The influence of sampling strategies and spatial variation on the detected soil bacterial communities under three different land-use types

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Abstract

To determine the influence of pooling strategies on detected soil bacterial communities, we sampled 45 soil cores each from a eucalypt woodland, a sown pasture and a revegetated site in an Australian landscape. We assessed the spatial variation within each land-use plot, including the influence of sampling distance, soil chemical characteristics and, where appropriate, proximity to trees on the soil bacterial community, by generating terminal restriction fragment length polymorphism profiles of the bacterial 16S rRNA genes. The soil bacterial community under the revegetated site was more similar to the original woodland than the pasture, and this result was found regardless of the soil- or the DNA-pooling strategy used. Analyzing as few as eight cores per plot was sufficient to detect significant differences between the bacterial communities under the different plots to be distinguished. Soil pH was found to be most strongly associated with soil bacterial community composition within the plots and there was no association found with proximity to trees. This study has investigated sampling strategies for further research into the transitions of soil microbial communities with land-use change across broader temporal and spatial scales.

Introduction

Extensive vegetation clearing associated with agricultural expansion in Australia has contributed to several severe and highly visible environmental problems including increased dry-land salinity, soil erosion and reduced water quality (Beeton et al., 2006). Consequently, the re-establishment of deep-rooted native perennial plants is a major restoration focus in many regions across the country. The success of these efforts is often assessed by plant germination and growth or animal colonization (Lindemayer et al., 2000; Thrall et al., 2005). However, recent studies have begun to include other ecosystem components in their assessment of ecological function. For example, Colloff et al. (2010) found that significantly more invertebrate soil macropores were present in soils 11–20 years after revegetation, which resulted in a doubling of water infiltration rates and indicated positive changes in the soil structure following the restoration of native vegetation.

Although soil microorganisms are known to play a critical role in biogeochemical cycling (van der Heijden et al., 2008), little information exists to determine whether the restoration of native vegetation leads to positive changes in microbial diversity and function. Anthropogenic land-use change has previously been shown to alter the structure and function of belowground bacterial communities (Buckley & Schmidt, 2001; Bossio et al., 2005; Berthrong et al., 2009; Jesus et al., 2009). In Australia, differences between microbially mediated biogeochemical processes in forested areas compared with pasture sites have illustrated that changes in soil function occur following the conversion of agriculture to forestry (Allen et al., 2009), but studies explicitly focusing on the transition of soil communities between native vegetation and agricultural soils are relatively scarce. It is important that we begin to understand how soil microbial communities respond to changes in land management both spatially and temporally, because one goal of revegetation
efforts should be to restore animal, plant and microbial biodiversity and function to ensure the reinstatement of multiple ecosystem services and the development of a resilient self-sustaining ecosystem.

While studies suggest that aboveground vegetation can strongly influence belowground microbial communities (Mitchell et al., 2010), there is also considerable evidence that soil chemistry, for example pH, drives bacterial community composition (Fierer & Jackson, 2006; Högborn et al., 2007; Lauber et al., 2009; Rousk et al., 2010). Understanding the relationships between soil chemistry, microbial composition and plant community structure during the transition from agricultural land to functional native vegetation is critical to improving the success and cost-effectiveness of restoration efforts. Procedures for sampling soil to assess microbial community composition are an important consideration. In this study, our first aim was to assess the relative influence of spatial separation, proximity to vegetation and edaphic soil chemistry on the soil bacterial community composition (Fierer & Jackson, 2006; Högborn et al., 2007; Lauber et al., 2009; Rousk et al., 2010). Understanding the relationships between soil chemistry, microbial composition and plant community structure during the transition from agricultural land to functional native vegetation is critical to improving the success and cost-effectiveness of restoration efforts. Procedures for sampling soil to assess microbial community composition are an important consideration. In this study, our first aim was to assess the relative influence of spatial separation, proximity to vegetation and edaphic soil chemistry on the soil bacterial communities under native vegetation, agricultural pasture and restored woodland within an Australian landscape. Secondly, we investigated appropriate sampling methodologies for comparing soils under various land-use types for future studies into the effect of land-use change across a range of spatiotemporal scales.

