Title

Riparian reforestation: are there changes in soil carbon and soil microbial communities?

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Key Words:

C sequestration; Soil ecology; Phospholipid fatty acids (PLFA); Fungi to bacteria ratio.

Abstract

Reforestation of pastures in riparian zones has the potential to decrease nutrient runoff into waterways, provide both terrestrial and aquatic habitat, and help mitigate climate change by sequestering carbon (C). Soil microbes can play an important role in the soil C cycle, but are rarely investigated in studies on C sequestration. We surveyed a chronosequence (0-23 yr) of mixed-species plantings in riparian zones to investigate belowground (chemical and biological) responses to reforestation. For each planting, an adjacent pasture was surveyed to account for differences in soil type and land-use history among plantings. Two remnant woodlands were included in the survey as indicators of future potential of plantings. Both remnant woodlands had significantly higher soil organic C (SOC) content compared with their adjacent pastures. However, there was no clear trend in SOC content among plantings with time since reforestation. The substantial variability in SOC sequestration among plantings was possibly driven by differences in soil moisture among plantings and the inherent variability of SOC content among reference pastures adjacent to plantings. Soil microbial phospholipid fatty acids (PLFA, an indicator of microbial biomass) and activities of decomposition enzymes (β-glucosidase and polyphenol oxidase) did not show a clear trend with increasing planting age. Despite this, there were positive correlations between total SOC concentration and microbial indicators (total PLFA, fungal PLFA, bacterial PLFA and activities of decomposition enzymes) across all sites. The soil microbial community compositions (explored using PLFA markers) of older plantings were similar to those of remnant woodlands. There was a positive correlation between the soil carbon:nitrogen (C:N) and fungal:bacterial (F:B) ratios. These data indicate that in order to maximise SOC sequestration, we need to take into account not only C inputs, but the microbial processes that

regulate SOC cycling as well.

1. Introduction

Reforestation is a recognised means to sequester carbon (C, IPPC 2000) and potentially mitigate climate change (Canadell and Raupach, 2008). Consequently, reforestation of marginal agricultural land, particularly pastures, is becoming more economically viable (Polglase et al., 2013). Mixed-species woody plantings on pastures are increasingly common in many regions of the world, not only for sequestering C, but to increase habitat for native flora and fauna, and improve water quality (Cunningham et al., 2015a). While aboveground C sequestration following reforestation is well understood, both in terms of the amounts of C sequestered and the processes involved, this is not the case for belowground C sequestration (Guo and Gifford, 2002). Although the amount and rate of soil C sequestration is known to be influenced by factors such as climate, soil type, forest type and previous land use (Guo and Gifford, 2002). It is difficult to predict how much (if any) C will be stored in soil following reforestation of agricultural land (Paul et al., 2002).

Comparisons between pastures and nearby remnant forests provide an indication of the potential of reforestation to sequester SOC. For example, pasture soils have been found to have higher soil C content compared with forest soils in North and South America (Brown and Lugo, 1990; Franzluebbers et al., 2000). Similar results have been observed in New Zealand systems where higher soil C content in pastures was related to higher C inputs and higher recalcitrance of C in pasture soils compared with montane beech forest soils in New Zealand (Tate et al., 2000). Conversely, whereas remnant woodlands in temperate, south-eastern Australia can have higher soil C content than their adjacent pastures (Hoogmoed et al., 2012), no significant differences in soil C between developing (<30 yr) plantings and pastures have been observed (e.g. Cunningham et al., 2015b; Hoogmoed et al., 2012). Together, these examples highlight the need for region and context specific (e.g. landscape position, soil type, etc) investigations into soil C sequestration.

