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Life at the interface: above- and below-ground responses of a grazed pasture soil to reforestation

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Title: Life at the interface: above- and below-ground responses of a grazed pasture soil to reforestation.

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Running title:

Life at the interface

Abstract:

20 Conversion of agricultural lands to mixed species woody plantings is increasingly being undertaken as a means of sequestering C and increasing biodiversity. The implications of such changes in landuse for soil communities, and the ecosystem services they provide (e.g. nutrient and C cycling), are relatively little understood. Results of a detailed study of vegetation, soil physicochemical properties and soil

25   communities (primarily microbial) to reforestation of a pasture (15 years post

reforestation), and its immediately adjacent un-restored pasture, are presented.

Whereas the reforested portion of the site had significantly higher levels of tree

canopy cover and a well-developed litter layer than the immediately adjacent

pasture, the reverse was true for grass biomass. Although there were no differences

30   in total root biomass between the sampling zones, the pasture zone was dominated

by fine roots and the reforested zone by coarse roots. Reforestation had a significant

impact on soil physicochemical properties, with soil C, C:N and mineral N being

higher than in the pasture. The reforestation also supported a greater microbial

PLFA, a higher Fungal:Bacterial PLFA ratio and a different microbial community

35   (based on PLFA profiles) from that of the adjacent pasture. There were also

difference in earthworm abundance, with earthworms present and absent in soils

from the pasture and reforested zones, respectively. All of the changes in vegetation,

soil physicochemical properties and biotic communities occurred abruptly at the

interface between the land-use types, with no evidence of an interaction between

40   side of fence (reforested versus pasture zones) and distance from the fence. Results

are discussed in the context of changes in land-use on soil ecology and their

potential functional significance.

1 Introduction

50   There is great potential to sequester C in the soil (Lal, 2004). This can be achieved in

many ways, including the addition of C-rich materials to the soil, changes in specific

farming practices, and land-use change (Cunningham et al., 2015a; Minoshima et al.,

2007; Ng et al., 2014; Paul et al., 2002; Quilty and Cattle, 2011). One approach that is

receiving increasing attention is the conversion of agricultural lands, especially those

55   that are marginal or are expected to become so under climate change, to mixed

species woody plantings (Cunningham et al., 2015b). This approach to C

sequestration can also provide additional environmental benefits, such as the

provision of habitat, improving soil stability, and reducing the risk of point source

pollution (Bradshaw et al., 2012; Burger et al., 2010; Cunningham et al., 2015b).

60   Reforestation can have a profound impact on soil properties. For example,

soil N levels are generally lower following reforestation compared to agricultural

lands due to the addition of fertilizers in fields (Garten and Ashwood, 2002), and the

large N demand of growing trees (Berthrong et al., 2009). In contrast, P

mineralization and availability can be higher in tree plantings than in agricultural

65   lands (Chen et al., 2008; Wilson et al., 1997). Increases in the amounts and stability

of soil C have also been reported following reforestation of agricultural lands

(Cunningham et al., 2015a; de Alcântara et al., 1996; Smith et al., 2012). These

changes in soil C are likely due differences in the amount and chemical nature of

plant litter inputs from trees compared to crop and pasture species (Aerts and

70   Chapin, 2000). Soil C:N ratios can also increase following reforestation of pastures

(Berthrong et al., 2009; Cunningham et al., 2015a; Cunningham et al., 2012). These

changes in soil chemistry are often associated with changes in soil microbial communities and their functioning.

Shifts in microbial community composition following reforestation have been reported (e.g. Bossio et al., 2005; Hedlund, 2002; Wu et al., 2013). An increase in soil fungal:bacterial (PLFA) ratios, as high as 50%, has also been found following reforestation of pastures (MacDonald et al., 2009). These increases in fungal:bacterial (PLFA) ratios can be explained by a positive relationship between soil fungal:bacterial (PLFA) and soil C:N ratios (Busse et al., 2009; Högberg et al., 2007; Waring et al., 2013). Together, such changes in soil microbial community composition and bacterial and fungal biomass can have implications for soil nutrient and carbon cycling as soil microbes play an important role in these processes (Bardgett and Wardle, 2010; Jackson et al., 2008; Paul, 2006).

Although soil ecological responses to reforestation of agricultural lands have been studied (e.g. Bossio et al., 2005; Hedlund, 2002; Singh et al., 2007), relatively little is known about patterns of change at the interface between these land-use types. However, some insights have been gained. For example, in a study of soil and vegetation properties at the interface between a reforested pasture and its immediately adjacent un-restored pasture, an abrupt change in both the amounts and forms of C (by ^{13}C solid-state NMR) was found (Smith et al., 2012). The same was also true for rates of nutrient cycling processes (specifically potential N mineralization), which were higher in the reforested zone. Impacts on soil communities were not considered in this earlier work. Given their importance in soil C and nutrient cycling (see Bardgett and Wardle, 2010; Jackson et al., 2008; Paul, 2006, for detailed review), this is an important knowledge gap.

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Here results of a study of soil ecological responses of a pasture soil to reforestation are presented. The study focused on the interface between an area that had been converted from a pasture to a tree planting 15 years prior to sampling, and a contiguous pasture of a similar size that had been managed in the same way as

100   the tree planting prior to its establishment. Particular emphasis was placed on changes in soil microbial community composition, soil C stocks and aspects of soil N cycling. It was hypothesised that planting trees on the pasture would result in:

1. An abrupt change in soil physicochemical properties at the interface between the two land-uses;

105   2. An increase in the fungal:bacterial (PLFA) ratio in the reforested portion of the site compared to the pasture; and

3. The development of microbial community in the reforested portion of the site that was different from that of the pasture.

2 Materials and Methods.

2.1 Field site and survey design

Soils were collected from a grazed (sheep) pasture farm in Archie's Creek, in the West Gippsland region of Victoria, Australia. The region has a temperate climate with a mean maximum temperature in the hottest month of 23.4°C, and a mean minimum temperature in the coolest month of 5.9°C, and an annual mean rainfall of 1095 mm/year ([http://www.bom.gov.au/climate/, last accessed June, 2015](http://www.bom.gov.au/climate/)). Prior to European settlement, this region was covered predominantly in woodlands and forests dominated by *Eucalyptus* species. These woodlands and forests have been extensively cleared since the 1840s for pasture and stock production.

The field site included an area that had been converted from a pasture to a tree planting 15 years prior to sampling. The tree planting was 2 ha in size, and was immediately adjacent to a pasture (3 ha in size) that had been managed in the same way as the tree planting prior to its establishment (i.e. previously part of the same field). The tree planting was established by fencing out grazing stock and hand planting tubestock seedlings into furrows/rip-lines at 3 m spacing. The site contained a mixture of native plant species and was dominated by *Eucalyptus globulus* spp. *globulus* and *E. obliqua*, with a tree density of 690 trees ha^{-1} and a basal area of 23.8m^2 ha^{-1} (Cunningham, unpublished).