**Materials and methods**

**Site selection and sampling regime**

Three adjacent plots were sampled on a mixed farming enterprise near Gundaroo, NSW, Australia (35°03′S, 149°14′E), 35 km north of Canberra: a eucalypt woodland remnant (Woodland); a managed pasture of phalaris and subclover (Pasture); and an area revegetated with mixed acacia and eucalypt species (Revegetation). The site is representative of many local restoration efforts and was chosen to minimize confounding factors such as slope and soil gradients across the plots. The managed pasture had been established for more than four decades on land cleared of the native species and received regular (annual or biannual) applications of phosphorus fertilizer according to normal district practices (equivalent to 10 kg P ha⁻¹ yr⁻¹), with at least two applications of either incorporated or surface-applied lime. The Revegetation plot was established in 1988 on pasture soil that was replanted with a range of locally adapted eucalypt and acacia species in rows separated by 5 m. The area was fenced to prevent grazing and had received no additional fertilizer inputs since establishment.

In May 2008, 45 soil cores (10 cm depth, 4.5 cm diameter) were collected at 10-m interval across three 90 × 50 m grids centered in each of the three land-use types. The land-use plots were each separated by approximately 100 m with the Pasture located between the Woodland and Revegetation plots. Cores in each plot were collected along the five 90 m columns of each grid in a serpentine path. In the Woodland, the presence of trees within the immediate vicinity of the core (≤ 2 m) was noted for each sample. For the Revegetation plot, cores in successive triplets of cores were sampled (in random order within each triplet) from locations adjacent to a tree; 0.5 m from a tree; or more than 1 m from a tree. The individual soil cores were kept intact and cool until being sieved the following day through a heat-sterilized 2 mm brass sieve, followed by thorough mixing. Sieved soil (50 g) from each of 15 cores within a plot was systematically pooled into three composite soil samples. To achieve this, the sieved subsamples from each core in successive triplets were randomly assigned to one of the three composite soil samples, ensuring that each composite was representative of conditions across the entire plot. The remainder of each soil core was kept for individual analysis. In the Revegetation plot, replicates of a Latin square design were used to randomly assign ‘adjacent’, ‘0.5 m’ and ‘> 1 m’ cores to the composite samples, ensuring exactly five of each core type in each composite. Soil chemical analysis and terminal restriction fragment length polymorphism (T-RFLP) profiling of the bacterial community was conducted on both the nine composite soil samples (three samples for each of the three land-use plots) and the 135 individual cores (45 samples from each of the three land-use plots).

**Analysis of soil chemistry**

The soil was classified as a brown to yellow Chromosol (Isbell, 1996). Soil chemical analysis was performed on air-dried composite soil samples and individual core samples. The gravimetric water content was determined by oven-drying the freshly sieved soil at 105 °C for 4 days. The soil was acidic, with pH determined on CaCl₂ extracts (0.1 M; 1:5 w/v; 30 min). Soil total organic matter was approximated using the loss on ignition method of Rowell (1994). Total carbon and nitrogen were determined using gas chromatograph mass spectrometry (20-20 Europa Scientific) according to Dumas (1981) and total, inorganic and organic phosphorus was determined using the ignition–extraction method of Olsen & Sommers (1982).

