [ ] Submitted for Publication [ ] Unpublished and Unsubmitted work written in manuscript style
Publication details	Seneviratne, M., Marschner, P., 2020. Soil respiration and nutrient availability after heating are influenced by salinity but not by prior drying and rewetting. Biology and Fertility of Soils

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Name of Principal Author (Candidate)	Mihiri Seneviratne		
Contribution to the paper	Performed the experiment, analysed the samples, data analysis and interpretation of data, writing the manuscript. I hereby certify that the contribution is accurate		
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 subjected 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	Mihiri Seneviratne	Date	15/02/2020

Co-Author contributions

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Name of Co-Author	Petra Marschner		
Contribution to the paper	Supervised development of work, data interpretation, manuscript evaluation and correction, acted as the corresponding author		
Signature		Date	15/02/2020

Seneviratne, M., Marschner, P., 2020. Soil respiration and nutrient availability after heating are influenced by salinity but not by prior drying and rewetting. Biology and Fertility of Soils https://doi.org/10.1007/s00374-020-014450

Biology and Fertility of Soils
https://doi.org/10.1007/s00374-020-01445-0

ORIGINAL PAPER

Soil respiration and nutrient availability after heating are influenced by salinity but not by prior drying and rewetting

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Received: 5 November 2019 / Revised: 10 February 2020 / Accepted: 13 February 2020

Abstract

Semi-arid and arid regions are characterised by drying and rewetting events, salinity as well as fires. The effects of these stresses on soils are all related to water availability and have been investigated extensively, but usually separately. To study the impact of two stresses, we combined a short heating event as it may occur in fast-moving grass fires with either a drying and rewetting event or salinity. In the first experiment, soils were incubated air-dried, moderately dried and constantly moist for 7 days after which the dried soils were rewetted. Seven days after rewetting, half of the replicates from each treatment were heated and then maintained at 60 °C for 30 min followed by rapid rewetting, while others remained unheated. The rewetting flush of respiration after 7 days was greater in previously air-dried soil than moderately dry soil. Regardless of prior water treatment, heating followed by rewetting compared to constantly moist soil increased respiration threefold and available N and P by about 20–30%. In the second experiment, NaCl was added to non-saline soil ($EC_{1:5}$ 0.01 dS m^{-1}) to achieve $EC_{1:5}$ 1 and 4 ds m^{-1} (referred to as NS, S1 and S4). Soils were incubated moist for 1 month and then amended with pea residue at 10 g kg^{-1}. Five days after residue amendment, half of the replicates from each treatment were heated and rewetted as in the first experiment. In unheated S4 compared to NS, cumulative respiration was 30% lower and available N and P were threefold and 30% higher. Heating reduced cumulative respiration by 10% in NS and S1 but by 30% in S4. Compared to unheated treatments, available N in heated NS was up to tenfold higher, but in S4, heating increased available N only up to threefold. In all salinity treatments, heating increased available P by about 15%. It can be concluded that the impact of short-term heating on nutrient availability and soil respiration was not affected by prior drying and rewetting. High salinity on the other hand reduced the impact on nutrient availability whereas the effect on soil respiration was exacerbated compared to non-saline soil.

Keywords Air-dried · Rewetting · Heating · Salinity · Water availability

Introduction

In semi-arid and arid regions, soils are exposed to water stress induced by drying and rewetting events, salinity or heating. The effects of these stresses individually have been studied before. For example, we showed that a short heating event (60 °C for 30 min) followed by rewetting induces a flush of respiration and increases N and P availability up to 14 days after heating (Seneviratne et al. 2019; Seneviratne and Marschner 2019). As soil dries, the water film around soil particles decreases, restricting water availability and substrate diffusion, which reduces microbial activity compared to constantly moist soil and leads to substrate accumulation (Ilstedt et al. 2000; Stark and Firestone 1995). Rapid rewetting increases substrate availability and induces a short-lived flush of respiration and nutrient availability (Birch 1958; Dinh et al. 2018; Xiang et al. 2008). The size of the rewetting flush is influenced by a number of factors, e.g., water content before rewetting and by the number of drying and rewetting events (Fierer and Schimel 2002; Meisner et al. 2017; Shi and Marschner 2014).

Whereas drying and rewetting events are short-term changes in water availability, water stress due to salinity can be sustained. The high salt concentration in the soil solution reduces water availability to microbes which reduces microbial activity and biomass and also alters microbial community composition compared to non-saline soil (Chowdhury et al. 2011). Water loss from saline soils can exacerbate salinity

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Published online: 20 February 2020

stress because it increases the salt concentration in the soil solution (Rath et al. 2017).

In semi-arid and arid regions, heating may follow drying and rewetting or occur in saline soils. Previous studies showed that response of microbial activity and community composition to water availability or drying and rewetting events is influenced by prior water availability (Banerjee et al. 2016; Evans and Wallenstein 2012). This suggests that the effect of heating may also be influenced by water availability.

This study was carried out to answer the question if the effect of heating on soil respiration and nutrient availability is influenced by prior exposure to drying and rewetting or by soil salinity. In the drying and rewetting experiment, soil was strongly or moderately dried. The first hypothesis was that drying and rewetting before heating will reduce the effect of heating on soil respiration and nutrient availability, with a greater effect after strong drying than moderate drying. This hypothesis was based on previous studies that showed that the flush of respiration after rewetting decreases with number of drying and rewetting events (Shi and Marschner 2014). The second hypothesis was that the effect of heating on respiration and nutrient availability compared to unheated soil will be smaller in saline than non-saline soil. We assumed that salinity constraints the ability of microbes to access substrate released by heating and rewetting.

Materials and methods

A loamy soil was collected from 0 to 10 cm depth from an area on Waite campus, University of Adelaide, South Australia (longitude 138° 38′ 3.2″ E, latitude 34° 58′ 0.2″ S). The climate in this region is Mediterranean. The soil is classified as Chromosol in Australian soil classification and as Rhodoxeralf in US Soil Taxonomy. After collection, the soil was air-dried and sieved to < 2 mm. The soil had following properties: sand 48%, silt 27% and clay 25%, pH (1:5 soil:water) 5.3, EC (1:5 soil:water) 0.01 dS m^{-1}, total organic C 22 g kg^{-1}, total N 1.6 g kg^{-1}, total P 400 mg kg^{-1}, available N 53 mg kg^{-1}, available P 13 mg kg^{-1}, maximum water holding capacity (WHC) 330 g kg^{-1} and bulk density 1.3 g cm^{-3}. For analytical methods see Table 1.

Experimental design

Drying and rewetting experiment

Thirty grams of air-dried soil were moistened to 165 g kg^{-1} (50% maximum water holding capacity) with reverse osmosis (RO) water and mixed thoroughly in a plastic bag. After mixing, the soil was filled into 70-ml plastic containers and adjusted to a bulk density of 1.3 kg cm^{-3}. The soil containers were kept in dark at 20–22 °C for 7 days to activate the microbes and stabilise soil respiration. Soil water content was maintained at 165 g kg^{-1} by checking the weight in every second day and adding RO water if necessary.