Most research on soil C following reforestation of pastures has been conducted on upland (i.e. non-riparian) systems (Laganière et al., 2010). Given that soil C sequestration increases with increasing soil moisture and primary productivity (Post et al., 1982), it is plausible that riparian plantings can sequester soil C faster than those on uplands. In addition, riparian plantings can act as buffer strips between agricultural lands and waterways, improving water quality (e.g. Osborne and Kovacic, 1993). Burger et al. (2010) found that soil C concentrations (not stocks) in restored riparian zones 6-12 years post-restoration were intermediate to those of riparian zones that were un-restored or were in remnant conditions. Conversely, working in the same region Cunningham et al. (2015b) found that riparian plantings had similar rates of soil C sequestration to upland plantings, when both were standardised for local variation in soil C stocks using their adjacent upland pastures. There has yet to be a direct investigation of soil C sequestration in riparian plantings where local variation in soil C stocks has been standardised for using pastures in the riparian zone (i.e. rather than an upland comparison).

Reforestation alters the form of plant inputs to the soil (Smith et al., 2012), which could have a profound effect on soil microbial communities utilising this resource. For example, wooded lands can support distinctly different microbial communities compared with agricultural lands (Bossio et al., 2005; Singh et al., 2007), as can reforested sites compared with pastures (Cavagnaro, 2016). More specifically, the fungal:bacterial (F:B) ratio of the soil microbial community can be correlated positively with the carbon:nitrogen (C:N) ratio of the soil (Fierer et al., 2009; Högberg et al., 2007), which often increases following reforestation (Cunningham et al., 2015b; Macdonald et al., 2009). An increase in the C:N ratio of soil can be driven by an increase in soil C and/or a decrease in soil N, both of which may occur following reforestation of grazing pastures (Cunningham et al., 2012).

Given the importance of soil microbes in the C cycle (Bardgett and Wardle, 2010; De Deyn et al., 2008), it is important to determine how soil communities respond to reforestation. Soil microbes play an important role in the breakdown of litter and the incorporation of litter C into soil C (Attiwill and Adams, 1993). Due to the chemical complexity of litter, a wide range of microbial enzymes are involved in its decomposition. For example, β-glucosidase catalyses the final steps of degradation of cellulose into glucose (Sinsabaugh, 2010) whereas polyphenol oxidases are extracellular enzymes that mediate lignin degradation (Sinsabaugh, 2010). Thus, quantification of the activity of these enzymes provides mechanistic and functional knowledge of litter decomposition, which is currently lacking for reforestation.

Here, we surveyed paired plantings and pastures to determine the effect of riparian plantings on soil physicochemical and biological properties. It was hypothesised that following reforestation: (1) soil C:N would increase due to an increase in soil C and a decrease in soil N, (2) soil microbial biomass and activity would increase and would correlate with soil C and (3) the microbial community composition would change, with an increase in fungi and a correlation between F:B and C:N.

2. Materials and Methods

2.1 Study area and site description

Tree plantings in the riparian zones of grazed pastures were surveyed in the Goulburn Broken catchment in northern Victoria, Australia (37°S 146°E). This region has been cleared extensively since the 1840s and converted to cropping and pasture-based grazing systems, although many individual trees and patches of trees remain, particularly along creeks and roadsides. Historically, the plains of this catchment were mainly covered in woodlands (10-30 m tall, 10-30% projective foliage cover, Specht, 1981) dominated by *Eucalyptus* species with grassy understoreys. The climate across the region is temperate 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 668 mm yr^{-1} (Benalla weather station; BOM 2015).

Ten sites, each on a different creek, were surveyed to explore the impact of land-use change on soil nutrients and microbes (Table 1). At each site, two 100 m transects were established parallel to the creek: a treatment transect (planting, pasture or remnant woodland, see below) and a reference transect (pasture, Figure 1). This paired sampling design is a commonly used approach for estimating soil organic C (SOC) sequestration after reforestation (e.g. Paul et al 2002). Reference transects were sampled to standardise for potential differences in soil characteristics and disturbance histories among sites. Measurements at the reference transects were used as an indicator of conditions if the plantings had not been established and not as an estimate of conditions prior to planting. To minimise differences in soil type and the influence of adjacent land uses, the reference transects were at least 100 m from the treatment transects, but no more than 1 km away, in the same topographic position as the treatment transects, and beyond the crown of any remnant trees. Further, the distance of transects from creeks were consistent within sites but

varied among sites (1-20 m away) due to the constraints of narrower plantings, the need to avoid remnant trees and steep terrain.

