The Effect of Afforestation on Soil Microbes and Biogeochemistry across Multiple Scales

by

Sean T. Berthrong

University Program in Ecology
Duke University

Date:_____

Approved:

_____
Robert B. Jackson, Supervisor

_____
Emily S. Bernhardt

_____
Daniel D. Richter

_____
Christopher W. Schadt

_____
Rytas J. Vilgalys

Dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor
of Philosophy in the University Program in Ecology
in the Graduate School
of Duke University

2009

ABSTRACT

The Effect of Afforestation on Soil Microbes and Biogeochemistry across Multiple Scales

by

Sean T. Berthrong

University Program in Ecology
Duke University

Date:_____

Approved:

_____
Robert B. Jackson, Supervisor

_____
Emily S. Bernhardt

_____
Daniel D. Richter

_____
Christopher W. Schadt

_____
Rytas J. Vilgalys

An abstract of a dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University Program in Ecology in the Graduate School of Duke University

2009

Abstract

Afforestation, the conversion of historically treeless areas into forests, is a rapidly spreading land-use change with the potential to sequester carbon. Afforested plantations typically feature fast growing exotic tree species that give landowners rapid returns. The efficient growth of plantations compared to less intensively managed forests also can provide greater timber yields in a smaller area. This increased efficiency in turn could require fewer acres to meet global forest product demands and could also reduce the need to log intact primary forests. Reduced primary forest harvest and high primary productivity make afforestation a highly efficient carbon sequestration tool.

However, the rapid growth and planting disturbance due to afforestation can have deleterious effects on soils and hydrology that undermine its benefits in some locations. The effects on hydrology include depletion of groundwater and reduced or complete elimination of surface water flow. Additionally, groundwater use can lead to increased concentrations of salts and trace metals in soil that could be deleterious for future plant productivity. Plantations have also been shown to acidify surface soils and stream water and to reduce soil carbon and nitrogen.

Despite the known effects of afforestation on soils, there has been little research on the mechanisms controlling these effects. For instance, there have been few studies on the effects of afforestation on soil microbes which mediate most biogeochemical processes. There is also little knowledge on what controls the effects of afforestation on soil carbon and nitrogen, vital indexes of soil quality, across regions with high levels of

afforestation. The overarching goal of this dissertation is to examine the effects of afforestation on soils, microbes, and biogeochemical processes across local, regional and global scales. Understanding the mechanisms by which afforestation alters soils and biogeochemical cycling and how these mechanisms change across different scales will aid in evaluating the true costs and benefits of afforestation. These results will be useful in determining if the benefits of afforestation will continue to outweigh its costs in the long-term.

The goal of Chapter 1 is to evaluate how afforestation across the globe affects mineral soil quality, including pH, sodium, exchangeable cations, organic carbon, and nitrogen, and to examine the magnitude of these changes in regions where afforestation rates are high. To control for different initial soil conditions across the globe, I examined paired sites of afforested plantations and controls. Controls included land-use types that are frequently afforested, such as grasslands, shrublands, and pastures. I also examined potential mechanisms to reduce the impacts of afforestation on soils and to maintain long-term productivity. Across diverse plantation types (153 sites) to a depth of 30cm of mineral soil, I observed significant decreases in nutrient cations (Ca, K, Mg), increases in sodium (Na), or both with afforestation. For the global dataset, afforestation reduced soil concentrations of the macronutrient Ca by 29% on average compared with native controls ($p<0.05$). Afforestation by *Pinus* alone decreased soil K by 23% ($p<0.05$). Overall, plantations of all genera also led to an average 71% increase of soil Na ($p<0.05$). Average pH decreased 0.3 units ($p<0.05$) with afforestation. Afforestation caused a 6.7% and 15%

($p<0.05$) decrease in soil C and N content respectively, though the effect was driven principally by *Pinus* plantations (15% and 20% decrease, $p<0.05$). Carbon to nitrogen ratios in soils under plantations were 5.7-11.6% higher ($p<0.05$). The major implication of these results are that in several regions with high rates of afforestation, cumulative losses of C, N, Ca, and Mg are likely in the range of tens of millions of metric tons. The decreases indicate that trees take up considerable amounts of nutrients from soils; harvesting this biomass repeatedly could impair long-term soil fertility and productivity in some locations. Based on this study and a review of other literature, I suggest that proper site preparation and sustainable harvest practices, such as avoiding the removal or burning of harvest residue, could minimize the impact of afforestation on soils. These sustainable practices could in turn slow erosion, organic matter loss, and soil compaction from harvesting equipment, maintaining soil fertility to the greatest extent possible.