After the 7-day pre-incubation, three different treatments were started, namely, air-dried (AD), moderately dried (MD) and constantly moist (CM). Containers with soil were placed individually into 1-L jars with gas-tight lids equipped with septa to allow quantification of the headspace CO_2 concentration. To dry the soil to < 5% (AD) and 25% WHC (MD), one pouch of self-indicating silica gel (BDH Chemicals, 8 g per pouch) was placed in each jar for AD or MD treatments and exchanged daily. Soil containers were weighed daily. It took 4 and 2 days to reach < 5% and 25% WHC, respectively. Then the soil in MD and AD treatments was maintained at this water content until day 7. CM soil was kept constantly moist at 165 g kg^{-1} until day 14.

On day 7, AD and MD soils were rapidly rewetted to 165 g kg^{-1} and maintained at this water content until day 14. On day 14, half of the soil containers of each treatment were heated to 60 °C within 1 h in a fan forced oven and then maintained at 60 °C for 30 min. This heating corresponds to soil temperatures in fast moving grass or shrub fires with low fuel load (Scotter 1970; Stoof et al. 2013). Thermocouples were placed in the soil to monitor the soil temperature during heating. The heated soils were allowed to cool to room temperature which took about 2 h and then rewetted from about 80 g kg^{-1} (25% WHC) to 165 g kg^{-1}. The other half of the containers remained unheated and at 165 g kg^{-1}. Respiration was measured daily from days 1 to 28. Soil was sampled destructively on day 9 (2 days after the first rewetting), day 14 (before heating), day 16, 21 and 28 (2, 7 and 14 days after heating) and analysed for available N and available P. For each sampling time and treatment, there were four replicates.

Salinity experiment

Soil was pre-incubated at 165 g kg^{-1} (50% maximum water holding capacity) at three salinity levels ($EC_{1:5}$ 0.01, 1 and 4 dS m^{-1}) for 1 month. The original soil was non-saline (0.01 dS m^{-1}). The other two salinity levels ($EC_{1:5}$ 1 and 4 dS m^{-1}) were achieved by adding different concentrations of NaCl, followed by thorough mixing. Initially the soils were adjusted to EC levels about 0.5 dS m^{-1} above the desired values because in our previous studies salinity decreased slightly over time. The EC of the soils was checked weekly, and after 1 month, the EC values were stabilised to the desired EC values (± 0.2). Salinity treatments are referred to as NS, S1 and S4, for $EC_{1:5}$ 0.01, 1 and 4 dS m^{-1}. The soil water content of the soils was maintained at 165 g kg^{-1} by checking the weight in every second day and adding reverse osmosis water if necessary. After 1 month, pea (*Pisum sativum* L.) residue with total organic C 474 g kg^{-1} and total N 7.9 g kg^{-1}, ground

Table 1 Soil analyses as described in (Marschner et al. 2015)

Parameter	Details	Reference
Soil pH and EC	1:5 soil:water ratio, 1-h shaking	(Rayment and Higginson 1992)
Soil maximum water holding capacity	At matric potential − 10 kPa	(Wilke 2005)
Total organic C	Wet oxidation and titration	(Walkley and Black 1934)
Total N	Digestion with H_2SO_4, measurement by modified Kjeldahl method	(Bremner and Mulvaney 1982)
Available N extraction	2 M KCl at a 1:5 soil extractant ratio, 1-h shaking	Bremner and Mulvaney (1982)
Exchangeable ammonium N measurement		(Willis et al. 1996)
Nitrate N measurement		(Miranda et al. 2001)
Available P extraction	Colwell P at a 1:40 soil extractant ratio	Colwell (1963)
Available P measurement		(Ohno and Zibilske 1991)
Soil respiration	CO_2 concentration in headspace of jars	(Setia et al. 2011)

and sieved (0.25–2 mm), was mixed into the soils at 10 g kg^{-1} to increase substrate availability. In a previous experiment with this soil, microbial activity was low after 4-week moist incubation (Seneviratne et al. 2019), likely because most available substrates had been decomposed. The low microbial activity would have limited the effect of salinity or heating on soil respiration and nutrient availability which were the focus of this experiment.

The residues were thoroughly mixed with 30 g soil in plastic bags and then filled into 70-ml plastic containers and packed to a bulk density of 1.3 g cm^{-1}. Soil containers were kept in dark at 20–22 °C. Four days after residue addition, soil from each salinity level was separated to three treatments namely heated (H), dried (D) and constantly moist (CM). In the heated treatment, soil was heated to 60 °C within 1 h in a fan-forced oven and then maintained at 60 °C for 30 min. Temperature during heating was monitored by thermocouples placed in the soil. The heated soils were allowed to cool into room temperature and then rewetted to 165 g kg^{-1}. The treatment names of heated soils were NSH, S1H and S4H. Heating reduced soil water content from 165 g kg^{-1} (50% WHC) to about 90 g kg^{-1} (28% WHC). Therefore in the dried treatment, soil from each salinity level was dried in a fan-forced oven at 30 °C until reaching 90 g kg^{-1}, which took 6 h. We did not use silica pouches as in the drying and rewetting experiment because drying would have taken several days which is much longer than drying during heating. After reaching the desired water content, soils were rewetted to 165 g kg^{-1} similar to the heated soils. This treatment was included to determine if the heat effect is due to water loss during heating or due to heat. The dried treatments were referred to as NSD, S1D and S4D. For the constantly moist treatment, soils were maintained at 165 g kg^{-1} throughout the experiment (referred to as NSCM, S1CM, S4CM). Respiration rate was measured daily for 14 days after heating and rewetting. Soil was sampled destructively 2, 7 and 14 days after heating for determination of available N and available P. Soil analyses were carried out as described in Seneviratne et al. (2019) except that available P was measured by the Colwell P method (Colwell 1963). The anion exchange resin method we used for available P in Seneviratne et al. (2019) is not suitable of saline soils because chloride competes with phosphate on the resin strip which leads to underestimation of available P.

Statistical analysis

There were four replicates per treatment and sampling time. Data was checked for normality using the Shapiro-Wilk test. Cumulative respiration was analysed by one-way ANOVA. The data of available N and P was transformed to log10 values to achieve normality and analysed by repeated measures ANOVA with between subjects factor-treatment, within subjects factor-time in SPSS statistics version 25. In the drying and rewetting experiment, there were more treatments after heating than before. Therefore, data of available N and P for days 9 and 14 was analysed separately from that of days 16, 21 and 28.

Results

Drying and rewetting experiment

During the drying period, the respiration rate declined, particularly in the treatment that was dried to air-dry (Fig. 1a). Rewetting of air-dried soil induced a flush of respiration. One day after rewetting, respiration rate was about threefold higher than in the constantly moist control. Two days after