Treatment transects were either in plantings, remnant woodlands or pastures. All plantings were planted with a mixture of species native to the region, predominantly *Eucalyptus* and *Acacia* species. The plantings were established by fencing out grazing, ripping the soil into furrows and hand planting tubestock seedlings into the furrows. Tree plantings underwent no further active management but at some sites after 5 yr the plantings were grazed briefly during the summer of dry years. Tree plantings included three age classes: 9-10 yr, 14 yr and 23 yr (see Table 1), which were representative of the current age of plantings in the region. Two remnant woodlands were included as a potential trajectory for plantings at maturity. While the exact age of the remnant woodlands was unknown, it is estimated that they were at least 100 yr. These remnant woodlands were some of the most mature native woodlands in the region, representative of the mono-dominant floodplain woodlands of *Eucalyptus camaldulensis* that occur along creeks of the region but with negligible native understorey or groundcover. Finally, two pasture sites were included to provide a time zero (0 yr) point and a measure of the differences in soil properties over small distances without land-use change. At these sites, both the treatment transect and the reference transect were located in pastures.

2.2 Sample collection

Fieldwork was completed during the Austral autumn in May, 2013. Four 10 m × 4 m plots were established randomly along each transect (Figure 1b). In each of these plots, four 25 cm × 25 cm quadrats were placed at randomly-located sampling points. Digital photographs were taken of the canopy directly above the quadrat for visual quantification of percentage cover of canopy. All leaf litter was collected from within the quadrat. Following this, one soil sample was collected within each quadrat from the 0-5 cm soil layer, where microbial activity is generally highest (Bolton Jr et al., 1993). This sampling intensity (i.e. samples per unit area)

has been shown to provide a representative sample of SOC in this region (Cunningham et al., 2012). Samples were taken by gently tapping a stainless steel ring (7 cm diameter) into the ground in the centre of the quadrat to a depth of 5 cm and collecting the soil within the ring. The four surface samples of soil within each plot were bulked and mixed carefully in the field to create one soil sample per plot. Each composite sample was then stored immediately at 4 ^{o}C in a portable car-refrigerator. The same soil samples were used for bulk density measurement (see below).

2.3 Soil analysis

In the laboratory, all soil samples were sieved to < 2 mm to remove stones, course roots and debris, and macroinvertebrates. Stones were retained to estimate stone volumes in each sample using displacement of water in a measuring cylinder. After sieving, a subsample from each sample was frozen at -20 ^{o}C. From the remaining soil, gravimetric moisture content was determined from the difference in mass after drying approx. 10 g of moist soil at 105 ^{o}C for 48 h, and from this the total dry soil mass for each composite sample was determined. Bulk density was determined by dividing the dry soil mass by the sample volume (i.e. four rings) minus the stone volume, following Minoshima et al. (2007). Leaf litter was dried in an oven at 60 ^{o}C until a constant mass was measured.

Duplicate soil samples (10 g fresh soil) were extracted with 2M KCl, and inorganic N content determined colorimetrically using a modification of the method for NO_3^--N (plus NO_2^--N) reported in Miranda et al. (2001) and the method for NH_4^+-N in (Forster, 1995). For each soil sample, potentially mineralizable N (PMN) was determined by anaerobic incubation for 7 days (Waring and Bremner, 1964). Plant available P was determined using the Colwell method (Colwell, 1963). Total SOC and total N were determined using a LECO IR Analyser, and the permanganate oxidisable C (POxC) pool was determined following the method of Blair et al. (1995). The pH of soil was determined on a 1:5 soil:water extract.