Patterns in soil properties at the pasture/tree-planting interface were studied at the site in September 2013 (Austral Spring). A 36 m × 36 m plot that (equally) spanned both sides of the fence line dividing the pasture and the tree planting was established (Figure 1); this spatially explicit sampling design is based on that of Smith *et al.* (2012). Importantly, the main plot was located on the site where all sampling

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zones were in a similar topographical position so as to avoid any gradients that may have existed across the site prior to reforestation. The main plot was divided into six contiguous sampling zones (referred to as zones A, B, C, D, E and F, hereafter), each of which was 36 m long (parallel to the direction of the fence line), and 6 m wide (perpendicular to the direction of the fence line). Thus, each sampling zone was divided into six equally sized (i.e. 6 m × 6 m) sampling plots, giving a total of 36 plots across the site.

2.2 Sample collection and analysis

Tree canopy cover (i.e. extent) was quantified in the center of each plot (following Burger et al., 2010). Surface litter was collected from each plot from a centrally located 0.25 m × 0.25 m quadrat. Grass biomass was also collected from within each of the litter sampling quadrats. Litter and grass dry weights were determined (separately) after drying of the samples at 60°C for 48 h.

After collection of grass and litter, three soil cores were collected from within each litter sampling quadrat by gently tapping metal cores (7.2 cm diameter) of known volume (203 cm^3) into the soil to a depth of 5 cm. This sampling zone was selected as this soil layer is where biological activity is greatest in these soils (Cavagnaro, un-published). The first core was used for measurement of bulk density and root biomass as follows. All soil was removed from the core and divided into two sub-samples. The first sub-sample was used to determine soil gravimetric moisture content following drying at 105°C for 48 h, and calculation of bulk density (see Smith et al., 2012), and the second for determination of root biomass. Roots were carefully washed from the soil, separated into to fine (<2 mm diameter) and coarse (>2 mm

diameter) roots, dried for 48 h at 60°C, and root biomass per g dry soil determined.

The remaining two soil cores were immediately combined in the field, place in a

160   plastic bag, and stored immediately at 4°C in a portable, battery powered

refrigerator. The refrigerated samples, all of which were collected on the same day,

were returned to the laboratory for immediate processing and analysis the following

day. Soil processing involved carefully mixing soil samples and passing them through

a 2 mm sieve to remove stones and any coarse woody debris. All earthworms were

165   also collected and counted. The sieved soil samples were then divided into four sub-

samples for the following analyses.

The first subsample was placed in a tube and immediately frozen at -20°C for

subsequent microbial analysis (see below). The second sub-sample was used to

determine gravimetric moisture content (as above). The third sub sample was use

170   for determination of mineral N and potentially mineralizable N (PMN), as follows.

Triplicate soil samples (30 g moist soil) were taken, extracted with 2 M KCl, and

inorganic N content determined colorimetrically using a modification of the method

reported in Miranda et al. (2001) for NO_3^--N, and in Forster (1995) for NH_4^+-N.

Potential mineralizable N (PMN) was determined by anaerobic incubation (following

175   Potthoff et al., 2005; Waring and Bremner, 1964). The fourth sub-sample was air

dried and analyzed for key physicochemical properties, including plant-available

(Colwell) P, pH and EC (1:5 water extracts), total C and N (by dry combustion) and

labile (permanganate oxidisable) carbon. These analyses were performed by the

Environmental Analysis Laboratory, Southern Cross University (see

180   http://scu.edu.au/eal/, for details of laboratory methods, last accessed November,

2015).

The soil sub-sample frozen at the time of processing was used for microbial analysis by PLFA. PLFA's were extracted and identified as described previously (see Mosse et al., 2012; Ng et al., 2014). Briefly, PLFAs were extracted using citrate buffer and alkaline methanolysis of phospholipids. The PLFA profile was then identified using a Varian CP 38/00 gas chromatograph fitted with a 5% phenyl:95% methylsiloxane column (Varian, Walnut Creek CA, USA). The fatty acids i15:0, a15:0, 15:0, i16:0, 16:1ω7, i17:0, a17:0, 17:0cy, and 17:0 were chosen as bacterial biomarkers and linoleic acid (18:2ω6,9) was chosen as the biomarker for decomposer fungi, based on Ng *et al*. (2014). These PLFA's where then used to calculate Fungal:Bactirial PLFA ratios.

2.4 Data analysis

Box plots were constructed for vegetation, soil physicochemcial, microbial community and worm abundance data, for each sampling location (A – F) using the *Boxplot* function in R (Murrell, 2005). Boxplots were selected for data presentation as they display the median, minimum, maximum, first and third quartiles, and any outliers in a single graphic.

As soil samples were taken at varying distances from the fence separating the paddock and the reforested zone, it is not valid to make direct comparisons between the two sampling zones using, for example, an ANOVA-based approach (Smith et al., 2012; Zar, 1999). To overcome this issue, spatial patterns in soil properties were described using piecewise linear (a.k.a. broken-stick) regression modelling. This approach allowed us to examine the relationships between soil variables and 'side of fence' (i.e. reforested-zone versus pasture-zone). This approach was also used to test

for interactions between side of fence and distance from the fence; however, there were no significant interactions, and so results of this analysis are not considered further. This analysis was performed using linear mixed effects models, using the Lem4 package in R (Bates et al., 2015). The Lem4 package provides an estimate, and

210 its associated standard error (S.E.), of the model intercept and slope of the parameter(s) of interest. We then used the pbkrtest package in R (Halekoh and Højsgaard, 2014) to use the Kenward-Roger approximation to get approximate degrees of freedom and the t-distribution to get p-values. These p-values were then used to identify significant differences in vegetation or soil properties on either side

215 of the fence. To aid in interpretation of the results, the p-values are presented on each data Figure, and are also presented in a summary Table, along with other output from this data analysis (Table 1).

Microbial community composition (PLFA; mol percent) data were analyzed with non-metric multidimensional scaling (NMDS) ordination. This analysis was

220 performed in R using the metaMDS function in the vegan package (Oksanen et al., 2012), with default parameters except for: autotransform = false, trymax = 100, pc = false, distance = bray. Only those PLFA's that were present in more than 10% of samples were included in this analysis. The final stress value in the NMDS was 0.12. Further, 95% confidence ellipses around locations were generated using the

225 ordiellipse() fucntion. Correlations between the NMDS ordination of the microbial community composition and soil variables were tested with 1000 permutations in the envfit() function in the vegan package in R. Soil variables included in this analysis were: soil moisture, NH_4^+-N, NO_3^--N, PMN, Colwell P, pH, EC, Total C (%), soil C:N and bulk density. Other soil variables were omitted because they were either highly

230   correlated with another variable included in the analysis (e.g. labile C, total N and

total C), or were highly correlated with land use – e.g. the presence and (complete)

absence of worms in the pasture and reforestated zones respectively. Permutational

multivariate analysis of variance (perMANOVA) was also employed to test

significance of the experimental factors (reforested zone versus pasture) microbial

235   community (PLFA) datasets and to assess the relative proportion of variation that

each factor contributed (Anderson, 2001). PerMANOVA analyses were performed in

R with the adonis function in the vegan package with the default parameters (Bray-

Curtis distance measure, 999 permutations). To explore changes in specific PLFA's

and their relationship to environmental variables, a second NMDS was constructed

240   in which specific PLFA's were ordinated and overlaid with the same vectors (of

environmental variables) as above. N.B. While this analysis used data for all PLFA's,

the resulting ordination had a large number of PLFA's clustered around the origin,

making it difficult to identify those individual PLFA's. Therefore, in the Figure (Figure

6b), these PLFA's at the origin have been removed for the sake of clarity of data

245   presentation.