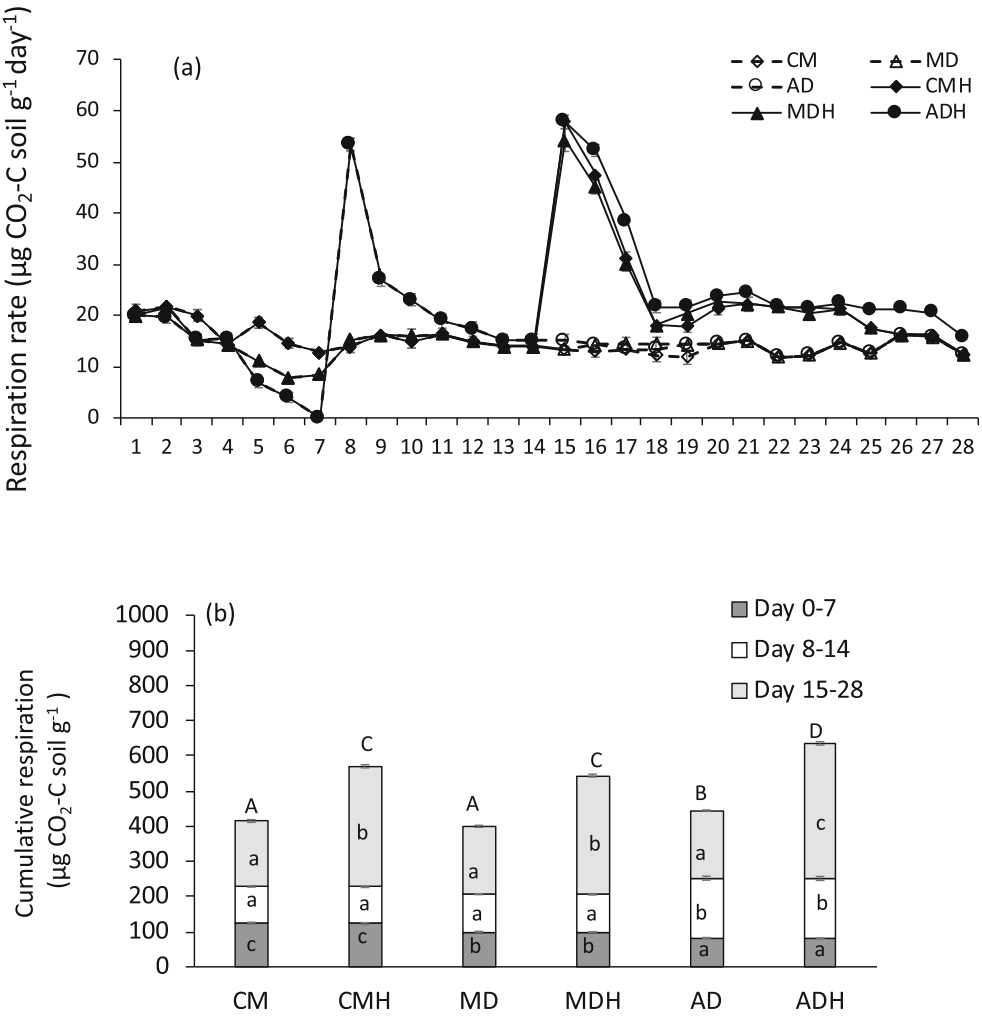

Fig. 1 **a** Respiration rate and cumulative respiration of soil maintained constantly moist (CM) or exposed to drying and rewetting in the first 14 days where soil was dried to air-dry or moderately dried (AD and MD). After day 14, soils were either maintained moist (CM, AD and MD) or heated and then rewet (CMH, ADH and MDH). In **b**, different lower case letters indicate significant differences in cumulative respiration in different periods. Different upper case letters show significant differences in total cumulative respiration ($n = 4$, means ± SE, $P \leq 0.05$)

rewetting of air-dried soil, respiration rate was only 50% higher than the control and further declined on the following days so that it was similar to the control after day 11 (4 days after rewetting). Rewetting of the moderately dried soil also increased respiration rate, but only to the level of the constantly moist control. In all prior water treatments, heating and rewetting on day 14 resulted in flush of respiration with respiration rates on day 15, threefold higher in heated than unheated soils. Respiration rates in heated soils then declined over the next 3 days. Respiration rate in heated soils remained higher than in unheated soils until day 26 in the constantly moist and moderately dried soils and until the end of the experiment on day 28 in previously air-dried soil.

Compared to constantly moist soil, cumulative respiration before the first rewetting (days 0–7) was 25% and 50% lower in moderately dried and air-dried treatments (Fig. 1b). But between the first rewetting and heating (days 8–15), cumulative respiration in previously air-dried soil was twofold higher than constantly moist or previously moderately dried soil. After heating (days 15 to 28), cumulative respiration was about twofold higher in heated than unheated soil, regardless of previous water treatment. Total cumulative respiration in unheated and heated treatments was about 20% higher in air-dried soil than the constantly moist and moderately dried soils.

Nitrate was the main form of available N (Fig. 2, Table 2). Treatments differed little in available N before heating (days 9 and 14, Fig. 2a, b). Water treatment before heating had little effect on available N after heating (Fig. 2c–e). On days 16 and 21 (2 and 7 days after heating and rewetting), available N was about 30% higher in heated than unheated soils which was due to a tenfold increase in exchangeable ammonium whereas nitrate was not affected by heating (Fig. 2c, d). Available N in all treatments increased by about 15% from days 16 to 21. Treatments did not differ in available N on day 28 (Fig. 2e).

Available P on days 9 and 14 was about 15% lower in moderately dried soil than constantly moist or air-dried soils (Table 3). Heating increased available P on day 16 by about 25% regardless of water treatment before heating. On day 21, available P was about 25% higher in heated soil that had been