Phospholipid fatty acids (PLFAs) were extracted from the soil to determine total PLFA, fungal PLFA, bacterial PLFA and a more detailed microbial community composition. Presence of PLFAs was estimated from the frozen soil sub-samples, following the methods of (Bossio and Scow, 1998), with slight modification (Ng et al., 2014). Briefly, PLFAs were extracted from 4 g of freeze-dried and finely ground soil samples, using a solvent containing citrate buffer (0.15 M, pH 4.0), chloroform and methanol, followed by transesterification of the polar lipid fraction containing the phospholipids. Individual PLFAs were separated using gas chromatography (5% Phenyl / 95% Dimethyl Polysiloxane column Agilent 6850). Peaks were identified and quantified by comparison with Supelco Bacterial Acid Methyl Ester (BAME) standard mix (product number 47080-U, Supelco, USA). Nomenclature of PLFAs followed that described by (Frostegard and Baath, 1996), with the fatty acids i15:0, a15:0, 15:0, i16:0, 16:1ω7, i17:0, a17:0, 17:0cy and 17:0 chosen as bacterial biomarkers and linoleic acid (18:2ω6,9) chosen as a fungal biomarker.

The activity of the enzymes β-glucosidase and polyphenol oxidase was determined from air-dried soil samples (10 g). Enzymes were assayed colorimetrically following a modification of the method reported by Allison and Jastrow (2006), as in Ng et al. (2014).

2.4 Data analysis

Data were explored to determine differences in C, N, C:N, microbial biomass, microbial enzyme activity and F:B among age classes. Soil C and N data were converted to content (i.e. mass per unit area) using the mean bulk density from the appropriate transects whereas biological data were analysed as concentrations. As our focus was changes in soil variables following reforestation, we calculated the mean change in properties at each site by subtracting the reference transect mean (i.e. the mean of the four plots in the reference transect) from the adjacent treatment transect mean. Comparisons among age classes were

analysed using one-way ANOVA. That is, age class was the fixed factor in the model, and for each age class (0 yr, 9-10 yr, 14 yr, 23 yr and remnant) there were two replicate sites.

As there were no significant differences in soil properties among planting age classes (see results), difference between land uses within sites were explored. *T*-tests were conducted to determine if the mean value of a soil variable at a treatment transect was significantly different ($P < 0.05$) to that at its adjacent reference transect ($N = 4$, i.e. four plots in each transect). Pearson's correlations were performed using plot level data to explore relationships between concentrations of soil physicochemical (total SOC, POxC, C:N and soil moisture) and biological (total PLFA, total bacteria, total fungi, F:B and enzyme activity) variables ($N = 80$, i.e. 10 sites × 2 transects × 4 plots).

Multivariate analyses were used to examine differences in microbial community composition (PLFAs) among transects. PLFA concentration values were range standardised [(x - minimum) / (maximum – minimum)] to avoid analyses being dominated by abundant PLFAs. Compositional differences among transects were estimated using the Bray-Curtis dissimilarity index (Bray and Curtis, 1957), which is known not to distort environmental patterns like many other distance measures (Faith et al 1987). Analysis of Similarity (ANOSIM, Clarke and Green, 1988) was used to determine if microbial composition was significantly dissimilar ($P > 0.05$) among transects ($N = 4$). We used ANOSIM to determine if there were differences in the microbial community composition: a) among the reference transects; and b) between the treatment transect and the adjacent reference transect at each site. There were insufficient replicates for informative comparisons of age class. Compositional differences among transects were visualised with non-metric multidimensional scaling (NMDS), creating an ordination from the dissimilarity values among transects. All analyses were performed in R, except for the NMDS plot which was created using Systat 10.

3. Results

3.1 Soil carbon and nitrogen

C:N ratios varied over a small range (10.5 – 14.8) across the sites (Table 2). The C:N ratio of the soil showed no significant differences among age classes (F = 0.61, P = 0.67). However, when sites were considered individually, both remnant woodland sites had significantly higher C:N ratios in their treatment transect compared with their reference transect (Table 1).

