



# Effects of wetting frequency and afforestation on carbon, nitrogen and the microbial community in soil



M. Hoogmoed<sup>a,1,\*</sup>, S.C. Cunningham<sup>b,c</sup>, P.J. Baker<sup>a,d</sup>, J. Beringer<sup>e,f</sup>, T.R. Cavagnaro<sup>g</sup>

<sup>a</sup> School of Biological Sciences, Monash University, 3800 VIC, Australia

<sup>b</sup> Centre for Integrative Ecology, Deakin University, Burwood, 3125 VIC, Australia

<sup>c</sup> Institute for Applied Ecology, University of Canberra, Canberra, 2601 ACT, Australia

<sup>d</sup> School of Ecosystem and Forest Sciences, University of Melbourne, Richmond, 3121 VIC, Australia

<sup>e</sup> School of Geography and Environmental Science, Monash University, 3800 VIC, Australia

<sup>f</sup> School of Earth and Environment, University of Western Australia, Crawley, 6009 WA, Australia

<sup>g</sup> School of Agriculture, Food and Wine, University of Adelaide, Waite Campus, Urrbrae, 5064 SA, Australia

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## ABSTRACT

Afforestation of agricultural land is increasing, partly because it is an important biological method for reducing the concentration of atmospheric CO<sub>2</sub> and potentially mitigating climate change. Rainfall patterns are changing and prolonged dry periods are predicted for many regions of the world, including southern Australia. To accurately predict land-use change potential for mitigating climate change, we need to have a better understanding of how changes in land-use (i.e. afforestation of pastures) may change the soils response to prolonged dry periods. We present results of an incubation study characterising C and N dynamics and the microbial community composition in soil collected from two tree plantings and their adjacent pastures under a baseline and reduced frequency. While the concentration of soil C was similar in pasture and tree planting soils, heterotrophic respiration was significantly lower in soil from pastures than tree plantings. Although there was little difference in the composition of the soil microbial community among any of the soils or treatments, differences in N cycling could indicate a difference in microbial activity, which may explain the differences in heterotrophic respiration between pastures and tree plantings. Soils from pastures and tree plantings responded similarly to a reduction in wetting frequency, with a decrease in microbial biomass (measured as total PLFA), and a similar reduction in heterotrophic respiration from the soil. This suggests that the responses to changes in future wetting cycles may be less dependent on land-use type than expected.

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## 1. Introduction

Afforestation of agricultural land is increasingly occurring for several reasons. For example, it is recognised as an important way of reducing levels of atmospheric CO<sub>2</sub> to mitigate climate change (IPCC, 2007), or it can reduce soil erosion (e.g. Chirino et al., 2006). Globally forests store large amounts of carbon (C) in their soil, biomass and litter and deadwood (373, 383 and 116 Pg, respectively, Pan et al., 2011). Mixed-species tree plantings are increasingly established because they have additional ecological

benefits over single-species tree plantations, such as habitat restoration and increasing biodiversity (Cunningham et al., 2015). In Australia, plantings of mixed native species (termed 'environmental plantings') accounted for up to 20% of the 1.14 Mha of afforestation between 1990 and 2012 (Paul et al., 2015). Soil moisture dynamics are a key factor affecting the sequestration of C in soils (e.g., Austin et al., 2004). Precipitation patterns are predicted to change under future climate projections (IPCC, 2007). In Australia, dry periods are predicted to increase (Whetton et al., 2015), affecting the wetting frequency of soil. In turn, this will alter C and nitrogen (N) cycling in the soil (e.g., Borken and Matzner, 2009) and may affect the potential of afforestation to increase C sequestration in soils.

Soil C cycling is affected predominantly by moisture (Borken and Matzner, 2009), temperature (e.g. Frey et al., 2008), and the quantity and quality (e.g. C:N ratio, lignin content, etc) of organic matter (e.g. Bending et al., 2002), through their impact on the

\* Corresponding author at: PIRSA-SARDI, 11a Hartley Grove, Urrbrae, 5064 SA, Australia.

E-mail address: [Marianne.hoogmoed@sa.gov.au](mailto:Marianne.hoogmoed@sa.gov.au) (M. Hoogmoed).

<sup>1</sup> Present address: South Australian Research and Development Institute, Waite Campus, Urrbrae, 5064 SA, Australia.

activity and composition of the soil microbial community. Wetting regimes (frequency and amount of rainfall) strongly affect the activity and mortality of the microbial population (Fierer et al., 2003; Borken and Matzner, 2009). When the soil dries, microbes produce osmolytes in order to reduce their internal water potential and prevent dehydration (Halverson et al., 2000). Soil microbes that are less adapted to water stress may die over the dry period. When the soil is rewetted, the surviving microbes rapidly excrete the osmolytes, before water enters the cells by osmosis and causes the microbial cells to rupture (Fierer and Schimel, 2003). A pulse in CO<sub>2</sub> emission after rewetting of dry soil, is caused by rapid mineralization of the excreted osmolytes in the soil, as well as mineralization of microbial biomass from microbes that died during the dry period or re-wetting of the soil (Steenwerth et al., 2005; Sponseller, 2007). The magnitude of this CO<sub>2</sub> pulse is dependent on the specific microbial community (e.g. bacterial vs fungal dominated) and how it responds to wetting and drying cycles (Aanderud and Lennon, 2011). In addition, swelling and shrinking of the soil may breakdown aggregates, which exposes organic material that was previously protected from microbial decomposition or chemical oxidation (van Gestel et al., 1991), which may contribute to the observed pulse of CO<sub>2</sub> after re-wetting of soil (Fierer and Schimel, 2003).