Soil microbes are highly diverse and control most soil biogeochemical reactions. Given the observed changes in Chapter 1, in Chapters 2 and 3 I examined how microbial functional genes and biogeochemical pools responded to the altered chemical inputs accompanying afforestation. I examined paired native grasslands and adjacent *Eucalyptus* plantations (previously grasslands) in Uruguay, a region that lacked forests before European settlement. Along with measurements of soil carbon, nitrogen, and bacterial diversity, I analyzed functional genes using the GeoChip 2.0 microarray that simultaneously quantified several thousand genes involved in soil carbon and nitrogen cycling. Plantations and grasslands differed significantly in functional gene profiles,

bacterial diversity, and biogeochemical pool sizes. Afforestation decreased both bacterial diversity and richness compared to grasslands, though diversity remained relatively high. Most grassland functional gene profiles were similar, but plantation profiles generally differed from grasslands due to differences in functional gene abundance across many microbial groups. Eucalypts decreased ammonification and N-fixation functional genes by 11% and 7.9% ($p<0.01$) which correlated with decreased microbial biomass N and more NH_4^+ in plantation soils. Chitinase, an important carbon polymer degrading enzyme, decreased in functional gene abundance 7.8% in plantations compared to grasslands ($p=0.017$), and C polymer degrading genes decreased by 1.5% overall ($p<0.05$), which likely contributed to 54% ($p<0.05$) more C in undecomposed extractable soil pools and 27% less microbial C ($p<0.01$) in plantation soils. In general, afforestation altered the abundance of many microbial functional genes corresponding with changes in soil biogeochemistry. These changes were driven by shifts in the whole community functional gene profile, not just one or two constituent microbial taxa. Such changes in microbial functional genes correspond with altered C and N storage and have implications for long-term productivity in these soils.

The area studied in Chapters 2 and 3 lies near the middle of a precipitation gradient that stretches across the Rio de la Plata grasslands. In Chapter 4 I studied if the effects of afforestation on soil C and N from Chapters 2 and 3 varied with different precipitation levels. The effect of afforestation on soil C has been shown to depend on mean annual precipitation (MAP), with drier sites gaining C and wetter sites losing C

with afforestation. This precipitation dependence of soil C changes with afforestation may be controlled by changes in soil nitrogen (N) cycling. In particular, loss of N due to leaching after afforestation could lead to soil C losses. However, the link between C and N changes due to afforestation has primarily been suggested by models and to my knowledge has never been explicitly tested across a precipitation gradient. The goal of this study was to test how precipitation affects changes in labile and bulk pools of soil C and N across a precipitation gradient, which will provide novel insight into the linkage between land-use change, different pools of soil C and N, and precipitation. I conducted this study across a gradient of precipitation in the Rio de la Plata grasslands of Argentina and Uruguay which ranged from 600mm to 1500mm of precipitation per year. The sites were all former grasslands that had been planted with *Eucalyptus*. I found that changes in bulk soil C and N were related to MAP with drier sites gaining and wetter sites losing C and N (R^2=0.59, p=0.003), which supports the idea that N losses are strongly linked to C losses with afforestation. C and N in microbial biomass and extractable pools followed similar patterns to bulk soil C and N. Interestingly, losses of C and N decreased as the plantations aged, suggesting that longer rotation times for plantations could reduce potential soil carbon and nitrogen losses. These results indicate that afforestation is still be a valuable tool for carbon sequestration, but calculations of the benefits of afforestation must take into account site factors such as age and precipitation to accurately calculate total sequestration benefit and ensure continued high productivity and carbon sequestration.

In conclusion, afforestation could be an effective tool for carbon sequestration. However, its benefits need to be carefully weighed against its costs for soil such as reduced microbial diversity, decreased soil microbial functional capacity, losses of soil organic matter, and nutrient depletion. Careful management and consideration of afforestation is needed to ensure the greatest benefits with the least long-term damage to soils.

Contents

List of Tables

List of Figures

Acknowledgements

I have been very fortunate to have had two excellent advisors who have helped shape my graduate career and who deserve many thanks for all their help over the years. Working with Adrien Finzi on my undergraduate thesis and Master's project was what first piqued my interest in soils and ecosystem ecology. Rob Jackson encouraged me to work abroad and gave me the intellectual and funding freedom to pursue my ideas.

My graduate committee also deserves many thanks for excellent mentorship. Emily Bernhardt taught me to think about ecosystems as interconnected wholes. Dan Richter helped me understand soils and their development along with ecosystems. Chris Schadt introduced me to the powerful techniques and intricacies of metagenomics. And Rytas Vilgalys taught me that fungi are far more than just mushrooms.