3 Results

3.1 Vegetation and litter

Fifteen years after reforestation there were substantial differences in the vegetation,

250   both above- and below-ground, and litter layers between the reforested and pasture

plots (Figure 2). Whereas the reforested portion of the site had an extensive tree

canopy (canopy cover = 56 ± 1.5 %) and a well-developed litter layer (Figure 1) than

the immediately adjacent pasture, the reverse was true for grass biomass. These

differences in vegetation extended below-ground with the pasture and reforested

255   zones having significantly higher (Table 1) fine root biomass and coarse root biomass

than one another, respectively (Figure 2). There were, however, no significant

differences in total root biomass between the two sides of the fence (Table 1).

Although the interaction between side of fence and distance from fence was not

significant, fine root biomass was notably lower immediately adjacent to the fence.

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3.2 Soil physicochemical properties

The concentrations of total C, labile C and soil C stocks (in the 0-5 cm soil layer) were

significantly higher in the reforested zone than in the pasture zone (Table 1, Figure

3a-c). Total soil N concentration was also significantly higher in the reforested zone

265   than the pasture zone (Table 1, Figure 3d), but the difference between the zones was

less than that for total C; nevertheless, soil C:N ratios were higher in the reforested

zone than the pasture zone (Table 1, Figure 3e). Soil bulk density did not differ

significantly between the two sampling zones (Table 1, Figure 3f).

      Mineral N, measured as both NH_4^+-N and NO_3^--N were significantly higher in

270   the reforested zone than in the pasture zone (Table 1, Figure 4a,b). There was,

however, no difference in PMN between the two sampling zones (Table 1, Figure 4c).

Levels of plant-available (Colwell) P were generally high and did not differ between

the two sampling zones (Table 1, Figure 4d). Where as soil pH was significantly lower

in the reforested zone than the pasture zone, the reverse was true for EC (Table 1,

275   Figure 4e,f). The differences in pH were, however, small and levels of EC low,

suggesting that the biological significance of these differences may be minimal.

3.3 Soil communities

There was a clear impact of reforestation on both vegetation and soil

280   physicochemical properties, providing a strong contrast for assessing soil ecological

responses to land-use change. Whereas total PLFA, fungal PLFA and bacterial PLFA

did not differ between the reforested and pasture zones (Table 1, Figure 5a-c), the

Fungal:Bacterial PLFA ratio did, with the ratio significantly higher in the reforested

zone than the pasture zone (Table 1, Figure 5d). This increase in Fungal:Bacterial

285   PLFA ratio was associated with an increase in soil C:N ratio, as indicated by a positive

correlation between C:N and Fungal:Bacterial PLFA ratios (P<0.001; *R2*=0.44). There

were also significantly more earthworms in the pasture zone than the reforested

zone (Table 1, Figure 5e). The soil microbial communities were also clearly different

between the two sampling zones (Figure 6), with the difference in communities on

290   either side of the fence (i.e. reforested versus pasture zone) significantly different

(perMANOVA p=0.001). The differences in the communities were associated with

soil mineral N, EC, Total (%) C and soil C:N ratio, as indicated by the vectors on Figure

6a. Further analysis of the PLFA data indicated no clear patterns between specific

PLFA's and the different environmental variables (Figure 6b) found to be associated

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295   with differences in microbial community composition at the site level (i.e. compare

Figure 6a, b). Although some PLFA's were strongly separated on the NMDS (Figure

6b), there was no clear relationship between the PLFA's (most of which are reported

to be bacterial markers) and the environmental variables on the ordination.

4. Discussion

Trees were well established with a dense canopy, a substantial litter layer and no grassy understory fifteen years after reforestation of the pasture. This contrasted the pasture which had no trees, very little litter and a dense pasture sward. This finding is consistent with earlier work showing a relatively rapid development of vegetation following reforestation (Burger et al., 2010; Cunningham et al., 2015b). Reforestation resulted in changes below-ground, with strong differences in soil physicochemical (hypothesis 1) and biological properties (hypotheses 2, 3) observed between the pasture and reforested sampling zones. Changes above- and below-ground occurred abruptly on either side of the fence-line, with no evidence of a gradient with increasing distance on either side of the fence, as indicated by a lack of interaction between side of fence and distance of fence in the piecewise linear regression modelling. Results are now discussed in the context of above- and below-ground impacts of reforestation of this pasture.

4.1 *Vegetation and litter*

Abrupt changes in vegetation on either side of the fence line were evident both above- and below-ground. Although there were no differences in root biomass between the two zones, the composition (i.e. fine roots versus coarse roots) differed greatly. This reflects the absence of a grassy understory (fine roots) in the reforested zone, and the absence of trees (coarse roots) in the pasture zone. It is important to note, however, that some coarse roots were observed in the pasture immediately adjacent to the fence. Given that the trees grew along the fence-line, and roots can

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extend large distances from the base of a tree (Ashton, 1975; Toky and Bisht, 1992), this was to be expected.

325   Substantial amounts of litter were found on the reforested portion of the site, but not the pasture. The rate of litter accrual in the reforested zone was ≥ 10 t ha^{-1} post-reforestation. This rate of accrual is almost twice that observed in eucalyptus-dominated mixed species woody plantings in lower rainfall region in northern Victoria, Australia (Cunningham et al., 2015a), but within the range

330   expected for forests (Cunningham et al., 2015a; Pregitzer and Euskirchen, 2004). Although litter stocks are less stable than soil C, and at greater risk of loss (e.g. due to fire), they represent an important store of C in these systems and can be considered as potential "future soil C". The rate at which C in the litter layer enters the soil C pool will vary depending on a range of factors, including the chemical

335   composition of the litter, environmental and edaphic conditions and rates of biological activity (Couˆteauxa et al., 1995; Melillo et al., 1992; Smernik and Oades, 2001). Future studies would benefit for more detailed studies of litter composition, both at the level of tissue types (e.g. leaves, sticks, etc.), but also at a chemical level (e.g. cellulose and lignin content, etc).