**DNA extraction**

The 135 individual core samples and nine composite soil samples were frozen in liquid nitrogen after sieving and stored at −20 °C until DNA extraction. DNA was extracted from 0.25 g of the composite soils using the MoBio PowerSoil DNA Isolation Kit according to the manufacturer’s instructions, with the following modifications: the samples were shaken in a Bio101 Savant on the maximum speed for 45 s instead of vortexing, all 4 °C incubation steps were...
conducted for 20 min and 100 μL of UV-treated TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) was used to resuspend the DNA on the spin column, which was incubated at 55 °C for 10 min before elution. DNA was extracted from 0.25 g of the 135 individual cores using the MoBio PowerSoil-htp 96 well Soil DNA Isolation Kit. The same modifications to the manufacturer’s instructions were carried out as above, except that the plates were shaken in a Qiagen TissueLyser for 20 min at 20 Hz. To compare the effects on the bacterial community analysis of pooling soil samples before or after the DNA extraction procedure, aliquots of the DNA extracts from each of the 135 individual cores were pooled into nine samples (pooled DNA) following the same allocation of cores as the composite soil samples. DNA was quantified from the extraction procedure by the PicoGreen dsDNA Quantitation Reagent (Invitrogen). This method was also used to quantify the amount of DNA from the nine composite soil and nine pooled DNA samples added to PCRs and T-RFLP restriction digests.

**Generation and analysis of T-RFLP profiles**

T-RFLP profiles of bacterial 16S rRNA genes present in each of the nine composite soil and nine pooled DNA extracts were performed in triplicate using 50 μL PCRs consisting of 1 ng of extracted DNA, 2.5 U of HotStarTaq DNA Polymerase (Qiagen) and the supplied buffer at 1 ×, 250 μM of each dNTP and 0.2 μM of each of the oligonucleotide primers, BAC519r and the 6-carboxyfluorescein (FAM)-labeled primer FAM27f (Osborne et al., 2006). PCRs were run in a PCR sprint thermal cycler (Hybaid), initially for 15 min at 95 °C to activate the HotStarTaq DNA Polymerase, followed by 25 cycles of 94 °C for 30 s, 48 °C for 30 s and 72 °C for 30 s, with a final extension step of 72 °C for 10 min. Triplicate reactions were pooled and purified using the QIAquick PCR Purification Kit (Qiagen), according to the manufacturer’s instructions. Twenty nanograms of the FAM-labeled PCR products were each added to four separate restriction digestions, in a volume of 25 μL that included 5 U of restriction endonuclease (RE), either AluI, Hinfl, MspI or Sau96I (New England Biolabs) and the supplied buffer at 1 ×. All restriction digestions were incubated at 37 °C for 4 h. T-RFLP profiles were generated by separating the fragments on an ABI 3130xl Genetic Analyzer (Applied Biosystems).

T-RFLP profiles from the 135 individual cores were generated using the same steps as above, with some modifications. Duplicate PCRs were carried out using 2 μL of a 1:10 dilution of each of the DNA extracts. The duplicate PCRs were pooled while purifying using the QIAquick 96 PCR Purification Kit (Qiagen), according to the manufacturer’s instructions. The restriction digestions contained 5 μL (1:20) of the purified, FAM-labeled PCR product.

Raw T-RFLP profile data was exported and analyzed using GENEMAPPER software (Applied Biosystems). Profiles generated with the different REs were kept in separate GENEMAPPER projects, and the 135 individual core samples were analyzed independent of the composite soil and pooled DNA samples. Within GENEMAPPER, the bin width was set to 1.5 nt and profiles were trimmed to between 50 and 550 nt. Four successful T-RFLP profiles were generated for 108 of the 135 individual core samples for the spatial comparison within the three land-use types (42 for woodland, 34 for pasture and 32 for revegetation). Sørensen’s pairwise dissimilarity coefficients, which score the presence of common peaks, or terminal restriction fragments (T-RFs), in the T-RFLP profiles, were calculated after concatenating the four profiles for each samples into a single ‘meta-profile’ and applying an optimal threshold (after Osborne et al., 2006).

