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Pastures to woodlands: changes in soil microbial communities and carbon following reforestation

T.R. Cavagnaro[a,*], S.C. Cunningham[b,c], S. Fitzpatrick[d]

[a] The Waite Research Institute and The School of Agriculture, Food and Wine, University of Adelaide, Waite Campus, PMB1 Glen Osmond, SA, 5064, Australia
[b] Centre for Integrative Ecology, School of Life and Environmental Sciences, Deakin University, Burwood, VIC 3125, Australia
[c] Institute for Applied Ecology, University of Canberra, Bruce, ACT 2617, Australia
[d] School of Biological Sciences, Monash University, Clayton, VIC 3800, Australia

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ABSTRACT

Reforestation of agricultural lands has the potential to sequester C, while providing other environmental benefits. It is well established that reforestation can have a profound impact on soil physicochemical properties but the associated changes to soil microbial communities are poorly understood. Therefore, the objective of this study was to quantify changes in soil physicochemical properties and microbial communities in soils collected from reforested pastures and compare then to remnant vegetation and un-reforested pastures. To address this aim, we collected soil from two locations (pasture and its adjacent reforested zone, or pasture and its adjacent remnant vegetation) on each of ten separate farms that covered the range of planting ages (0–30 years and remnant vegetation) in a temperate region of southeastern Australia. Soils were analysed for a range of physicochemical properties (including C and nutrients), and microbial biomass and community composition (PLFA profiles). Soil C:N ratios increased with age of tree planting, and soil C concentration was highest in the remnant woodlands. Reforestation had no clear impact on soil microbial biomass or fungal:bacterial ratios (based on PLFA's). Reforestation was associated with significant changes in the molecular composition of the soil microbial community at many farms but similar changes were found within a pasture. These results indicate that reforestation of pastures can result in changes in soil properties within a few decades, but that soil microbial community composition can vary as much spatially within pastures as it does after reforestation.
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1. Introduction

Carbon sequestration in vegetation and soils has substantial potential to help mitigate further climate change. Reforestation of pastures is an important means of sequestering C in the soil (Hoogmoed et al., 2012; IPCC, 2013). Reforestation can provide other environmental benefits, such as the provision of habitat for native flora and fauna, increasing habitat connectivity, and reducing non-point source pollution from agriculture (Cunningham et al., 2015b). For this reason, reforestation of marginal agricultural land is seen as an important form of land-use change (Mackey et al., 2013).

In addition to increasing soil C levels, reforestation can change the chemical nature of C inputs into the soil (de Alcântara et al., 1996; Smith et al., 2012). Trees being long-lived perennial plants typically produce nutrient poor and resistant to decomposition tissues, whereas agricultural plants typically allocate most of their C to photosynthetically active, high nutrient and readily decomposed tissues (Aerts and Chapin, 2000). This can have important implications for soil C cycling, as the residence time of C in the soil is linked closely to its chemical nature and its accessibility to microbes (Conte et al., 2010; de Alcântara et al., 1996; Smernik and Oades, 2001). Additionally, the cycling of C in soil is determined to some degree by its C:N ratio and management (e.g. Giardina et al., 2000). For example, an increase in soil C:N ratio is often associated with the conversion from pasture to woodland, due to increased C:N ratio of the litter inputs, and reduced disturbance and fertiliser inputs (Hoogmoed et al., 2014; Hoogmoed et al., 2012; Ussiri et al., 2006).

Reforestation can change physicochemical properties of the soil (see Cunningham et al., 2015b; and references therein). For example, soil nutrient levels (especially N) often decrease following reforestation due to cessation of fertiliser addition, reduced levels of biological N fixation associated with leguminous species

* Corresponding author.
E-mail address: timothy.cavagnaro@adelaide.edu.au (T.R. Cavagnaro).

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and increased nutrient immobilisation (Garten and Ashwood, 2002; Hooker and Compton, 2003). However, increases in soil nutrients (both N and P) have been reported following reforestation of highly-degraded soils (Jiao et al., 2012) and centuries after reforestation (Wilson et al., 1997). Removal of livestock associated with reforestation can change soil physicochemical properties due to reduced levels of nutrient redistribution and grazing effects on plant-soil feedbacks (Holland and Detling, 1990; Semmartin et al., 2008). These changes in soil properties may have significant effects on soil biotic communities, including those that regulate the cycling of C and nutrients in soils (Bardgett and Wardle, 2010; De Deyn et al., 2008; Ng et al., 2014b).

The biomass, activity and diversity of soil microbial communities is affected strongly by changes in soil physicochemical properties (Bossio and Scow, 1998; Ng et al., 2014b), with most of this information coming from agricultural systems. In contrast, few insights have been gained about how soil microbial communities respond to reforestation. Soil microbial communities can differ between forested (plantations and native woodlands) and agricultural lands (Bossio et al., 2005; Singh et al., 2007), among different types of agriculture (Drenovsky et al., 2010), and within a few years among different methods of revegetating agricultural lands (Hedlund, 2002). However, how reforestation of pastures with mixed-species, affects soil microbial communities remains largely unknown.