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4.2 Soil physicochemical properties

Reforestation of the pasture was associated with higher levels of mineral N (NO_3^--N and NH_4^+-N) compared to the adjacent pasture. This is in contrast to other studies showing lower levels of mineral N following reforestation of agricultural lands

345   (Berthrong et al., 2009; Garten and Ashwood, 2002). One possible explanation is that fertilizer inputs in the pasture may have been low, or non-existent (data not

available). Alternatively, the higher levels of mineral N following reforestation may be a result of the presence of a number of tree species that from associations with N-fixing bacteria, including members of the genera *Acacia* and *Allocasuarina*. An

350 earlier study at a different site, but with a similar botanical composition, found that levels of N can be higher under N-fixing trees, but impacts on soil N (and C) varied with tree species (Hoogmoed et al., 2014). The higher levels of mineral N observed in the present study may also reflect greater turn-over of N in the soil, although this was not reflected in levels of potentially mineralizable N measured at the time of

355 sampling. Longer term studies of N cycling will be important in helping us to understand the dynamics of N-cycling in these systems.

Soil C stocks and concentrations were higher in the reforested portion of the site compared to the pasture. The increase in soil C was both substantial and rapid, with an increase observed 15 years after reforestation, and in a soil already high (for

360 the region) in C (i.e. 4-5% total C in the pasture soil). These high rates of C sequestration are likely a reflection of the relatively high rainfall and net primary productivity at this site. In addition to a general increase in total soil C, labile (permanganate oxidisable) C was also higher in the reforested portion of the site. This increase in labile C likely reflects C released from recently deposited plant litter

365 and root exudates. Finally, soil C:N ratios were higher in the reforested portion of the site than the pasture. This increase, which is consistent with earlier work (Berthrong et al., 2009; Cunningham et al., 2012) but has not been previously demonstrated in these systems, is likely to have important impacts on soil communities, as will now be discussed.

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4.3 Soil communities

Reforestation of the pasture resulted in significant changes in soil communities at the Total PLFA and structural levels (diversity and community composition). Although total microbial, fungal and bacterial PLFA, did not differ between sampling

375   zones, there was a shift towards greater fungal dominance (increased Fungal:Bacterial PLFA ratio) of the soil microbial community with reforestation. This increase in the Fungal:Bacterial PLFA ratio, which can be explained by a small, albeit non-significant increase in fungal PLFA in the reforested zone and bacterial PLFA in the pasture, was positively correlated with the soil C:N ratio, as in earlier studies

380   (Fierer et al., 2009; Waring et al., 2013).

Reforestation resulted in the development of a microbial community that was compositionally different from that of the adjacent pasture, consistent with earlier studies (Bossio et al., 2005; Hedlund, 2002; Wu et al., 2013). These differences were associated with differences in soil C, mineral N pools, C:N ratios and

385   soil EC, all of which are known to have an impact on microbial community composition and activity (e.g. Ng et al., 2014; Smuckler et al., 2010; Steenwerth et al., 2003). Whereas clear differences in microbial community composition between the reforested and pasture zones were observed in the ordination of the PLFA data (Figure 6a), there was little variation between sampling locations (i.e. distance from

390   fence) within the sampling zones. That is, there was no evidence of the adjacent land-use having an impact on microbial community composition immediately adjacent to the fence. Given the abrupt changes in vegetation and soil physicochemical properties between the sampling zones, this was not unexpected. Despite the changes in microbial community composition at the site level (i.e.

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395   separation sites on basis of land-use in Figure 6a), there was no clear indication of

these changes being associated with specific PLFA's (Figure 6b). This suggests that

changes in community composition were due to complex changes in the relative

abundance of a range of PLFA's; this is worthy of further investigation.

400   **5 Conclusions.**

The results presented here show that 15 years after reforestation of a former

pasture, substantial changes can be observed both above- and below-ground. These

changes occurred abruptly at the interface between the two land-use types, with no

interaction between land-use and distance from fence observed. Changes in the

405   microbial community at the total PLFA, fungal:bacterial PLFA ratio, and whole

community composition levels point to the relatively rapid development of a

microbial community following reforestation that is different form that of the

adjacent pasture. The functional implications of these changes, especially at the level

of the ecosystem services provided by soil biota, are of particular interest and

410   worthy of further investigation. Although not a primary focus of this study, there was

also a very strong difference in earthworm abundance between the sampling zones;

this observation is also in need of further detailed investigation in these systems

given the important role of earthworms in soil processes (Paul, 2006). While it is

important to not make broad generalizations beyond this site (or sampling depth)

415   about changes that occur below-ground following reforestation, it was clear at this

site that dramatic changes were observed in the upper soil layer of this site. As

landscapes become increasingly fragmented, understanding changes in above- and

below-ground components of ecosystems, especially at the interface between

landuse types, will become increasingly important.

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References

Aerts, R., Chapin, F.S.I., 2000. The mineral nutrition of wildplants revisited: a re-
435   evaluation of process and patterns. Adv. Ecol. Res 30, 1-67.
Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of
variance. Austral Ecol. 26, 32-46.
Ashton, D.H., 1975. The root and shoot development of *Eucalyptus regnans* F. Muell.
Aust. J. Bot. 23, 867-887.
440   Bardgett, R.D., Wardle, D.A., 2010. Aboveground-Belowground Linkages: Biotic
Interactions, Ecosystem Processes, and Global Change. Oxford University Press,
Oxford.
Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R.H.B., Singmann, H., Dai,
B., Grothendieck, G., 2015. lme4: Linear Mixed-Effects Models using 'Eigen' and S4.
445   Berthrong, S.T., Jobbagy, E.G., Jackson, R.B., 2009. A global meta-analysis of soil
exchangeable cations, pH, carbon, and nitrogen with afforestation. Ecol. Apps. 19,
2228-2241.
Bossio, D.A., Girvan, M.S., Verchot, L., Bullimore, J., Borelli, T., A., A., Scow, K.M., Ball,
A.S., Pretty, J.N., Osborn, A.M., 2005. Soil microbial community response to land use
450   change in an agricultural landscape of western Kenya. Microb. Ecol. 49, 50-62.
Bradshaw, C.J.A., Bowman, D.M.J.S., Bond, N.R., Murphy, B.P., Moore, A.D.,
Fordham, D.A., Thackway, R., Lawes, M.J., McCallum, H., Gregory, S.D., Dalal, R.C.,
Boer, M.M., Lynch, A.J.J., Bradstock, R.A., Brook, B.W., Henry, B.K., Hunt, L.P., Fisher,
D.O., Hunter, D., Johnson, C.N., Keith, D.A., Lefroy, E.C., Penman, T.D., Meyer, W.S.,
455   Thomson, J.R., Thornton, C.M., VanDerWal, J., Williams, R.J., Keniger, L., Specht, A.,
2012. Brave new green world—consequences of a carbon economy for the
conservation of Australian biodiversity. Biol. Converv. 161, 71-90.
Burger, B., Reich, P., Cavagnaro, T.R., 2010. Trajectories of change: riparian
vegetation and soil conditions following livestock removal and replanting. Austral
460   Ecol. 35, 980-987.
Busse, M.D., Sanchez, F.G., Ratcliff, A.W., Butnor, J.R., Carter, E.A., R.F., P., 2009. Soil
carbon sequestration and changes in fungal and bacterial biomass following
incorporation of forest residues. Soil Biology & Biochemistry 41, 220-227.
Chen, C.R., Condron, L.M., Xu, Z.H., 2008. Impacts of grassland afforestation with
465   coniferous trees on soil phosphorus dynamics and associated microbial processes: a
review. Forest Ecol. Manage. 255, 396–409.
Couˆteauxa, M.-M., Bottnera, P., Berg, B., 1995. Litter decomposition, climate and
liter quality. TREE 10, 63-66.
Cunningham, S.C., Cavagnaro, T.R., Mac Nally, R., Paul, K.I., Baker, P.J., Beringer, J.,
470   Thomson, J., Thompson, R.M., 2015a. Reforestation with native mixed-species
plantings in a temperate continental climate effectively sequesters and stabilizes
carbon within decades. Glob. Change. Biol. 21, 1552.
Cunningham, S.C., Mac Nally, R., Baker, P.J., Cavagnaro, T.R., Beringer, J., Thomson,
J.R., Thompson, R.M., 2015b. Balancing the environmental benefits of reforestation
475   in agricultural regions. Prespect. Plant Ecol. 17, 301-317.
Cunningham, S.C., Metzeling, K.J., Mac Nally, R., Thomson, J.R., Cavagnaro, T.R.,
2012. Changes in soil carbon of pastures after afforestation with mixed species:
sampling, heterogeneity and surrogates. Ag. Ecosyst. Environ. 158, 58-65.