There was a large amount of variability in the difference in SOC within some age classes (especially the 23 yr age class, Figure 2a). Despite this variability, the difference in SOC between treatment transects and reference transects was close to zero for the 0 yr age class, negative in the 9-10 yr age class, and positive in all other age classes, with a trend (P = 0.1) for increasing SOC with age (Figure 2a). Both remnant woodlands sites had significantly higher SOC content in their treatment transects than their reference transects (Table 1). The difference in SOC content between treatment transects and reference transects was correlated negatively with the SOC content in reference transects (R = -0.69, P = 0.03). Soil organic C concentration was correlated strongly with soil N (R = 0.90, P < 0.001) and weakly with soil moisture content (R = 0.23, P = 0.04).

There were no significant differences in soil N among age classes (F = 2.12, P = 0.22). A 14 yr site, a 23 yr site and a remnant site were the only sites to have significantly more soil N in their treatment transect than their reference transect (Table 1). There was substantial variability among transects in NO_3^--N (1-125 kg ha^{-1}), NH_4^+-N (0.9-12.1 kg ha^{-1}) and PMN (3-96 kg ha^{-1}; Table S1), so none of these variables differed significantly among age classes.

3.2 Soil microbial biomass and activity

There were no significant differences among age classes in total PLFA (F = 2.28, P = 0.20, Figure 2b), fungal PLFA (F = 2.10, P = 0.22) or bacterial PLFA (F = 2.11, P = 0.22).

However, two planting sites and both remnant woodlands sites had significantly more total PLFA and bacterial PLFA in their treatment transects compared with their reference transects (Table 2). Also, two plantings and one remnant had significantly more fungal PLFA in their treatment transects compared with their reference transects (Table 2). Total PLFA, bacterial PLFA and fungal PLFA were positively correlated, either strongly ($0.60 > R > 0.79$) or very strongly ($0.80 > R > 1.00$) with SOC concentration, POxC concentration and soil moisture content (Table 3).

There were no significant differences among age classes in β-glucosidase activity ($F = 2.01$, $P = 0.23$) and polyphenol oxidase activity ($F = 1.28$, $P = 0.39$). However, in two plantings the activity of polyphenol oxidase was higher treatment transects compared with reference transects (Table 4). Moreover, activities of both enzymes were positively correlated, either moderately ($0.40 > R > 0.59$) or weakly ($0.20 > R > 0.39$) with SOC concentration and POxC concentration of soil (Table 3).

3.4 Soil microbial community composition

The fungal to bacterial ratio (F:B) showed no differences among age classes ($F = 0.03$, $P = 0.998$). Only one planting site had a significantly higher F:B ratio in its treatment transect compared with its reference transect. Across the plot data, F:B correlated weakly with C:N ($r = 0.29$, Table 3).

The microbial community composition of reference transects all differed significantly from each other ($R = 0.82$, $P = 0.001$). When treatment transects were compared with reference transects, differences in microbial community composition were observed at half of the sites (Table 5). The microbial community composition of reference transects in 23 yr plantings and remnant woodlands were either significantly ($P < 0.05$) or marginally significantly ($P < 0.08$) different from their adjacent reference transects (Table 5). This is shown in the NMDS plot (Figure 3), with the treatment transects of 23 year-old plantings and

remnant woodlands having distinct compositions from their adjacent reference transects, whereas other sites were much closer in composition.