All statistical analyses were carried out using R (R Development Core Team, 2010). Univariate ANOVA analyses were performed, when relevant, by multiple pairwise tests conducted using Tukey’s honestly significant difference (HSD) at the 5% family-wise level of significance. Multivariate analyses were performed using routines in the R package `vegan` (Oksanen et al., 2010). In particular, multivariate ANOVA (MANOVA) analyses were performed using a robust permutational alternative (Anderson, 2001) to standard parametric MANOVA provided by `vegan`’s `adonis` function. Unlike parametric MANOVA, the method of Anderson (2001) can be applied to non-Euclidean distance metrics such as Sørensen’s pairwise dissimilarity coefficient, used in this study to score the presence of common peaks. In addition, F tests for the significance of model terms are performed via permutation methods, making the analysis much more robust to violations of the assumptions of Gaussian random behavior that are required by the notoriously sensitive parametric MANOVA analysis. Quoted `MANOVA` P values are therefore F values for the permutational testing equivalence of an F test for the significance of a model term.

A canonical analysis of principle coordinates (CAP; Anderson & Willis, 2003) was performed to determine how soil chemistry related to the bacterial community similarities under each of the three land-use types. CAP, as implemented by the ‘capscale’ function in the `vegan` package, is a form of redundancy analysis (RDA; Legendre & Legendre, 1998), extending multiple linear regression to a multivariate response. RDA is a constrained ordination that maximizes the ability of linear combinations of environmental variables to explain variation in the multivariate T-RFLP dataset, while CAP generalizes RDA to apply to non-Euclidean distance metrics such as the Sørensen dissimilarity used in our analyses. The ‘capscale’ function provides a biplot of the ordination, with arrows reflecting correlations between environmental variables and the T-RFLP variation, as well as permutation tests for the significance of the ordination axes, and for the...
Results

Variation in the soil chemistry from the three land-use plots

The soil in the three land-use plots varied significantly with respect to soil pH, organic matter, carbon and inorganic phosphorus, according to the three composite soil samples from each plot (Fig. 1a–c, ANOVA \( P < 0.001 \) for each). The levels of inorganic phosphorus in the Woodland and Revegetation soils were not significantly different according to Tukey’s HSD test. The gravimetric water content was significantly higher for the Pasture soil (mean 9.1 ± 0.6%, ANOVA \( P = 0.02 \)) than the Woodland soil (mean 6.9 ± 0.2%), but the Revegetation soil was not significantly different from either the Woodland or the Pasture soil (mean 8.2 ± 1.0%), according to Tukey’s HSD test. Soil nitrogen (mean 0.33 ± 0.04%, ANOVA \( P = 0.10 \)) and organic phosphorus (mean 174.5 ± 32.4 μg g\(^{-1}\) soil, ANOVA \( P = 0.28 \)) were not significantly different across the three plots. Measurements of soil chemistry from the 45 individual cores from each plot yielded equivalent mean values, but higher variation than the composite soil samples for the three land-use plots (Fig. 1d–f). The levels of soil carbon in the individual cores within all three plots were highly correlated with organic matter as expected (Pearson’s correlation 0.95–0.99).

Fig. 1. Soil chemical characteristics (pH, % organic matter and total inorganic phosphorus) that differed significantly between land-use types (all ANOVA \( P < 0.001 \)). (a–c) Mean ± one SD for each soil parameter for the three land-use plots (Woodland, Pasture and Revegetation) from composite soil samples; (d–f) mean ± one SD from the 45 individual cores from each land-use plot. Open circles (in d–f) show the upper and lower values (2.5 and 97.5 percentile, respectively) for the 95 percentile range. The soil chemical measurements are: pH from the composite soil samples (a) and individual cores (d), percent organic matter from the composite soil samples (b) and individual cores (e), and inorganic phosphorus (μg g\(^{-1}\) dry soil) from the composite soil samples (c) and individual cores (f). Capital letters within each subfigure indicate significant groupings according to Tukey’s HSD test, with a 5% level of significance.
Variation in the soil bacterial community across the three land-use plots