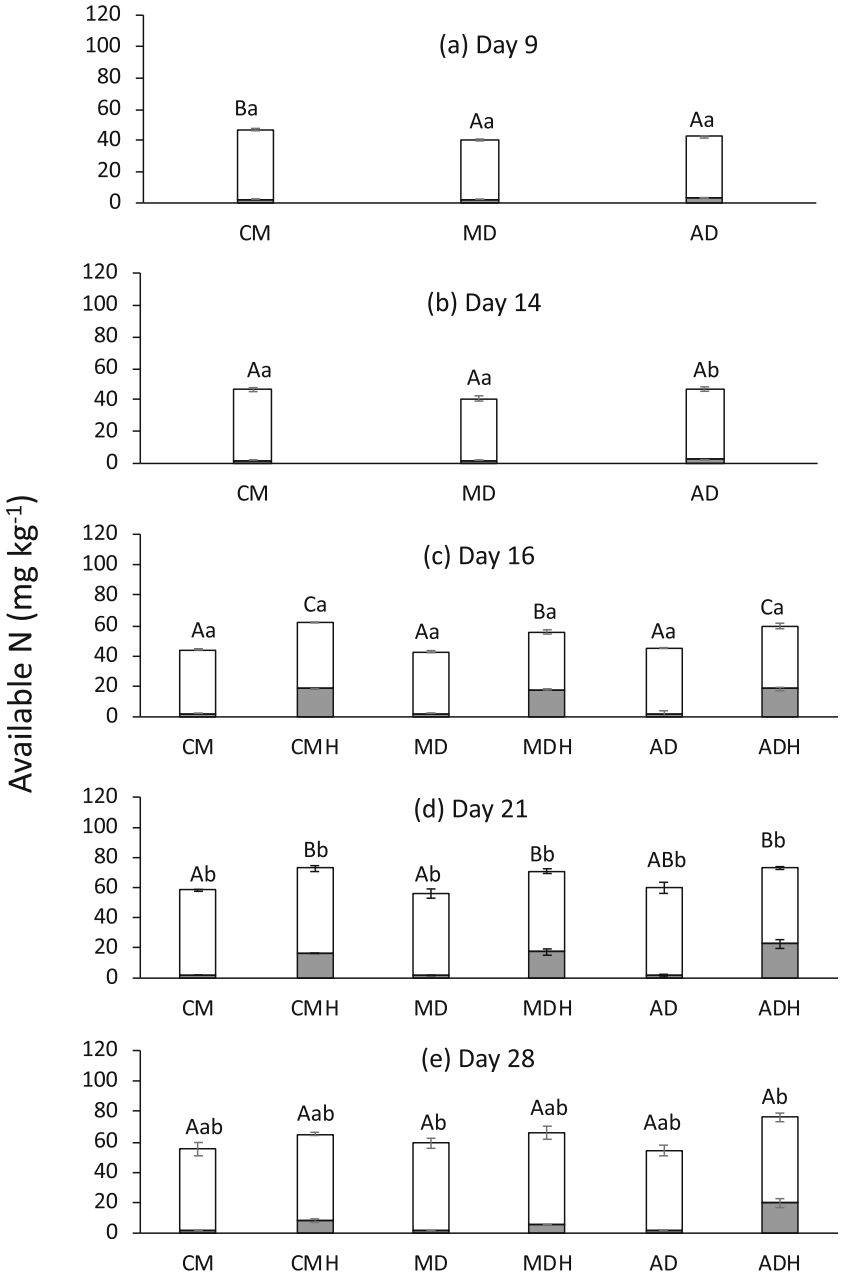

Fig. 2 Available N on days 9, 14, 16, 21 and 28 in soil maintained constantly moist (CM) or exposed to drying and rewetting in the first 14 days where soil was dried to air-dry or moderately dried (AD and MD). After day 14, soils were either maintained moist (CM, AD and MD) or heated and then rewet (CMH, ADH and MDH). Different upper case letters indicate significant differences between treatments at each sampling date. Different lower case letters show significant differences in a treatment over time ($n = 4$, means ± SE, $P \leq 0.05$). Shaded bars indicate exchangeable ammonium-N, white bars nitrate-N

constantly moist or air-dried before heating. But on day 28, heating increased available P only in previously air-dried soil.

Salinity experiment

Compared to S4, respiration rate on day 1 was two and threefold higher in S1 and NS in constantly moist and dried soils (Fig. 3a, b). Respiration rates in S1 and NS then declined and became similar to that in S4 after day 6. In heated treatments, respiration rate on day 1 (1 day after heating and rewetting) was about 50% higher in NS and S1 than S4 (Fig. 3c). Respiration rate in S4 gradually declined over time. But in NS and S1, respiration rates declined only until day 3 and then changed little or slightly increased. In NS, respiration rates in the first 3 days were about 40% lower in heated soil than constantly moist or dried soil. Respiration rate in S1 on day 1 was 10% lower in heated than constantly moist soil. However in S4, the respiration rate on day 1 in heated soil was about twofold higher than in constantly moist or dried. But from day 3 to about day 10, respiration rates in heated S4 were lower than constantly moist soil.

Cumulative respiration was highest in NS and lowest in S4, and differed little between constantly moist or air-dried treatments (Fig. 3d). In heated treatments, cumulative respiration

Table 2 Exchangeable ammonium and nitrate on days 9, 14, 16, 21 and 28 in soil maintained constantly moist (CM) or exposed to drying and rewetting in the first 14 days where soil was dried to air-dry or moderately dried (AD and MD). After day 14, soils were either maintained moist (CM, AD and MD) or heated and then rewet (CMH, ADH and MDH). Different upper case letters indicate significant differences between treatments at each sampling date. Different lower case letters show significant differences in a treatment over time ($n = 4$, means ± SE, $P \leq 0.05$)

Treatment		NH_4^+–N (mg kg^{-1})	NO_3^-–N
Day 9	CM	2.5 Aa	44.4 Ba
	MD	2.4 Aa	37.9 Aa
	AD	3.2 Bb	39.0 Aa
Day 14	CM	2.2 Aa	44.3 Aa
	MD	2.1 Aa	38.9 Aa
	AD	2.3 Aa	44.5 Ab
Day 16	CM	2.4 Ab	42.0 BCa
	CMH	18.3 Bb	43.8 Ca
	MD	2.5 Ac	40.5 BCa
	MDH	18.1 Bb	37.8 ABa
	AD	2.2 Aa	43.0 Ca
	ADH	18.3 Ba	41.5 BCa
Day 21	CM	1.9 Ab	56.2 Ab
	CMH	16.4 Bb	56.2 Ab
	MD	1.8 Ab	54.2 Ab
	MDH	17.3 Bb	53.7 Ab
	AD	1.7 Aa	58.2 Ab
	ADH	22.6 Ba	50.4 Ab
Day 28	CM	1.7 Aa	53.5 Ab
	CMH	8.2 Ba	57.0 Ab
	MD	1.6 Aab	57.4 Ab
	MDH	5.5 Ba	60.5 Ab
	AD	1.9 Aa	52.5 Ab
	ADH	19.7 Ca	56.4 Ab

Exchangeable ammonium and nitrate DRW

Table 3 Available P on days 9, 14, 16, 21 and 28 in soil maintained constantly moist (CM) or exposed to drying and rewetting in the first 14 days where soil was dried to air-dry or moderately dried (AD and MD). After day 14, soils were either maintained moist (CM, AD and MD) or heated and then rewet (CMH, ADH and MDH). Different upper case letters indicate significant differences between treatments at each sampling date. Different lower case letters show significant differences in a treatment over time ($n = 4$, means ± SE, $P \leq 0.05$)

Treatment		Available P (mg kg^{-1})
Day 9	CM	16.7 Ba
	MD	12.9 Aa
	AD	18.7 Ca
Day 14	CM	19.2 Bb
	MD	15.0 Ab
	AD	18.2 Ba
Day 16	CM	20.9 Ab
	CMH	23.9 Bb
	MD	19.9 Aa
	MDH	24.9 Bb
	AD	18.6 Aa
	ADH	24.2 Ba
Day 21	CM	18.6 Aa
	CMH	24.6 Bb
	MD	18.4 Aa
	MDH	20.6 Aa
	AD	20.0 Aa
	ADH	24.4 Ba
Day 28	CM	18.9 Aa
	CMH	21.1 ABa
	MD	20.0 Aa
	MDH	19.0 Aa
	AD	19.0 Aa
	ADH	24.0 Ba

in S4 was about 30% lower than NS from days 0 to 7, but 70% lower from days 8 to 14. Compared to NS, cumulative respiration in S4 at the end of the experiment was 20%, 30% and 50% lower in constantly moist, dried and heated treatments, respectively. Cumulative respiration in heated soil was 10% lower than constantly moist treatments in non-saline soil and S1, but 30% lower in S4.

Available N was lowest in NS and highest in S4, and it was higher in heated soils than constantly moist and dried treatments (Fig. 4, Table 4). In constantly moist and dried treatments, available N compared to NS was about twofold higher in S1 and fivefold higher in S4. Heating increased available N due to a three to 20-fold increase in exchangeable ammonium. On day 2, available N in heated soils was about 20% higher in S4 than NS; it was tenfold higher in heated soil than the constantly moist treatment in NS and S1 and twofold higher in S4 (Fig. 4a). Available N decreased from days 2 to 7 by about 30% in NS, but did not change in S1 and S4. On day 7, available N in heated soil compared to NS was 50% higher in S1 and twofold higher in S4 (Fig. 4b). Compared to constantly moist treatments, available N on day 7 was about twofold higher in heated NS and S1 and threefold higher in S4. In heated treatments on day 14, available N compared to NS was 50% higher in S1, but more than threefold higher in S4 (Fig. 4c). In all salinity levels, available N on day 14 was about two to threefold higher in heated soils than constantly moist treatments.