I have benefited greatly from the assistance and patience of many people in several different labs. Kelly Adamski, Cathy Johnson, Joe Williams, Sukhi Wahla, Tracey Crocker, Yael Erlitz, and Will Cook all assisted me over the years, and this dissertation would not have been possible without their help. Jennifer Reeve, Hector Castro, and Dawn Klingeman from Oak Ridge National Laboratory mentored me in my metagenomic projects. Jason Jackson, Jeri Parrent, Tim James, and Greg Bonito allowed me to share space with them in the Vilgalys lab and helped teach me the basics of soil molecular biology.

I cannot thank my colleagues from Argentina and Uruguay enough without whom I would have never been able to complete my field work. Marcelo Nosetto, Matías

Carambula, German Mazzo, Rozy Aragon, Juanma Piñeiro, Elke Noellemeyer, Camilo Bagnato all helped me navigate many backroads and out of the way sites. Gervasio Piñeiro and Esteban Jobbágy were invaluable colleagues in helping me arrange and plan my trips.

At Duke I had considerable administrative help without which I would have never been able to navigate my graduate years. Nancy Morgans, Anne Lacey, Jim Tunney, Meg Stephens, and Jim Haggard made sure my payroll was functional and managed my grants and awards. Susan Gerbeth-Jones, Andrew Turnier, David Friend, and John Beane provided invaluable computer help.

I also must thank all my friends and family. They are steadying influences, without which I would have never been able to weather the ups and downs of a Ph.D. I thank my parents, John and Evelyn, for all the years of encouraging and enabling me to pursue every educational opportunity. The Tuesday Lunch crowd, the daily lunch bunch, Biology Basketball team, and Wednesday squash club all helped to make graduate life more pleasant, and thanks especially to Libby for all her love and support.

Last, but not least, I thank the many people, agencies, and foundations that funded this work. I thank Milton Constantin, Jeff Gaffney, Nancy Marley, and all the people at the Department of Energy's Global Change Education Program. I also thank the Tinker Foundation, the Mellon foundation, the National Science foundation, the Keever endowment, and the Duke University Biology Department, and Graduate School for funding my research and field work.

The research in chapter 1 was conducted in collaboration with Esteban G. Jobbágy and Robert B. Jackson. Daniel D. Richter, the Jackson lab, and two anonymous reviewers assigned by *Ecological Applications* provided helpful suggestions on the manuscript. Will Cook helped assemble the database, and Justin Baker provided valuable statistical advice.

The research in chapters 2 and 3 was conducted in collaboration with Christopher W. Schadt, Gervasio Piñeiro, and Robert B. Jackson. I thank the Martirena Family, the Ferber Family, Elvecia Huelmos de Pereira, Eduardo Coria, Enrique Rodas, Anibal and Cristina Fernandez, Pedro Marmo, and Aldo Rudi for allowing us to conduct research on their properties. We thank Kathleen Farley, Esteban Jobbágy, and Sheila Palmer for valuable help locating and developing the sites and field work. Three anonymous reviewers from *Applied and Environmental Microbiology* provided useful feedback on a previous version of this chapter.

The research in chapter four was conducted in collaboration with Gervasio Piñeiro, Esteban Jobbágy, and Robert B. Jackson. I also thank our other Argentine and Uruguayan colleagues for valuable field assistance for this chapter.

1. Afforestation Decreases Soil Exchangeable Cations, pH, Carbon, and Nitrogen: A Global Meta-analysis

1.1 Introduction

Afforestation, planting trees on land that has not previously been forested for at least 50 years, has been featured as a potential mechanism to sequester carbon dioxide (Vitousek 1991, Houghton and Hackler 1999, Wright et al. 2000, McCarl and Schneider 2001, Hoffert et al. 2002, Jackson et al. 2002, Jackson and Schlesinger 2004, Pacala and Socolow 2004, Lal 2008). Afforestation has also gained attention as a means for developed countries to mitigate their carbon emissions through offset programs such as the Clean Development Mechanism of the Kyoto Protocol. However, the fast growth rates of plantations compared to other vegetation types can lead to higher demand for soil nutrients (Mendham et al. 2003b, Merino et al. 2004, Zhang et al. 2004). Depending on how sustainably the harvested biomass is managed, its frequent removal can deplete soil nutrients from these ecosystems, lowering primary productivity of future rotations and reducing their long-term potential as carbon sinks (Bi et al. 2007). The goal of our study was to quantify the effects of afforestation on soil nutrients and to suggest forestry practices that can ameliorate any negative impacts of afforestation.