4. Discussion

4.1 Soil carbon and nitrogen

While some plantings had higher SOC content compared with adjacent reference pastures, clear temporal patterns in SOC were not observed. Alternatively, there was high variability in SOC content, both within and among sites (Table 1). Large variabilities in C sequestration following reforestation have been observed previously in this region (Cunningham et al., 2015b) and elsewhere (e.g. Guo and Gifford, 2002) and have been attributed to variability in tree density, basal area, site productivity, soil moisture, and soil N in adjacent pastures (Cunningham et al., 2015b; Guo and Gifford, 2002; Paul et al., 2002). Here, SOC concentration was correlated positively with soil moisture, regardless of planting age (R = 0.23, P = 0.04). This is consistent with increased primary productivity, and potentially C sequestration, in soils with higher moisture contents (Post et al., 1982). A negative correlation was also observed between the difference in SOC within sites and SOC at reference pastures. This is possibly because when pastures have high SOC content, they have a higher potential for initial loss of SOC when plantings are established (Paul et al., 2002). Another explanation is that pastures with a high SOC content are already close to maximum carbon storage, so planting trees on these pastures will provide minimal increase in SOC content (Guo and Gifford, 2002)

An initial decrease in SOC following reforestation is common (Paul et al. 2002; Cunningham et al. 2012; Hoogmoed et al. 2012) and we found the young (9-10 yr) tree plantings had a lower SOC content than their adjacent pastures. This decrease has been attributed to the effect of the disturbance during soil preparation (i.e. ripping of furrows), which can break up soil aggregates, exposing organic matter to microbes for decomposition (Turner and Lambert 2000), thereby reducing SOC. If pastures with a higher SOC content have a higher potential for initial SOC loss, it will take longer for these plantings to sequester

SOC than plantings on pastures with low SOC. However, there is evidence that after several decades, even tree plantings on pastures with relatively high SOC will sequester SOC (Cunningham et al., 2015b).

The largest differences in SOC were measured here between the remnant woodlands and their adjacent pastures (Table 1). This is consistent with an earlier study of remnant woodlands in the same region (e.g. Cunningham et al., 2015b). Together, these studies suggest that tree plantings in temperate Australia, even those on pastures with initially high SOC contents, have the capacity to sequester additional SOC. The same may not be true for systems where remnant forests contain less SOC than pastures (Brown and Lugo, 1990; Franzluebbers et al., 2000). This highlights the need for regional studies of SOC sequestration following reforestation of pastures.

Here, we only sampled the top 5 cm of soil because most microbial activity occurs in the upper soil layer (Bolton Jr et al., 1993) and, therefore, changes in SOC are often first observed there (Paul et al., 2002, Richter et al., 1999). However, changes in SOC in lower soil layers can occur (Cunningham et al., 2015b) and should be investigated in studies that include plantings older than those studied here.

Nitrogen often decreases following reforestation, and this has been attributed to cessation of fertiliser inputs, less faecal inputs by grazing animals, greater demand for N of growing trees compared with pasture grasses, increased immobilization of N by soil microbes and increased N emissions from soil (Berthrong et al., 2009; Garten and Ashwood, 2002). However, no change in soil N was observed here or in other plantings in south-eastern Australia, even when plantings contained N-fixing trees (Cunningham et al., 2015b; Hoogmoed et al., 2012). Nitrogen fluxes in riparian systems may be more complex compared with upland systems, as N is very mobile in soils and often moves towards waterways

(Likens et al., 1970; Puckett et al., 1999). Therefore, management of upland areas, as well as riparian areas, can contribute to soil N concentration of riparian areas (Burger et al., 2010).

4.2 Soil microbial biomass and activity

While there were no significant differences among age classes in total PLFA, bacterial PLFA or fungal PLFA, all three of these variables were correlated strongly with both SOC and permanganate oxidisable C (POxC, Table 3), indicating that C is a strong determinant of microbial biomass. When C:N is below 20, as it was in our soils, both fungi and bacteria are often C-limited (Waring et al., 2013). Consequently, any increase in SOC is likely to have a large effect on microbial biomass.

Soil microbial activity is important for the decomposition of the litter layer and soil organic matter. However, there is limited research on the activities of decomposition enzymes in riparian forests. The activities of β-glucosidase and polyphenol oxidase were predicted to increase following planting due to the expected increase in SOC. These enzymes are known to play important roles in degrading cellulose and lignin, respectively (Ng et al., 2014; Sinsabaugh, 2010). While there were no significant differences in SOC or enzyme activity among age classes, both β-glucosidase activity and polyphenol oxidase activity correlated with SOC and POxC. These results suggest that higher SOC concentrations, regardless of planting age, are associated with an increase in decomposition.