Available P was generally higher in S4 than NS, but differed little between NS and S1 (Fig. 5). Drying had little effect on available P compared to the constantly moist treatment, but heating increased available P. Available P changed little over time. On day 2, available P was about 30% higher in S4 than

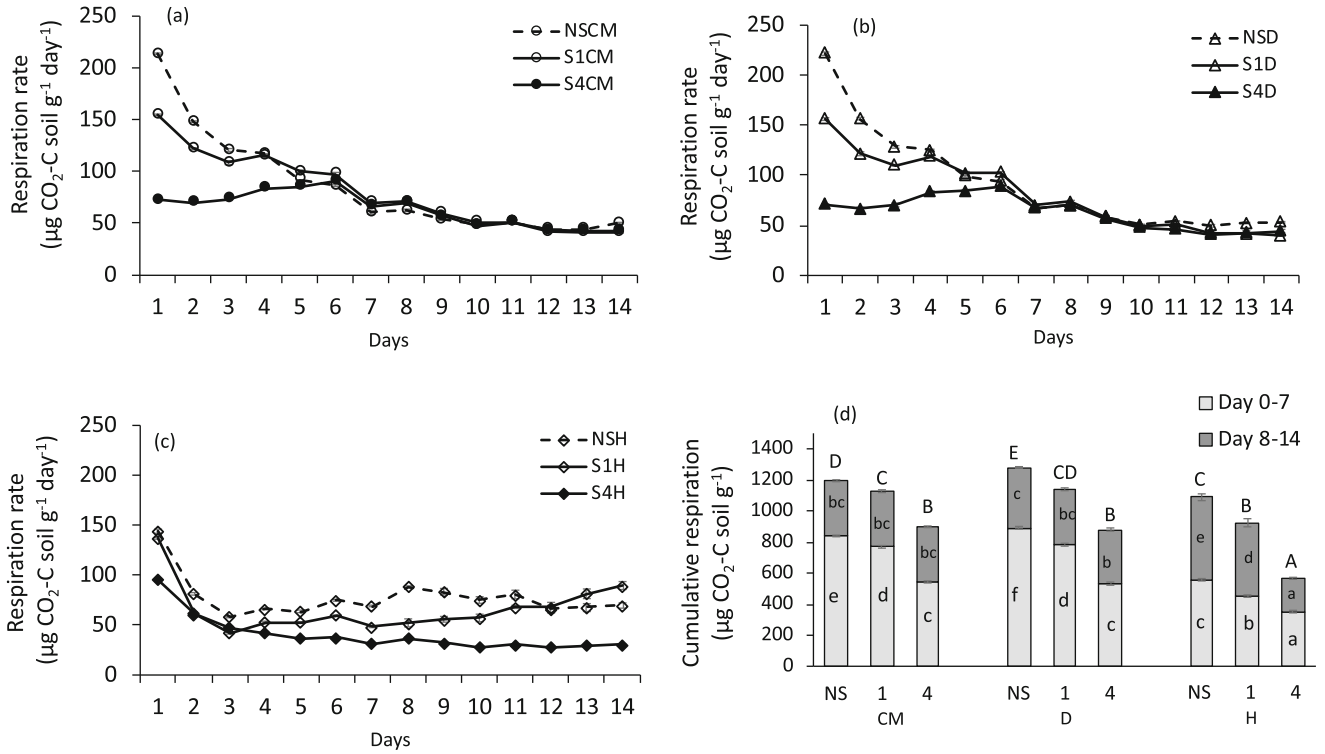

Fig. 3 Respiration rate in constantly moist (**a**), dried (**b**) and heated (**c**) treatments and cumulative respiration (**d**) of non-saline soil (NS) and saline soils with $EC_{1:5}$ 1 and 4 dS m^{-1} (S1 and S4). In **d**, different lower case letters indicate significant differences in cumulative respiration in different periods. Different upper case letters show significant differences in total cumulative respiration ($n = 4$, means ± SE, $P \leq 0.05$)

NS in constantly moist and dried treatments, but was not affected by salinity in heated treatments (Fig. 5a). Available P on days 7 and 14 was about 20% higher in S4 than NS. Heating increased available P on days 7 and 14 compared to constantly moist treatments in NS and S4 by about 15% (Fig. 5b, c).

Discussion

This study showed that the impact of heating was altered by salinity, but not by prior drying and rewetting. The impact of drying and rewetting on soil respiration was similar as in previous studies. Rewetting of air-dried soil induced a flush of respiration compared to constantly moist soil whereas rewetting of moderately dry soil had little effect on respiration (Meisner et al. 2017). The strong flush after rewetting of air-dried soil can be explained by substrate release upon rewetting from osmolytes, killed microbes and exposure of previously occluded organic matter by aggregate breakdown (Denef et al. 2001; Warren 2014). Substrate release upon rewetting of moderately dried soil is smaller because the difference in water availability before and after rewetting is smaller (Meisner et al. 2013). The flush of respiration after rewetting of air-dried soil compensated for the lower respiration during drying compared to the constantly moist soil. Thus on day 14 (before heating), cumulative respiration as well as N and P availability differed little among water treatments. This is likely the reason for the finding that drying and rewetting had no effect on respiration, N and P availability after heating. Therefore the first hypothesis (drying and rewetting before heating will reduce the effect of heating on soil respiration and nutrient availability, with a greater effect after strong drying than moderate drying) has to be declined. However, repeated drying and rewetting events or shorter periods in moist conditions prior to heating may affect the impact of heating if they change cumulative respiration and thus substrate availability, microbial community composition or nutrient availability (e.g. Fierer and Schimel 2002; Shi and Marschner 2014). The heating-induced increase in respiration rate, available N and P is in agreement with our previous studies (Seneviratne et al. 2019; Seneviratne and Marschner 2019) and can be explained by greater substrate availability and heat-induced N and P mineralisation (Esque et al. 2010; Merino et al. 2019; Prokushkin and Tokareva 2007). However, the effect of two heating events each followed by rewetting is different from the results of this experiment where heating followed drying and rewetting. In our previous study, a soil was exposed to either one or two short heating events (60 °C for 30 min) which were separated by 4, 8 or 16 days (Seneviratne and Marschner