According to the T-RFLP profiles of bacterial 16S rRNA genes, the bacterial communities under the three land-use plots were significantly different in composition (MANOVA \( P < 0.001 \); Fig. 2). Neither of the two pooling strategies (i.e. composite soil samples or pooled DNA extracted from the individual cores) made a significant difference to the detected bacterial community (82–84% of the T-RFs/peaks were detected in both pooling strategies). An average of Sørensen’s pairwise dissimilarity coefficients from the 18 profiles (generated using both pooling strategies) revealed that the Woodland and Pasture communities were the least similar (55% mean similarity, \( n = 36 \)). The Revegetation bacterial community was more similar to the community in the Woodland soil (mean 65%, \( n = 36 \)) than the Pasture soil (mean 60%, \( n = 36 \), MANOVA \( P < 0.001 \)).

The T-RFLP profiles generated from the 108 individual cores (i.e. from the 135 samples where T-RFLP profiles were successfully generated for all four restriction enzymes) also had a significantly different composition across the three representative plots (Fig. 3, MANOVA \( P < 0.001 \)), despite higher within-plot variation. The mean pairwise similarity between plots was similar to that observed in the 18 samples derived from composite soil and pooled DNA, with the lowest similarity occurring between the Pasture and the Woodland soil cores (mean 54%, \( n = 1326 \)) and the highest similarity between the Revegetation and the Woodland soil cores (mean 62%, \( n = 1209 \), MANOVA \( P < 0.001 \)).

A simulation approach was used to explore the effect of sampling effort on the ability to detect statistically significant differences between the bacterial communities. We randomly sampled subsets (of a specified size) of cores from each land type in the 108 individual core T-RFLP dataset and obtained MANOVA \( P \)-values for each of the three possible pairwise comparisons. These steps were iterated to produce 1000 simulated \( P \)-values for each pairwise comparison, and the observed percentages of \( P \)-values significant at \( P < 0.001 \), \( P < 0.01 \) and \( P < 0.05 \) were recorded. The simulations were carried out for sample sizes of 3, 5, 8, 10, 15 and 20 cores from each land type (Table 1). Observed percentages of significant differences for all pairwise comparisons were <100% for sample sizes of three or five cores per plot. For sample sizes of eight cores per plot, the proportion of \( P \)-values significant at \( P < 0.01 \) reached 100% for comparisons between the Pasture plot and either the Woodland or the Revegetation. The Woodland and Revegetation plots, being more similar, required a larger sampling effort to consistently achieve significance. With the analysis of 15 soils cores per plot, the MANOVA analysis consistently detected significant differences between all three land-use types at the \( P < 0.01 \) significance level. Determining how the spatial separation of samples within a site affects the soil bacterial community represented was then further investigated.

Spatial variation of the soil bacterial community within each land-use plot

The relationship between bacterial community composition in the individual cores (measured by the Sørensen
dissimilarity coefficient), soil chemical characteristics and physical distance (measured in meters) was explored independently in all three land-use types. A CAP (Anderson & Willis, 2003) was performed to determine how soil chemistry related to the bacterial community similarities under each of the three land-use types. CAP analyses indicated that in all cases, pH was strongly and significantly aligned with CAP axis 1 for all three land-use types (P < 0.001), suggesting that it is the most important driver of variation in the individual T-RFLP profiles under each land use (Figs 3 and 4a–c). According to the CAP plots, organic matter was highly significant in the Woodland and Revegetation soil (P < 0.01), but marginally significant in Pasture soil (P = 0.054). Soil water content was not significant for any land-use type (P > 0.15). Mantel tests indicated that the only other chemical characteristics explaining a significant amount of the observed variation among bacterial communities were organic matter in the Pasture soil (Pearson’s correlation 0.21, Mantel P = 0.04) and organic phosphorus.