4.3 Soil microbial community composition

While there was no increase in F:B with planting age, F:B was positively correlated with C:N. There was also a correlation between C:N and fungal PLFA, but not bacterial PLFA, suggesting the correlation between C:N and F:B is driven by changes in fungal biomass. The weak relationship between C:N and F:B observed was probably due to the low values and small range of C:N ratios observed (Waring et al., 2013). In contrast, strong correlations (e.g.

r^2 = 0.65) between C:N and F:B have been found at the scale of global biomes, which provides a wide range of C:N ratios (Fierer et al., 2009).

The microbial community composition of the soil, based on PLFA analysis, was different at each site's pasture (Figure 3), which reflects the variability in SOC, NH_4^+-N and NO_3^--N contents among pastures. Despite this variability, the microbial community compositions of plantings were increasingly different from adjacent reference pastures with increasing planting age (Table 5). Microbial community compositions of forests are generally different from those of pastures (Bossio et al., 2005; Singh et al., 2007) because of the different forms of C in forest soils compared with pastures (Smith et al., 2012), which may be differentially available to different microbial functional groups (Ng et al., 2014). The changes observed in the soil microbial community composition could be an early indication of the effects of reforestation on SOC, confirming our hypothesis that reforestation will slowly lead to SOC sequestration.

5. Conclusion

There were strong correlations linking SOC with soil microbial biomass (fungal and bacterial) and activity. Furthermore, there was a correlation between C:N and F:B, despite the low level and range of C:N ratios observed. Older plantings had distinct soil microbial communities compared with their adjacent pastures, indicating an effect of woody inputs on the soil microbial community.

While SOC was higher in some plantings compared with their adjacent pastures, there was no consistent change with time since reforestation. However, both remnant woodlands had significantly higher SOC content compared with their adjacent pastures. This suggests that while reforestation of riparian zones has the potential to sequester C in the soil, it may take more than two decades to be consistently higher than that found in pastures and to reach levels similar to those in remnant riparian zones.

Taken together, these data indicate that belowground changes following reforestation in riparian zones are complex, and both soil C data and C cycling data are needed to better understand soil C sequestration.

Acknowledgments

We wish to thank the land-holders for access to their farms. Thanks to Ms Lisa Oborne for assistance in the field and Dr Precilla 'Pree' Johnson, Dr EeLing Ng and Ms Alicia Brown for technical assistance in the laboratory. This research was funded by the Australian Research Council Linkage Program (LP0990038), Victorian Department of Sustainability and Environment, Goulburn-Broken Catchment Management Authority (CMA), Victorian EPA, Kilter Pty Ltd and the North Central CMA. TRC gratefully acknowledges the ARC for supporting his research via the award of a Future Fellowship (FT120100463).

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Fig. Captions

Fig. 1. Schematic diagram of (a) a typical site (of which there were 10), including the location of a treatment transect (in a planting) and a reference transect near the creek (dashed lines), and (b) a transect, showing four randomly placed plots along the transect. Diagrams are not drawn to scale. Note, at sites in the 0 yr age category, both transects were placed in a pasture, and one transect was randomly allocated as the treatment transect, while the other was the reference transect.

Fig. 2. Difference (treatment transect – reference transect) in (a) soil C content and (b) total PLFA for each planting age class (0-5 cm soil layer, $N = 2$). Error bars represent one standard error. Results of one-way ANOVAs comparing age classes are provided.

Fig. 3. Non-metric multidimensional scaling ordination of the microbial community composition (PLFAs) of soils. Closed circles indicate treatment transects and open circles indicate reference transects. Numbers indicate age of planting at the site, R indicates remnant sites. Transects from the same site are linked by lines.