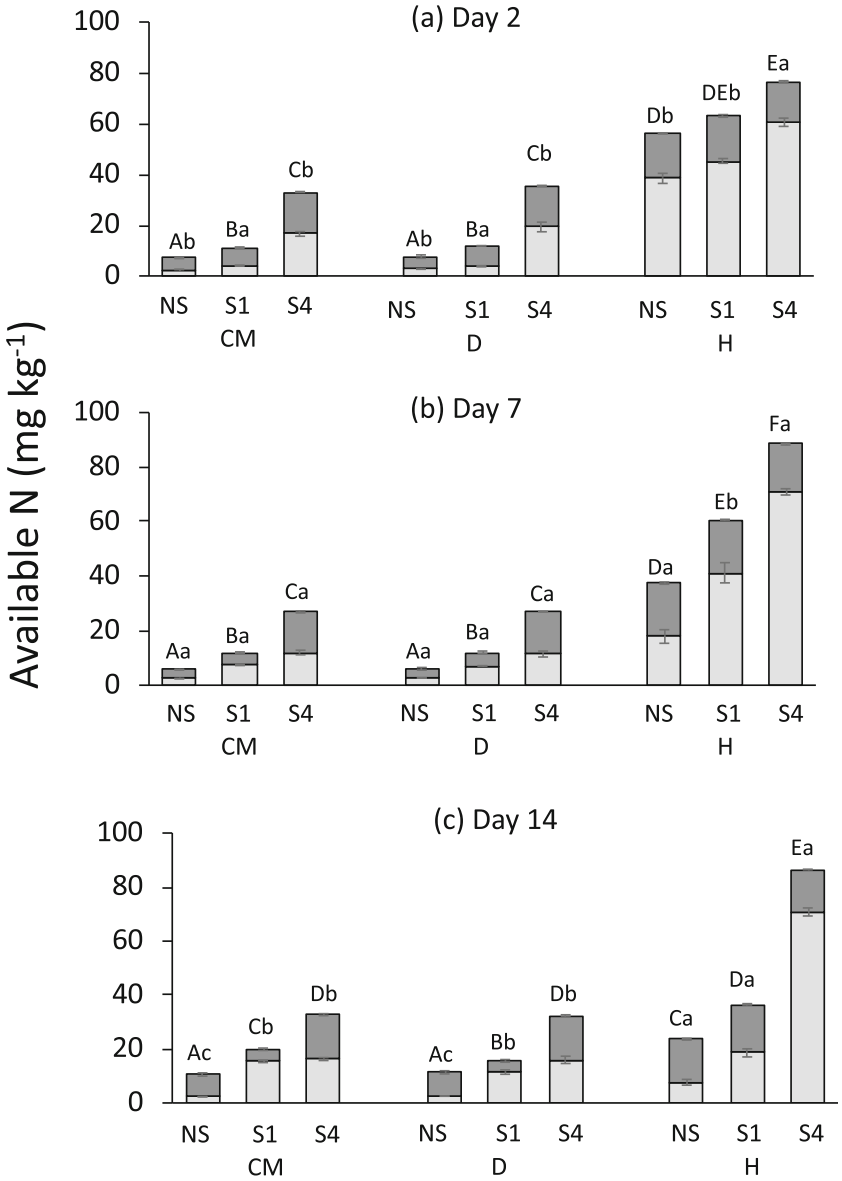

Fig. 4 Available N in constantly moist (CM), dried (D) and heated (H) treatments on days 2, 7 and 14 in nonsaline soil (NS) and saline soils with $EC_{1:5}$ 1 and 4 dS m^{-1} (S1 and S4). Different upper case letters indicate significant differences between treatments at each sampling date. Different lower case letters show significant differences in a treatment over time ($n = 4$, means ± SE, $P \leq 0.05$). Shaded bars indicate exchangeable ammonium-N, white bars nitrate-N

2019). The second heating event induced a further increase in available N and P compared to the first heating. Thus, prior drying combined with heating may have a different effect on the impact of the subsequent heating on nutrient availability than prior air-drying and rewetting.

In the salinity experiment, the measured parameters were similar in non-saline soil and the low salinity (S1) treatment whereas high salinity (S4) influenced the measured parameters compared to the non-saline soil in constantly moist and heated treatments. This indicates that, in the soil used here, there is a salinity threshold above which the impact of heating is altered. In the following, only the effect of S4 will be discussed.

Respiration in S4 was lower than in non-saline soil which can be explained by the osmotic stress in the saline soil which reduces microbial activity (Rietz and Haynes 2003; Yuan et al. 2007). In agreement with Seneviratne et al. (2019), drying of soil to the same water contents as in heated soils had little effect on the measured parameters. This indicates that the effect of heating is not only due to water loss during heating but also to heating itself. Both available N and P were higher in S4 than in non-saline soil. Since soil respiration was lower in S4 than in non-saline soil, the higher N and P availability is unlikely to be due to increased mineralisation in the saline soil. More likely explanations are reduced microbial N and P uptake and lower N and P binding capacity in saline soils. Salinity has been shown to reduce microbial growth and thus N and P immobilisation (Zhou et al. 2017). High salt concentrations in the soil solution can reduce exchangeable ammonium by competition for binding sites on soil particles

Table 4 Exchangeable ammonium and nitrate in constantly moist (CM), dried (D) and heated (H) treatments on days 2, 7 and 14 in non-saline soil (NS) and saline soils with $EC_{1:5}$ 1 and 4 dS m^{-1} (S1 and S4). Different upper case letters indicate significant differences between treatments at each sampling date. Different lower case letters show significant differences in a treatment over time ($n = 4$, means ± SE, $P \leq 0.05$)

Treatment		NH_4^+–N (mg kg^{-1})	NO_3^-–N
Day 2	NSCM	2.6 Aa	4.5 Ab
	1CM	4.0 Ba	7.0 Bb
	4CM	16.5 Cb	16.5 Ca
	NSD	2.7 Aa	5.0 Ab
	1D	3.8 Ba	8.1 Bb
	4D	19.3 Cb	16.2 Ca
	NSH	38.4 Dc	17.7 Cab
	1H	45.3 Db	17.7 Ca
	4H	60.5 Ea	15.9 Ca
Day 7	NSCM	2.4 Aa	3.3 Aa
	1CM	7.5 Bb	4.4 Aba
	4CM	12.0 Ca	14.9 Ca
	NSD	2.6 Aa	3.4 Aa
	1D	7.1 Bb	4.9 Ba
	4D	11.4 Ca	15.6 Ca
	NSH	17.9 Db	19.6 Cb
	1H	41.2 Eb	19.5 Ca
	4H	70.9 Fa	17.5 Ca
Day 14	NSCM	2.3 Aa	8.2 Bc
	1CM	15.4 CDc	4.5 Aa
	4CM	16.3 Db	16.4 Ca
	NSD	2.5 Aa	8.8 Bc
	1D	11.4 Cc	4.1 Aa
	4D	16.0 CDb	16.3 Ca
	NSH	7.7 Ba	16.0 Ca
	1H	18.6 Da	18.0 Ca
	4H	70.8 Ea	15.7 Ca

(Rysgaard et al. 1999) Competition may also occur between chloride and phosphate. This competition likely increases N and P extractability.

The second hypothesis (the effect of heating on respiration and nutrient availability compared to unheated soil will be smaller in saline than non-saline soil) can be accepted for available N, but not for respiration and available P. Compared to unheated soil, heating reduced the initial respiration rate in non-saline soil, but increased it slightly in S4. The reduction of the initial respiration rate by heating in the non-saline soil is in contrast to the drying and rewetting experiment where heating increased respiration rate. However, in the salinity experiment, residues were added shortly before the start of the experiment to ensure a strong salinity effect. Organic amendments typically increase microbial activity (e.g. Marschner et al. 2015), which was also the case in this experiment where residue addition resulted in tenfold higher initial respiration rate of the non-saline soil than in the unamended soil in the drying and rewetting experiment. The reduction of initial respiration in heated non-saline soil is likely because of the activation of microbes by residue addition which made them more susceptible to stress than inactive microbes (Schimel et al. 2007). However, from 5 after heating, the respiration rate was higher in heated non-saline soil than the unheated treatments which indicates recovery of microbes from heat stress and compensated to some extent for the lower respiration in the first 7 days. Thus, in the salinity experiment, the increased substrate availability after heating could not be utilised by the microbes initially. In the drying and rewetting experiment on the other hand, the microbes were less active because of low organic matter availability before heating and therefore susceptible to heat stress. This allowed them to rapidly decompose substrate that became available after heating.