Despite the tremendous complexity of soil microbial communities, predictions can be made about how different groups of soil microbes, such as fungi and bacteria, will respond to revegetation. For example, following reforestation and afforestation (i.e. planting trees on areas that were historically treeless) of agricultural lands, soil C:N ratios generally increase (Berthrong et al., 2009; Cavagnaro, 2016; Mackay et al., 2016), which is likely to cause a shift from bacterial to fungal dominance in soil communities (Busse et al., 2009; Fierer et al., 2009; Högberg et al., 2007). Given that soil communities play an important role in soil C and nutrient cycling (Bardgett and Wardle, 2010; De Deyn et al., 2008; Ng et al., 2014b), it is valuable to determine how reforestation alters the microbial composition of soils.

Here, we quantify changes in the microbial community and soil physicochemical properties following the conversion of pastures to mixed-species plantings dominated by species belonging to the genera *Eucalyptus* L'Hér. and *Acacia* Mill. We selected mixed-species plantings because they are planted increasingly instead of single-species plantings, and their higher above-ground biodiversity potential. We hypothesized that with time, the soil physicochemical properties and microbial community composition of tree plantings would become increasingly divergent from that of the adjacent pasture. To test this hypothesis, we surveyed a replicated chronosquence of sites ranging from treeless pastures through to remnant woodlands on ten farms in a temperate region of southeastern Australia. In order to account for differences in soil properties among farms, at each farm we sampled soils from both the reforested or remnant vegetation zones and an adjacent unreforested pasture.

2. Materials and methods

2.1. Study area and design

This study focused on tree plantings on formerly-grazed pastures in northern Victoria, Australia (Table 1). Prior to European settlement in the 1840s, the region was dominated by *Eucalyptus* woodlands (10–30 m tall, 10–30% projective foliage cover (i.e. percentage of the sky blocked out by leaves and stems), Specht, 1981) with grassy understoreys. Since European settlement the land has been cleared extensively and converted predominantly to dryland cropping and pasture-based grazing systems. Consequently, this region offers substantial opportunities for reforestation. The region has a temperate climate with seasonal changes in mean monthly maximum temperature (12.8–31.0 °C) and minimum temperature (3.2–14.9 °C), and a winter-dominant annual precipitation of 500–700 mm year^{-1} (Table 1).

This study involved a survey of ten grazing farms that were selected to cover a representative range of time since reforestation (Table 1). At each of the 10 farms two sites were established, one of which was a 'reference pasture site' and the other was a 'treatment site' (Fig. 1). The two sites on each farm were located 50 m apart from one another, but were in the same topographic position and on the same soil type (see below), and had the same management prior to re-forestation. The treatment sites were of the following classes: reforested patches, remnant woodland patches, or pastures. The reforested sites were planted with Trees 10, 18 or 30 years prior to sampling (i.e. there were two farms per age class) and were included to provide an indication of changes in soil properties with time since tree planting. The remnant sites were included to represent a potential trajectory for plantings at maturity (two farms). The reference pasture –pasture comparison (two farms) was included to provide a temporal reference without reforestation (0 years) for soil properties, and a spatial reference for the variability of soil properties across a field. This paired design allowed us to assess changes in soil properties under various stages of reforestation (i.e. treatment sites) relative to a conventional pasture management scenario (i.e. reference pasture sites). It also allowed us to partially account for differences among farms due to variation in land-use history and local soil properties.

The treatment sites on each farm included the whole tree planting or patch of remnant vegetation (approx. 2 ha), with an equivalent area sampled in the adjacent reference pastures. The

Table 1
Environmental characteristics of the survey sites from the ten farms.

Farm	Land Use	Age (yr)[a]	Latitude (°S)	Longitude (°E)	Rainfall (mm yr^{-1})	Max temp. (°C)	Elevation (m)	Landform	Soil Texture	Basal area (m^2 ha^{-1})	Tree density (trees ha^{-1})
1	Pasture	0	36.65	145.58	581	21.3	150	plain	sandy loam	0	0
2	Pasture	0	36.39	145.95	563	22.0	225	gentle slope	sandy loam	0	0
3	Planting	9	36.46	145.77	556	21.8	145	plain	sandy loam	3.6	456
4	Planting	10	36.50	146.13	629	21.6	180	gentle slope	sandy loam	7.4	474
5	Planting	17	36.00	145.91	487	22.5	120	plain	clay loam	39.3	604
6	Planting	18	36.58	146.11	684	20.6	240	gentle slope	sandy loam	9.9	493
7	Planting	30	36.53	145.75	581	21.6	175	gentle slope	sandy loam	9.7	389
8	Planting	31	36.17	146.95	510	22.2	190	plain	sandy loam	39.1	581
9	Remnant	na	36.58	145.62	580	21.3	160	gentle slope	sandy loam	13.5	342
10	Remnant	na	36.68	145.03	566	20.9	140	gentle slope	loam	10.2	263

[a] Age = years since planting. Age for the pastures was zero as they were not reforested and was unknown (na) for the remnant woodlands (see text).

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