**Table 1. Results from simulating MANOVA P-values for pairwise comparisons between plots, applied to subsamples of n = 3, 5, 8, 10, 15 and 20 cores per plot.**

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<tr>
<th># Cores sampled</th>
<th>Pasture vs. Revegetation (%)</th>
<th>Woodland vs. Revegetation (%)</th>
<th>Woodland vs. Pasture (%)</th>
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<tr>
<td>3</td>
<td>&lt; 0.001 65.4</td>
<td>40.9</td>
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<td></td>
<td>&lt; 0.01 68.5</td>
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<td></td>
<td>&lt; 0.05 84.4</td>
<td>58.6</td>
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<tr>
<td>5</td>
<td>&lt; 0.001 60.9</td>
<td>36.6</td>
<td>58.9</td>
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<td></td>
<td>&lt; 0.01 94.5</td>
<td>57.8</td>
<td>85.8</td>
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<td></td>
<td>&lt; 0.05 100.0</td>
<td>78.1</td>
<td>99.7</td>
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<td>8</td>
<td>&lt; 0.001 97.4</td>
<td>50.7</td>
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<td>&lt; 0.01 100.0</td>
<td>87.1</td>
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Reported values are the proportion of P-values from 1000 simulations that were observed to be significant at the stated significance level. A result of 100.0% indicates that all 1000 simulated P-values suggested statistically significant differences between bacterial community profiles at the relevant significance level.

Fig. 4. CAP biplot of the soil chemistry explanatory variables (arrows) and the bacterial community T-RFLP profile dissimilarity coefficients (circles) for the three land-use types: (a) Woodland, (b) Pasture and (c) Revegetation soil core samples. The soil chemical variables modeled are pH, organic matter (OM), water content (WC), inorganic phosphorus (Pi) and organic phosphorus (Po). CAP axis 1 is significant for all three biplots and axis 2 is only significant for Woodland (a) and Revegetation (c).
in the Woodland soil (Pearson’s correlation 0.18, Mantel $P = 0.01$).

The significant correlations between the difference in soil pH between any two samples and bacterial community pairwise dissimilarity for each land use are illustrated in Fig. 5a–c (Mantel $P$-values are indicated on each graph). To explore the effect of spatial autocorrelation within sites, an analysis of intersample distance in meters and difference in microbial community composition was also undertaken. This revealed significant correlations between spatial separation and bacterial community similarity for the Woodland and Revegetation land-use types, although spatial separation explained less variation than pH (Fig. 5d–f). In addition, there was no correlation between the spatial distance of sampling and the pH difference between samples (pairwise Pearson’s correlation $-0.04$ to $0.01$, Mantel $P = 0.42$–0.70), suggesting that the effect of pH on the bacterial community is independent of spatial distance. In fact, some aspects of

![Fig. 5. Correlations between the Sørensen dissimilarity coefficients of the bacterial community T-RFLP profiles with (a–c) pH change or (d–f) spatial distance (measured in meters), for the three land-use types: Woodland (a, d), Pasture (b, e) and Revegetation (c, f). Pearson’s correlation coefficient and Mantel test $P$-values are reported.](image-url)
soil chemistry did correlate significantly with the spatial distance; in the Pasture soils, organic matter and phosphorus were significantly correlated with distance (Pearson’s correlation 0.13–0.36, all Mantel $P$-values $< 0.04$), as was organic matter in the Revegetation site soil (Pearson’s correlation 0.18, Mantel $P = 0.01$). The effect of proximal eucalyptus or acacia trees on the bacterial community was investigated in the Woodland and Revegetation sites to determine whether this explained the slight, but significant spatial autocorrelation found in these plots (Fig 5d and f). No significant relationships were found between bacterial community similarity and the relative proximity to trees for either the Woodland (Fig. 6a, MANOVA $P = 0.83$) or the Revegetation plots (Fig. 6b, MANOVA $P = 0.25$).