de Alcântara, F.A., Buurman, P., Curi, N., Furtini Neto, A.E., van Lagen, B., Meijer,
480   E.L., 1996. Changes in soil organic matter composition after introduction of riparian
vegetation on shores of hydroelectric reservoirs (Southeast of Brazil). Soil Biol.
Biochem. 36, 1497-1508.
Fierer, N., Strickland, M.S., Liptzin, D., Bradford, M.A., Cleveland, C.C., 2009. Global
patterns in belowground communities. Ecol. Letts. 12, 1-12.
485   Forster, J.C., 1995. Soil nitrogen., in: Alef, K., Nannipieri, P. (Eds.), Methods in Applied
Soil Microbiology and Biochemistry. Academic Press San Diego, CA., pp. 79–87.
Garten, C.T., Ashwood, T.L., 2002. Landscape level differences in soil carbon and
implications for soil carbon sequestration. Glob. Biogeochm. Cy. 16, 61/61–61/14.
Halekoh, U., Højsgaard, S., 2014. pbkrtest: Parametric bootstrap and Kenward-
490   Roger-based methods for mixed model comparison.
Hedlund, K., 2002. Soil microbial community structure in relation to vegetation
management on former agricultural land. Soil Biol. Biochem. 34, 1299–1307.
Högberg, M.N., Högberg, P., Myrold, D.D., 2007. Is microbial community composition
in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three?
495   Oecologia 150, 590-601.
Hoogmoed, M., Cunningham, S.C., Baker, P.J., Beringer, J., Cavagnaro, T.R., 2014. N-
fixing trees in restoration plantings: effects on nitrogen supply and soil microbial
communities. Soil Biol. Biochem. 77, 203-212.
Jackson, L., Burger, M., Cavagnaro, T., 2008. Roots, nitrogen transformations, and
500   ecosystem services. Ann. Rev. Plant Biol. 59, 341–363
Lal, R., 2004. Carbon sequestration in dryland ecosystems. Environ. Manage. 33,
528–544.
MacDonald, C.A., Thomas, N., Robinson, L., Tate, K.R., Ross, D.J., Dando, J., Singh,
B.K., 2009. Physiological, biochemical and molecular responses of the soilmicrobial
505   community after afforestation of pastures with *Pinus radiata*. Soil Biol. Biochem. 41,
1642–1651.
Melillo, J.M., Aber, J.D., Muratore, J.F., 1992. Nitrogen and lignin control of
hardwood leaf litter decomposition dynamics. Ecology 63, 621-626.
Minoshima, H., Jackson, L.E., Cavagnaro, T.R., Sánchez-Moreno, S., Ferris, H., Temple,
510   S.R., Mitchell, J.P., 2007. Soil food webs and carbon dynamics in response to
conservation tillage in legume rotations in California. Soil Sci. Soc. Am. J. 71, 952-963.
Miranda, K.M., Espey, M.G., Wink, D.A., 2001. A rapid, simple spectrophotometric
method for simultaneous detection of nitrate and nitrite. Nitric Oxide 5, 62-71.
Mosse, K.M.P., Patti, A.F., Christen, E.W., Cavagnaro, T.R., 2012. Physicochemical and
515   microbiological effects of long- and short-term winery wastewater application to
soils. J. Hazard. Mat. 201, 219-228.
Murrell, P., 2005. R Graphics. Chapman & Hall/CRC Press., London, UK.
Ng, E., Rose, M.T., Schefe, C.R., Wilkinson, K., Smernik, R.J., Cavagnaro, T.R., 2014.
Does the chemical nature of soil carbon drive the structure and functioning of soil
520   microbial communities? Soil Biol. Biochem. 70, 54-61.
Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., Hara, R.B., Simpson,
G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2012. vegan: Community Ecology
Package.
Paul, E.A., 2006. Soil microbiology, ecology and biochemistry. Academic Press.

525 Paul, K.I., Polglase, P.J., Nyakuengama, J.G., Khanna, P.K., 2002. Changes in soil carbon following afforestation. Forest Ecol. Manage. 168, 241-257.

Potthoff, M., Jackson, L.E., Steenwerth, K.L., Ramirez, I., Stromberg, M.R., Rolston, D.E., 2005. Soil biological and chemical properties in restored perennial grassland in California. Restor. Ecol. 13, 61-73.

530 Pregitzer, K.S., Euskirchen, E.S., 2004. Carbon cycling and storage in world forests: biome patterns related to forest age. Glob. Change. Biol. 10, 2052-2077.

Quilty, J.R., Cattle, S.R., 2011. Use and understanding of organic amendments in Australian agriculture: a review. Soil Res. 49, 1-26.

Singh, B.K., Tate, K.R., Kolipaka, G., Hedley, C.B., Macdonald, C.A., Millard, P.,
535 Murrell, C.J., 2007. Effect of afforestation and reforestation of pastures on the activity and population dynamics of methanotrophic bacteria. Appl. Environ. Microb. 73, 5153–5161.

Smernik, R.J., Oades, J.M., 2001. Background Signal in Solid State ^{13}C NMR Spectra of Soil Organic Matter (SOM)--Quantification and Minimization. Solid State Nuclear
540 Mag. Reson., 74-84.