Although heating increased respiration rate in S4 initially, it was lower than in unheated soil later. This suggests that microbes in saline soil did not recover from the heat stress. Consequently, heating reduced cumulative respiration by 30% in S4, whereas the reduction was only about 10% in non-saline soil. This indicates that microbes exposed to salt stress were vulnerable to the additional stress imposed by heating. The reduction in respiration in saline cannot be explained by the increase in osmotic potential due to soil drying because cumulative respiration was not reduced by drying compared to constantly moist soil.

Heating increased N and P availability compared to unheated treatments with a greater increase in non-saline soil than S4. Increased nutrient availability after heating has been explained by abiotic mineralisation, microbial death, reduced microbial nutrient uptake and increased substrate availability (Bárcenas-Moreno and Bååth 2009; Prokushkin and Tokareva 2007; Seneviratne and Marschner 2019). In the non-saline soil, heating only transiently reduced respiration rates. The greater cumulative respiration from days 8 to 15 in heated soil than unheated treatments indicates strong mineralisation of substrate in the second week after heating. This was apparently accompanied by microbial N uptake as available N in heated soil was lower on day 14 than day 2. In S4, the increase in available N in heated compared to unheated soil was smaller than in non-saline treatments. Since respiration rates were low in S4, increased mineralisation after heating or release of substantial amounts of N from dead microbes are unlikely to contribute to the higher N availability. Thus, the increase in available N (and also available P) by heating in saline soil is likely due to abiotic mineralisation. The lack of change in available N over time after heating in S4 indicates that immobilisation of nutrients by microbes remained low.

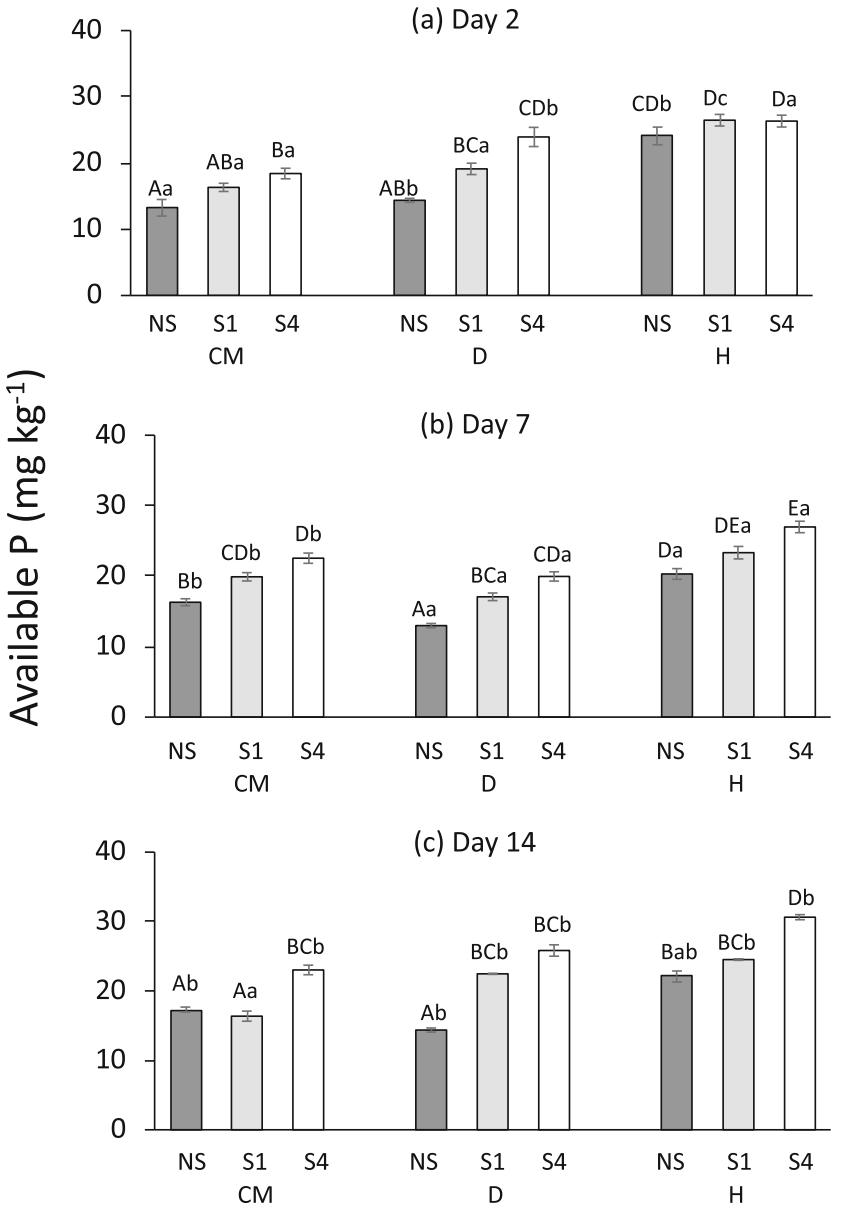

Fig. 5 Available P in constantly moist (CM), dried (D) and heated (H) treatments on days 2, 7 and 14 in nonsaline soil (NS) and saline soils with $EC_{1:5}$ 1 and 4 dS m^{-1} (S1 and S4). Different upper case letters indicate significant differences between treatments at each sampling date. Different lower case letters show significant differences in a treatment over time ($n = 4$, means ± SE, $P \leq 0.05$)

Conclusion

This study showed that the impact of a short heating event followed by rewetting is influenced by salinity, but not by prior drying and rewetting. Prior drying and rewetting likely had no effect because substrate utilisation before heating was similar as in constantly moist soil. Other prior scenarios such as multiple drying and rewetting events or longer dry periods could influence substrate availability and thus the impact of heating. High salinity which affected microbes both before and after heating influenced the impact of heating. In strongly saline soil, the heating-induced reduction of soil respiration was greater than in non-saline soil whereas the increase in available N was smaller. This suggests that microbes that are already stressed by salinity, are more susceptible to heat stress than microbes in non-saline soil. Future experiments could be carried out with more salinity levels between $EC_{1:5}$ 1 and 4 dS m^{-1} to determine this threshold. In the salinity experiment, plant residues were added to enhance microbial activity and thus the impact of heating. However, organic matter content is often low in saline soils. In the future, the effect of heating in saline soils without addition of organic materials could be investigated. To gain more detailed insights into the interactive effect of salinity or drying and rewetting with heating, additional measurements including gross N mineralisation, microbial biomass C, N and P as well as microbial community composition could be carried out. Sun et al. (2019) showed that the response to drought differed between autotrophic and heterotrophic respiration. Future experiments could investigate if they also differ in response to a short heating event.

Acknowledgements M. Seneviratne received a Turner Family postgraduate scholarship.

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Chapter 7

Conclusions and Future Research

Conclusions and future research

In this study, the effects of a short heating event to 60 °C followed by rewetting of soil on soil respiration and N and P availability were investigated as well as various factors that modulated the heating effect. In general, heating followed by rewetting increased respiration and N availability.

The experiment in Chapter 1 showed that the heating effect on respiration and nutrient availability was not only due to drying of soil. When soil was dried at 30 °C to the same water content as the heated soils, rewetting induced no or a smaller flush of respiration and nutrient availability than rewetting after heating. This indicates that heating increases substrate availability not only by rewetting-induced aggregate breakdown and microbial death but also by thermal processes such as heat-induced mineralisation.