Afforestation of agricultural land can alter the biomass, activity and composition of soil microbes (e.g., Singh et al., 2007; Carson et al., 2010). For example, the soil microbial communities of afforested soils and mature forests typically contain larger fungal biomass compared with pastures. This difference is attributed to fungi decomposing woody material quicker than bacteria (e.g., Fierer et al., 2009; Macdonald et al., 2009) and forest ecosystems generally experience lower levels of soil disturbance, favouring fungi over bacteria (Six et al., 2006). Furthermore, fungi are thought to sequester more C than bacteria, due to the higher C use efficiency of fungi (Bailey et al., 2002; Jastrow et al., 2007) and are thought to be less sensitive to drought stress than bacteria (Schimel et al., 2007; Blazewicz et al., 2014). Whether a drier climate leads to less C sequestration after afforestation of pastures has yet to be determined.

A meta-analysis on the effect of different types of land-use change on soil C sequestration showed a trend of higher soil C sequestration after afforestation in low rainfall areas compared with high rainfall area's (Guo and Gifford, 2002). In addition, a study investigating woody plant invasion along a precipitation gradient also showed that dryer sites showed an increase in soil organic carbon, while wetter sites lost soil organic carbon after woody plant invasion (Jackson et al., 2002). A doubling of the length of a dry-period was found to reduce soil respiration flux after re-wetting by approximately 17% (Fay et al., 2000). Because of the changes in quantity and quality of organic matter inputs into the soil after afforestation of pastures, and consequently changes in soil microbial communities, soil from pastures and adjacent tree plantings may respond differently to wetting and drying cycles (Gordon et al., 2008; Zhao et al., 2010). While afforestation effects on soil C sequestration is widely studied, research on (interactive) soil responses to both prolonged dry periods and land-use change (afforestation of pastures) are rare (e.g., Fierer and Schimel, 2003).

Given that drying climates are predicted for many regions of the world, including southern Australia, we need to have a better understanding of how prolonged dry periods may affect C sequestration and the degree to which the response is dependent on the land-use context: do soils in pastures and tree plantings respond differently? Here, we present results of an incubation study that aimed to answer the question: "Are the effects of an increase in the time between wetting events on C sequestration and soil respiration context-dependent? And, specifically, do

different land-use types exhibit different responses? We measured soil respiration during a 100-day laboratory-based incubation study in which soils collected from tree plantings and their adjacent pastures at two farms were subjected to two different wetting frequencies. We also characterize soil microbial community composition and C and N dynamics.

Fungal biomass was expected to be relatively higher in the afforested pastures than pastures. Consequently, we expected lower heterotrophic respiration in soils from tree plantings than the pastures due to the larger mass of low quality (high C:N) inputs and the higher C use efficiency of fungi than bacteria (Bailey et al., 2002; Jastrow et al., 2007). Finally, because of the lower sensitivity of fungi to wetting and drying cycles than bacteria (Schimel et al., 2007; Blazewicz et al., 2014) we hypothesize that there will be a (relatively) greater reduction in respiration with reduced wetting frequency in pasture soils than in afforested soils.

## 2. Materials and methods

### 2.1. Soil collection

Soil was collected for the incubation experiment in the austral winter (June 2012), from two mixed-species, restoration tree plantings and their adjacent pastures. All sites were riparian, with the pasture plots immediately downstream of the tree planting (Fig. 1). The sites were located around Benalla (−36.74°S, 145.99°E and −36.70°S, 145.88°E), Victoria, Australia. Tree plantings were established in 1994 and 1990 (i.e. this study was conducted 18 and 22 years after planting) respectively by tubestock, and both were dominated by the tree species *Eucalyptus camaldulensis* Dehnh. and *Acacia melanoxylon* R.Br. No additional fertilizers were applied during or after establishment. The plantings were fenced and stock kept out. The pastures were cleared at least by 1860, and covered in native grassy woodlands before that. Soils were sandy loam sodosols (Australian soil classification) with pHs of  $5.3 \pm 0.09$  in the 18-year-old planting and  $4.9 \pm 0.08$  in the adjacent pasture, and  $4.5 \pm 0.1$  in the 22-year-old planting and  $4.1 \pm 0.5$  in the adjacent pasture. The climate across the region is temperate with seasonal changes in mean monthly maximum temperature ( $12.3\text{--}29.7^\circ\text{C}$ ) and minimum temperature ( $4.1\text{--}15.3^\circ\text{C}$ ), and an annual precipitation of 650 mm that is winter-dominant, ranging from 30 to 80 mm month<sup>−1</sup> (Bureau of Meteorology, 2013).

A 20 m × 10 m plot was established in each pasture and tree planting, with the pasture and adjacent tree planting plots ca 50–100 m apart at each farm. Ten soil samples of approx. 500 g were taken randomly within each plot, with samples at least 1 m away from the stems of trees within the plantings (Fig. 1). Samples were taken using a trowel, after removing any vegetation and litter (litter layer was not well developed, some dry leaves and twigs), from the top 10 cm of the soil, which is the layer of highest microbial activity in these soils (Cavagnaro, unpublished). The soil was stored immediately at 4 °C in a portable refrigerator for 2 days until further processing in the laboratory.

### 2.2. Incubation pre-treatment

Soil samples were coarsely sieved (5 mm mesh) to remove large pieces of organic matter and stones. Soil moisture was determined from the proportional difference in mass of subsamples of approx. 10 g of field-moist soil before and after drying at 105 °C for 48 h until a constant weight. Each soil sample (10 replicates per land-use at a farm, referred to as a 'soil core' in the statistical model, see below) was then each divided over three 250 cm<sup>3</sup> jars, so that each contained approx. 100 g dry weight equivalent soil (Fig. 1). The soil was packed in the jar to a bulk density of 1 g cm<sup>−3</sup>, which is similar to bulk densities at the sites, giving a headspace of approx. 150 cm<sup>3</sup>.

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