Smith, M., Conte, P., Berns, A.E., Thomson, J., Cavagnaro, T.R., 2012. Spatial patterns of, and environmental controls on, soil properties at a riparian-paddock interface. Soil Biol. Biochem. 49, 39-45.

Smuckler, S.M., Sánchez-Moreno, S., Fonte, S.J., Ferris, H., Klonsky, K., O'Geen, A.T.,
545 Scow, K.M., Steenwerth, K.L., Jackson, L.E., 2010. Biodiversity and multiple ecosystem functions in an organic farmscape Ag. Ecosyst. Environ. 139, 80-97.

Steenwerth, K.L., Jackson, L.E., Calderon, F.J., Stromberg, M.R., Scow, K.M., 2003. Soil microbial community composition and land use history in cultivated and grassland ecosystems in coastal California. Soil Biol. Biochem. 35, 489–500.

550 Toky, O.P., Bisht, R.P., 1992. Observations on the rooting patterns of some agroforestry trees in an arid region of north-western India. Agroforest. Sys. 19, 245-263.

Waring, B.G., Averill, C., Hawkes, C.H., 2013. Differences in fungal and bacterial physiology alter soil carbon and nitrogen cycling: insights from meta-analysis and
555 theoretical models. Ecol. Letts. 16, 887-894.

Waring, S.A., Bremner, J.M., 1964. Ammonium production in soil under waterlogged conditions as an index of nitrogen availability. Nature 201, 951-952.

Wilson, B.R., Moffatt, A.J., Nortcliff, S., 1997. The nature of three ancient woodland soils in southern England. J. Biogeog. 24, 633-646.

560 Wu, J.P., Liu, Z.F., Sun, Y.X., Zhou, L.X., Lin, Y.B., Fu, S.F., 2013. Introduced Eucalyptus urophylla plantations change the composition of the microbial community in subtropical China. Land Deg. Develop. 24, 400-406.

Zar, J.H., 1999. Biostatistical Analysis, 4th ed. Prentice Hall, New Jersey.

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Table 1. Summary of analysis of vegetation, soil physicochemical and biological properties by piecewise linear (a.k.a. broken-stick) regression modelling (see text).

Property	Estimate	S.E.	*t*-value	*p*-value (KR)
Vegetation				
Canopy Cover (%)	55.8	1.5	37.9	0.000006
Litter Mass (g dry weight)	1216.7	127.6	9.5	0.0007
Grassy Biomass (g dry weight)	-152.7	20.2	-7.6	0.002
Coarse Root Biomass (g dry weight)	2.9	0.6	4.7	0.009
Fine Root Biomass (g dry weight)	-4.4	1.1	-4.0	0.02
Total Root Biomass (g dry weight)	-1.6	1/2	-1.3	0.3
Soil Physicochemical				
Total C (%)	1.5	0.3	5.1	0.007
Labile C (%)	0.5	0.1	5.4	0.006
Soil C stock (t ha^{-1})	6.4	1.7	3.7	0.02
Total N (%)	0.06	0.02	2.9	0.04
Soil C:N	1.8	0.3	2.8	0.004
Bulk Density (g cm^{-3})	-0.04	0.06	-0.7	0.5
NH_4^+-N (μg g^{-1})	1.0	0.3	3.6	0.02
NO_3^--N (μg g^{-1})	7.5	1.4	5.4	0.006
PMN (μg g^{-1})	-3.1	8.2	-0.4	0.7
Colwell P (μg g^{-1})	0.3	2.1	0.1	0.9
pH	-0.15	0.05	-2.9	0.05
EC	0.06	0.01	9.5	0.0007
Soil microbes				
Total PLFA (nmol g^{-1})	-115.0	55.2	-2.1	0.1
Fungal PLFA (nmol g^{-1})	6.0	2.7	2.2	0.09
Bacterial PLFA (nmol g^{-1})	-60.5	25.2	-2.4	0.07
Fungal:Bacterial PLFAratio	0.05	0.008	6.4	0.003
Worm abundance (worms kg^{-1})	-6.6	1.4	-4.6	0.01

570

Figure Captions

Figure 1. Schematic diagram of field site and sampling regime. All soil and vegetation samples were taken from the center of each plot. N.B. diagram not drawn to scale.

575   The fence was a barbed wire fence 1 m in height and the width of a single line of wire.

Figure 2. Key above- and below-ground vegetation properties, including (a) litter mass, (b) grassy biomass, (c) coarse root biomass, (d) fine root biomass, and (e) total

580   root biomass, at each sampling location. N.B. sampling locations A, B and C, and D, E and F are located in the reforested, and pasture zones respectively (see text and Figure 1). Box plots display median, minimum, maximum, first and third quartiles, and any outliers; N = 6. Significant differences between land-use types (i.e. reforested, and pasture zones) were identified using piecewise linear regression

585   modelling (see text) and exist where P<0.05; see also Table 1 for full details of data analysis.

Figure 3. Soil physicochemical properties, including (a) total (%) C (b) labile (%) C, (c) soil C stock, (d) total (%) N, (e) soil C:N ratio, and (f) bulk density, at each sampling

590   location. N.B. sampling locations A, B and C, and D, E and F are located in the reforested, and pasture zones respectively (see text and Figure 1). Box plots display median, minimum, maximum, first and third quartiles, and any outliers; N = 6. Significant differences between land-use types (i.e. reforested, and pasture zones) were identified using piecewise linear regression modelling (see text) and exist

595   where P<0.05; see also Table 1 for full details of data analysis.

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Figure 4. Soil physicochemical properties, including (a) NH_4^+-N (b) NO_3^--N, (c) potentially mineralizable N (PMN), (d) Plant available (Colwell) P, (e) pH, and (f) EC, at each sampling location. N.B. sampling locations A, B and C, and D, E and F are located in the reforested, and pasture zones respectively (see text and Figure 1). Box plots display median, minimum, maximum, first and third quartiles, and any outliers; $N = 6$. Significant differences between land-use types (i.e. reforested, and pasture zones) were identified using piecewise linear regression modelling (see text) and exist where $P<0.05$; see also Table 1 for full details of data analysis.

Figure 5. Soil biological properties, including (a) total PLFA (b) bacterial PLFA, (c) fungal PLFA, (d) fungal:bacterial PLFA ratio, and (e) worms, at each sampling location. N.B. sampling locations A, B and C, and D, E and F are located in the reforested, and pasture zones respectively (see text and Figure 1). Box plots display median, minimum, maximum, first and third quartiles, and any outliers; $N = 6$. Significant differences between land-use types (i.e. reforested, and pasture zones) were identified using piecewise linear regression modelling (see text) and exist where $P<0.05$; see also Table 1 for full details of data analysis.