**Discussion**

Buckley & Schmidt (2001) suggested from their study into the effect of land-use change on soil bacterial communities that cultivation of a field, no matter how long ago, leaves an indelible mark on the soil bacterial community. In this study, we found significant differences between the bacterial community compositions under three plots representing land-use change within an Australian landscape from Woodland to Pasture to Revegetation. Significantly, the Revegetation plot was more similar in both community composition and soil chemical properties to the original woodland as compared with the pasture site, despite being returned to native vegetation only 20 years earlier. In this context, it would be useful for future studies to monitor soil chemistry and microbial communities at multiple time points following restoration. This would explicitly allow the evaluation of whether the soil is being restored to its ‘native state’ over time or whether it has stagnated at an ‘alternative stable state’ that is intermediate between its life histories (Beisner *et al.*, 2003). Of practical relevance to such studies is the intensity of sampling required to adequately characterize communities over space and time. Analyzing subsets of our dataset indicated that as few as eight or 10 soil cores per plot were sufficient to show significant differences ($P < 0.05$ or $< 0.01$, respectively) between the bacterial communities under the different land-use plots. The pooling strategy we used, based on compositing 15 individual soil cores (across 90 m x 50 m plots) to create composite soil samples, was sufficient to demonstrate significant differences ($P < 0.001$) in soil chemistry and bacterial community similarities based on T-RFLP profiles across all three land-use plots.

Pooling of soil cores into composite samples is routinely used to minimize variation (Baker *et al.*, 2009), although under some circumstances, this may also generate other analysis issues, such as the loss of rare taxa in uneven communities (Manter *et al.*, 2010). Although we found more variation in the T-RFLP profiles from the 108 individual cores than the nine composite soil or nine pooled DNA T-RFLP profiles, the three different ways of comparing the cores (compositing before or after the DNA extraction, or not at all) did not affect the conclusions drawn about the similarity of the bacterial communities under the three plots in this soil system.

Analysis of the soil chemistry and bacterial communities in 45 soil cores per plot allowed us to investigate the specific effects of spatial variation within each of the land-use plots. Our results suggest that the proximity of established eucalypt or acacia trees to the collected cores did not significantly influence the bulk soil bacterial community, although explicit rhizosphere samples were not collected in this study. Although some studies suggest that single trees can significantly influence the microbiota (Mitchell *et al.*, 2010), another study in a native Australian remnant woodland has found that soil fungal diversity is not greater under trees compared with intertree areas (Bennett *et al.*, 2009). In contrast, we did find a small, but significant effect of spatial separation on bacterial community similarity in each of the
three land-use types, although the basis of this variation presently remains unclear. For the Pasture and Revegetation sites, there was a significant correlation between soil organic matter and distance, whereas no such relationship was evident with soil pH, which alone was found to be the major soil chemical factor that contributed to differences in microbial diversity. Recently, Bissett et al. (2010) found evidence for a significant spatial component to bacterial community structure that varied with scale (from meters to 100 km), but this was clearly dependent on microorganism life history, such as dispersal and survival ability.

In the present study, soil pH was found to be the key determinant of community similarity across all three land-use types, as has been found in previous studies conducted on a variety of soil types and spatial scales, using many different methods (Fierer & Jackson, 2006; Högberg et al., 2007; Jesus et al., 2009; Lauber et al., 2009; Rousk et al., 2010). There are a number of biological reasons why subtle gradients of pH in soil can have dramatic influences on microbial cells. These include the impact of low pH on metal toxicity and nutrient availability (Delhaize & Ryan, 1995) and the potentially narrow pH ranges that are optimal for bacterial growth (Rousk et al., 2010).

The effect of spatial variability of soil chemistry and proximity to vegetation on the soil bacterial community was specifically assessed in this study by independently analyzing the soil cores collected from three land-use plots. We found that abiotic soil factors, especially soil pH, were more important for shaping the soil bacterial community than proximity to trees. We also thoroughly assessed appropriate sampling methodologies for comparing soils under various land-use types for future studies into the effect of land-use change across time and wider spatial scales.

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References