Consistent across several experiments (Chapter 2, 4, 5), heating increased N availability due to a strong increase in ammonium N. Heating increased ammonium N between two and eight-fold whereas nitrate was generally not affected. Thus, organic N released by heating and rewetting was utilised by ammonifiers, but not subsequently converted into nitrate. This suggests that heating stimulated ammonifiers, but reduced nitrifier activity, which is consistent with the greater heating tolerance of ammonification compared to nitrification (Myers, 1975). The sustained increase in available N after heating suggests that microbial N uptake was low. Initially, because a proportion of microbes were killed by heating and rewetting, and later due to low substrate availability as indicated by low respiration rates. Heating also increased available P which was likely due to mineralisation of organic matter released by heating. Some of the available P may also be from microbial biomass because heating reduced microbial biomass P. Microbial biomass P remained low after heating which supports the conclusion that microbial nutrient uptake remained low due to limited substrate availability after the initial flush.

The effect of heating on citrate P was variable, but in general, citrate P was higher in heated than unheated soil. This is likely due to increased P mineralisation and the binding of proportion of mineralised P to soil particles in heated soil. Citrate P may also have increased after aggregate breakdown following rewetting which would expose previously occluded P binding sites.

The effect of heating on respiration differed between unamended and amended soil as shown in Chapter 3 and in the comparison of non-saline soils in the drying and rewetting experiment and the salinity experiment in Chapter 6. In the unamended soil, heating increased the respiration rate for several days and cumulative respiration was greater in heated than unheated soil. This indicates that although some microbes are killed by heating and rewetting, a large proportion survived and rapidly decomposed the organic substrates released by heating. The low respiration rate before heating suggests that most microbes were inactive and

possibly C-starved. Inactive microbes are less susceptible to stress than active ones (Schimel et al., 2007), thus a large proportion of the microbial biomass survived after heating. Further, starved microbes will rapidly decompose freshly available substrate (Berg, 2000).

However, in soil amended with pea residue shortly before heating (Chapter 3), initial respiration rates after heating and cumulative respiration were lower in heated than unheated soil. More active microbes, as indicated by high respiration rates before heating, are susceptible to stress (Scotter, 1970). Therefore it is likely that a large proportion of microbes died and the heat-induced increase in substrate availability was not used by microbes. Another possible reason for the lack of increase in respiration was that substrate availability prior to heating was high, thus microbes were not starved.

In drying and rewetting studies, the flush of respiration is often highest after the first rewetting and then decreases gradually with subsequent rewetting events (Mikha et al., 2005). Possible reasons for the lower flush in rewetting events following the first include death of microbes and smaller release of substrates because aggregate breakdown occurred mainly in the first rewetting (Wu and Brookes, 2005). The hypothesis was that this would also be the case when soil was exposed to two heating events. However, in the experiment in Chapter 4, the second heating event had similar effects on respiration and nutrient availability as first heating event. This suggests that the second heating event released similar amounts of decomposable compounds as the first heating event which were rapidly utilised by surviving microbes. These results confirm that the effect of short-term heating followed by rewetting on respiration and nutrient availability is not only due to rewetting.

The experiment in Chapter 5 showed that the presence of plants prior to heating changed the impact of heating. Compared to unplanted soil, prior plant growth resulted in a greater increase in respiration and available N and P after heating. The greater increase in respiration and available N after heating of planted soil is likely due to the higher microbial activity induced by substrate supply from roots. The increase in respiration by heating in previously planted soil is in contrast to the experiments where residues were added shortly before heating. Both growing plants and residue amendment increased microbial substrate supply. But after residue amendment, heating initially reduced respiration rates compared to unheated soil. However, the residues were added shortly before heating, inducing a strong increase in respiration rate, but there may not have been sufficient time for an increase in microbial biomass. Thus, the highly active microbes in the residue-amended soil were susceptible to heat stress. In planted soil on the other hand, microbial biomass could build up over four weeks. By the time the soil was heated, microbes were likely no longer very active because of the depletion of available substrates and thus not strongly affected by heating (Schimel et al., 2007). The large surviving microbial biomass could rapidly mineralise substrates released by heating.

Chapter 6 included two experiments which examined the effect of drying and rewetting before heating and the impact of salinity. Drying and rewetting before heating had no effect on the influence of heating. This is

probably because in the air-dried soil, the low respiration rate during the dry period was compensated by the flush of respiration after rewetting. Therefore cumulative respiration as well as N and P availability were similar to the constantly moist soil just before heating. In the second experiment, high salinity changed the effect of heating compared to unheated treatments. In the salinity experiment, pea residue was added. In agreement with Chapter 3, heating temporarily reduced respiration in the non-saline soil, but later the respiration rate increased. In contrast, heating slightly increased respiration rate one day after rewetting in the highly saline soil, followed by a gradual reduction. . Consequently, heating reduced cumulative respiration to a greater extent in the highly saline soil than in the non-saline soil. The stronger reduction of soil respiration in highly saline soil indicated that salinity exacerbated the effect of heating and rewetting, likely because of the more dramatic changes in water potential compared to non-saline soil. In agreement with the other experiments, heating increased N and P availability. However, the increase was greater in non-saline soil than highly saline soil probably because salinity reduced microbial activity and thus mineralisation of organic substrates released by heating. Another possible reason for the smaller increase in N availability in highly saline soil is that in the constantly moist treatments, available N was higher in the saline soil than non-saline soil which may be due to reduced microbial N uptake and ammonium binding to soil particles.

The experiments in this thesis revealed new information regarding the impact of a short heating event on soil respiration and nutrient availability. Future studies could investigate the mechanisms in greater detail. Examples for future studies include:

To gain more detailed information about gross N transformation processes after heating, ^{15}N ammonium and nitrate could be added before heating. Dilution of ^{15}N ammonium and nitrate could then be used to calculate gross N transformation rates (Müller et al., 2002).

The fate of nutrients added with organic amendments could investigated by adding residues labelled with ^{13}C and ^{15}N before heating (Knicker et al., 1996). Then ^{13}C in CO_2 and microbial biomass as well as ^{15}N in ammonium, nitrate and microbial biomass could be measured.

Heating is likely to change microbial community composition and function. Microbial community composition could be determined by phospholipid fatty acid analysis (Klamer and Bååth, 1998) and, in greater detail, whole genome sequencing (Fraser et al., 2000). Abundance of genes involved in different nutrient transformation processes could be determined by quantitative PCR (Arya et al., 2005).

In this study, the effect of heating to 60 °C was studied. However, in rapidly moving grass fires, temperatures can range from 50 to 100 °C (Scotter, 1970). The effect of short heating events to other temperatures within this range could be investigated. Higher temperatures may result in greater microbial death and slower recovery of microbial activity after heating.

In the experiments in this study, soils were immediately rewet after cooling. In the field, soils may remain dry for extended periods after fire. Future studies could investigate how the length of time between heating and rewetting influences the impact of heating on soil respiration and nutrient availability.

The experiments in the thesis were laboratory studies. To assess if the results can be transferred to the field, a field experiment in a pasture could be conducted. Plots would be burned or left unburnt and rewet, then soil respiration could be measured in-situ and soil samples taken to measure soil available N and P. Temperature probes should be inserted into the soil at 5 cm to record the temperature during the fire to be able to relate the observed effects to specific temperatures.

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