Figure 6. Nonmetric multidimensional scaling ordination of soil microbial communities (PLFA) (a) at all sampling locations, and (b) for specific PLFA's. N.B. sampling locations A, B and C, and D, E and F are located in the reforested, and pasture zones respectively (see text and Figure 1). 95% confidence ellipses are given for each sampling zone (A-F). Correlations of key soil properties with microbial

620   community composition are depicted by the vectors. The length and angle of the

vector represent the strength and direction of the relationship to the microbial

community. All vectors depict statistically significant correlations ($p < 0.001$; see

text). N.B. the first axis of the ordinations differs between plots (a) and (b); see also

Materials and Methods for additional details on data presentation and analysis.
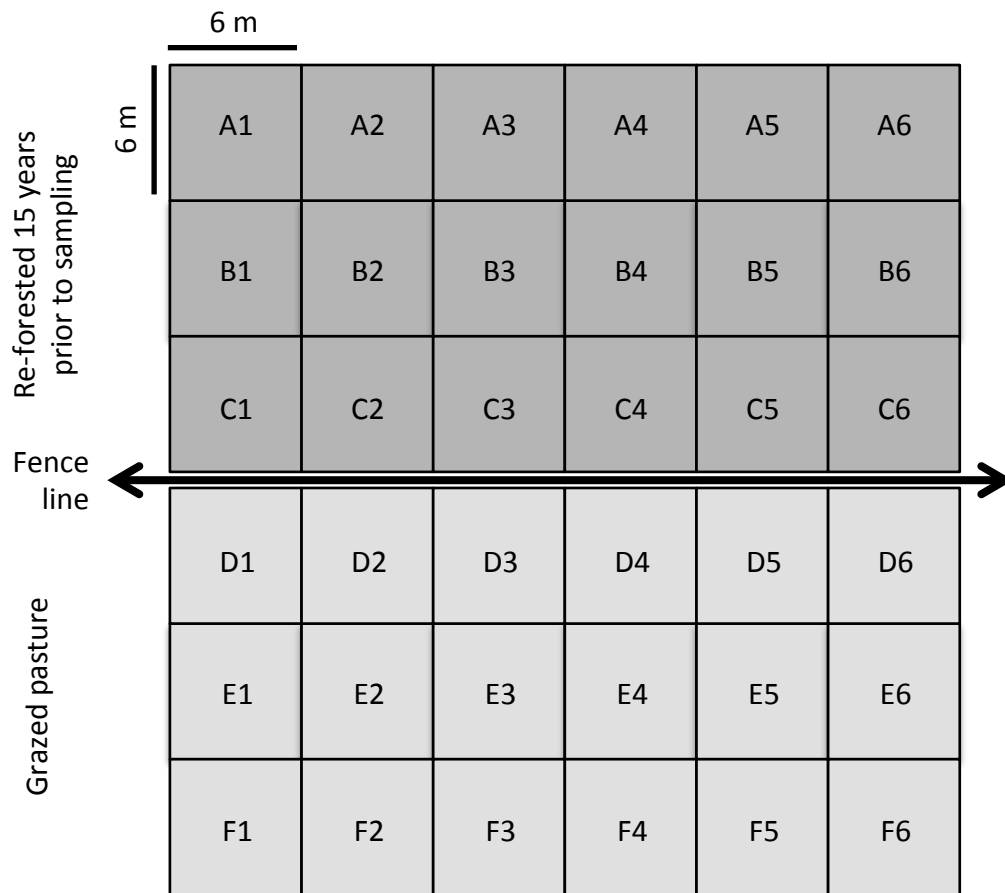
Figure 1.
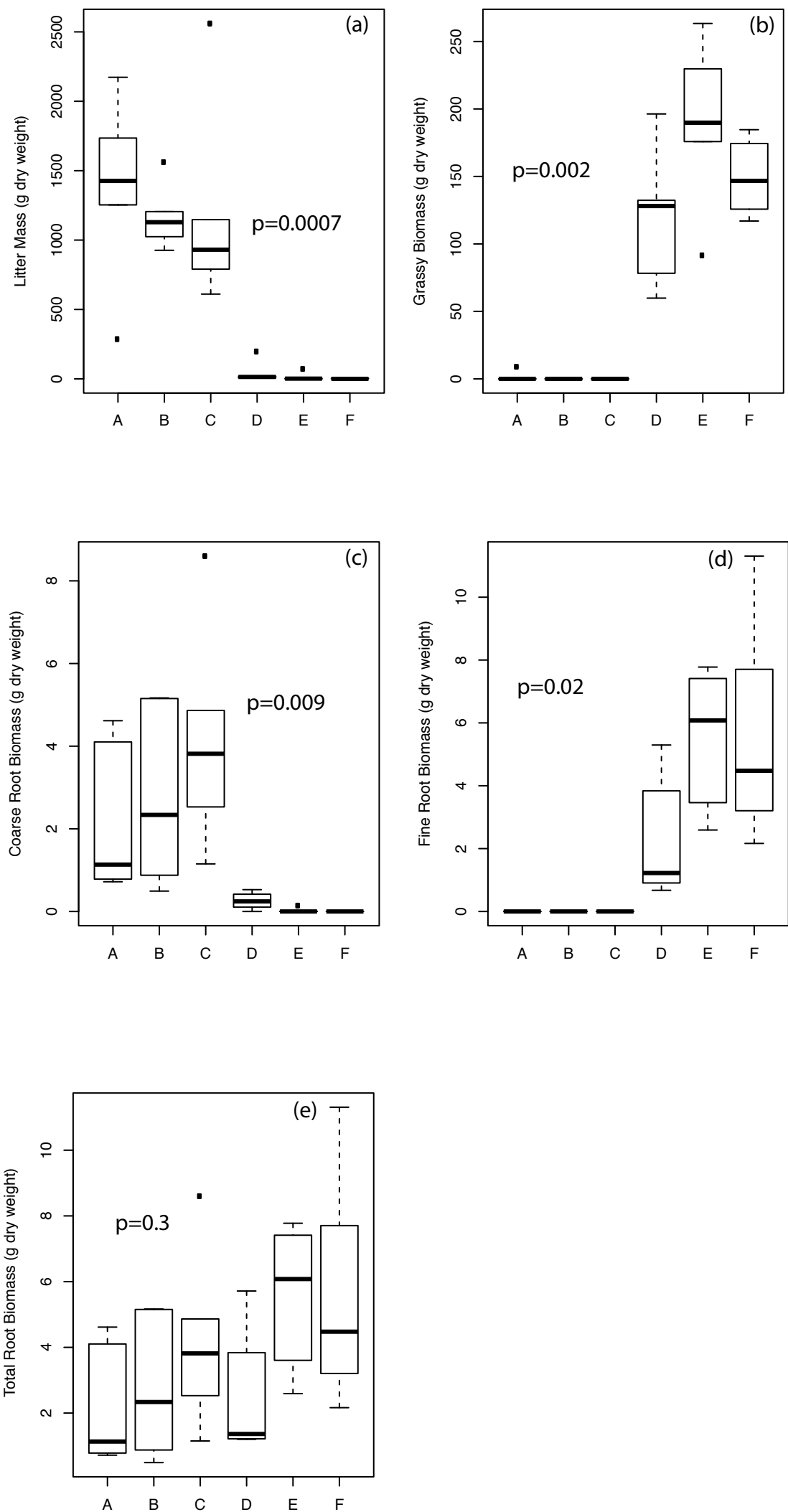
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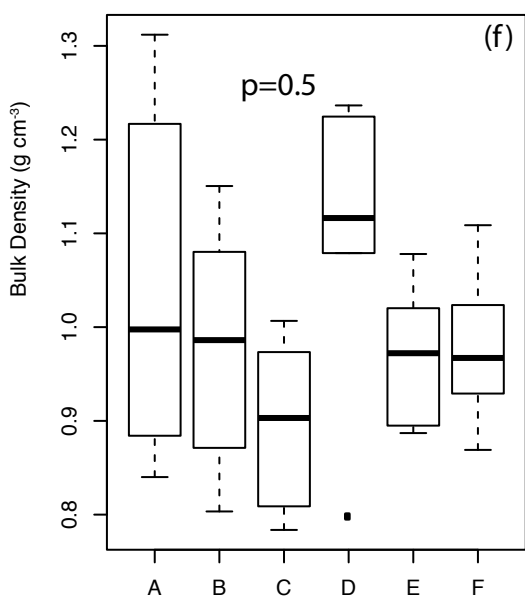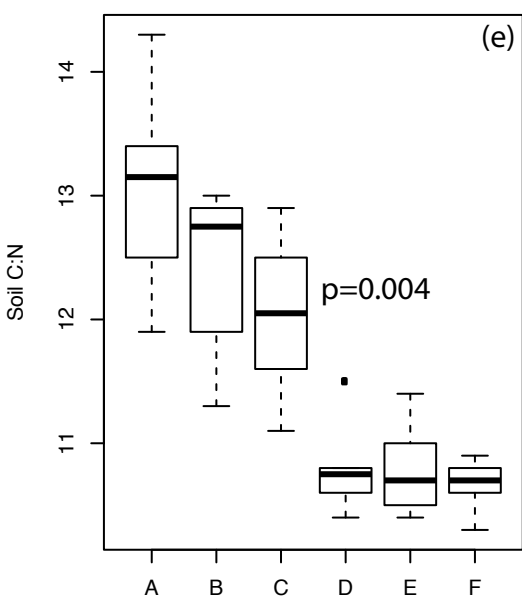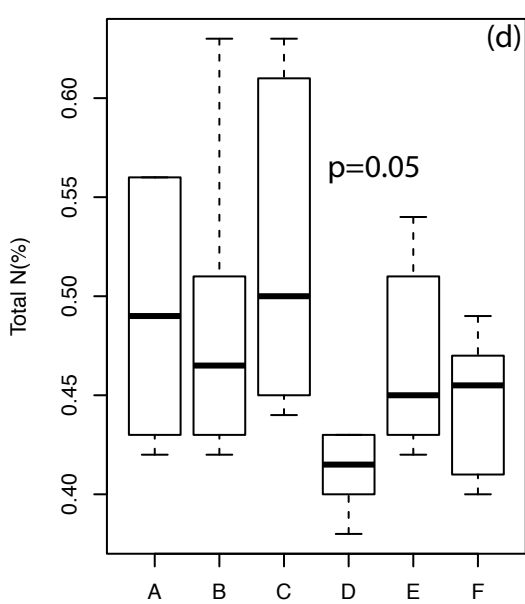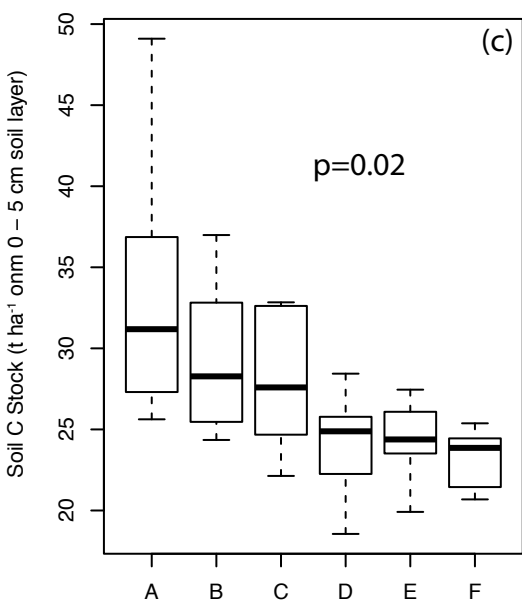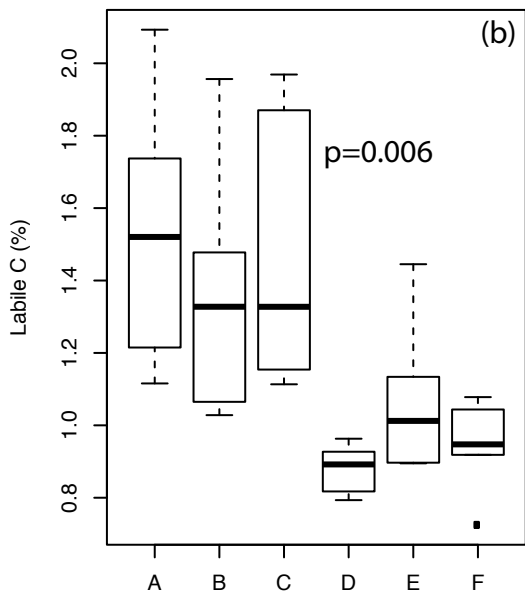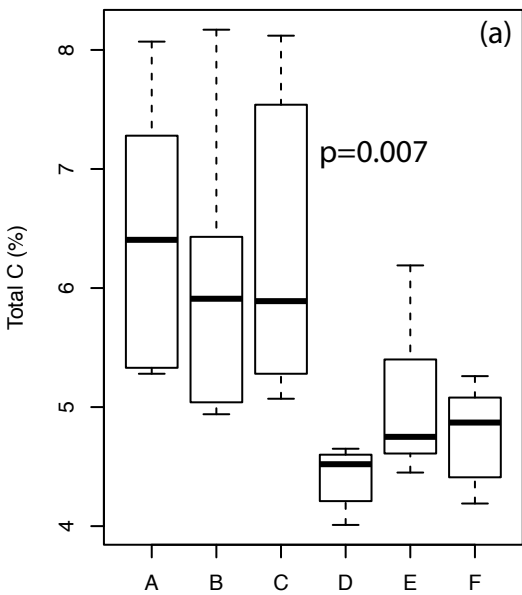
Figure 3

Figure 4

Figure 5

(a)

EC
C:N
Total C
NO$_3^-$-N
NH$_4^+$-N

A
C
B
E
D
F

Stress=0.12

NMDS2

NMDS1

(b)

EC
C:N
Total_C
NO$_3^-$-N
NH$_4^+$-N

2OHC10:0
3OHC12.0

2OHC16.0 a17.0

C12.0

C16.1_7trans

NMDS2

NMDS1

Figure 6