Globally, the scope of afforestation has rapidly increased in recent decades. As of 2005, roughly 140 million ha were grown as afforested plantations, with ~2.8 million more hectares afforested per year (FAO 2006b). The afforestation rate is likely to increase, since many plantations typically produce greater economic returns than native forests, particularly those of non-native *Pinus* and *Eucalyptus* (Cubbage et al. 2006).

With a potential 34 million more hectares afforested by 2020, managers need to understand the long-term effects of plantation establishment on soils and how this could affect long-term productivity.

Plantations have many potential economic and ecological benefits beyond wood products and carbon sequestration. For example, afforestation of marginal agricultural and grazing lands can reduce soil erosion and diversify and improve revenues (Geary 2001, Cubbage et al. 2006). Plantations may grow faster than natural forests and produce more timber products per year, reducing the amount of land needed to meet wood demand globally (Wright et al. 2000). Sustainable harvest of afforested plantations could therefore reduce the loss of primary forest, preserving biodiversity (ABARE and Jaako-Poyry 1999, FAO 2001b). Forested plantations already contribute >35% of the world's industrial wood products, even though plantations account for only ~4% of the global forested area (ABARE and Jaako-Poyry 1999, FAO 2001b).

Afforestation can occur in many different forms. The UN Food and Agriculture Organization (FAO) defines afforestation as either the establishment of forests on historically treeless areas or on land cleared of native forests for at least 50 years. Although afforestation is typically conducted with fast growing, exotic tree species, native species are used in a smaller subset of afforested areas. These different scenarios (treeless vs. deforested regions, exotic vs. native species) can potentially lead to different trajectories of ecosystem change, including different rates of C storage, nutrient depletion, and biomass increment; nevertheless, convergent trends such as the redistribution of soil nutrients to tree biomass and soil acidification may emerge. In this

study we include data on many different pathways of afforestation in order to find effects that are common across afforestation scenarios.

By redistributing nutrients from soils to biomass, afforestation has potentially strong effects on plant macronutrients (Jobbágy and Jackson 2003, 2004b, Farley et al. 2008b). Nutrient uptake and subsequent harvest and removal of biomass can deplete cations, including calcium (Ca), magnesium (Mg), and potassium (K) (Richter et al. 1994, Mendham et al. 2003a, Zhang et al. 2004). Jobbágy and Jackson (2003) showed that the redistribution of base cations from soils to biomass acidified the surface soil of *Eucalyptus* plantations in Argentina; this phenomenon was also observed globally for *Pinus* and *Eucalyptus* plantations (Jackson et al. 2005). Sodium redistribution caused by afforestation with *Eucalyptus* plantations can even salinize soils in some locations; in the Argentine pampas, for instance, afforestation caused a 4-19 fold salinization of soils and ground water compared to native grasslands (Jobbágy and Jackson 2004a).

Previous research, primarily in New Zealand and Australia, has shown that afforestation can significantly alter both soil carbon (C) and nitrogen (N) stocks. *Pinus* and *Eucalyptus* afforestation was shown to decrease soil carbon content by an average of 10% (Davis and Condron 2002, Guo and Gifford 2002). In the same region, a study of afforestation with *Pinus radiata* found a reduction in total soil N by more than 45% (Parfitt et al. 2003a, Parfitt et al. 2003b). In addition, afforestation in Australia was shown to slow the rate of N supply by soil (N mineralization) to plant-available forms (O'Connell et al. 2003). The potential loss of C and N from soils with afforestation

suggests that future plantation productivity on these soils might be less than in the initial rotations.

This study examines the effects of afforestation on soils through a formal meta-analysis of soil changes, including soil cations, acidity, carbon, and nitrogen. Our study examines data from numerous different families and genera of plantation species from globally distributed sites. Based on the results of site-specific and regional studies, we predict that afforestation will lead to more acidity and Na in soils and lower nutrient cations, carbon, and nitrogen contents. We discuss potential management tools to reduce the long-term impacts of afforestation on soil and to enable plantations to continue as productive, sustainable sinks for carbon sequestration.

1.2 Methods

1.2.1 Literature search and Calculations

Data sources on the effects of afforestation on soil were assembled from the scientific literature through the end of 2007. We contacted investigators and searched the online databases Web of Science (http://isiknowledge.com) and Agricola (http://agricola.nal.usda.gov) for available papers. We limited the search parameters to papers whose title, abstract, or keywords referred to afforestation or plantation; and soil; and grass, grassland, pasture, or shrubland. Of the papers returned by the search, we selected those that had a paired-sample or chronosequence design; the final data set contained 71 papers with 153 independent sites (Table 1).

The data set contains analyses of afforestation with many different tree species, which we grouped into the following categories: *Eucalyptus* spp., *Pinus* spp.,

angiosperms other than *Eucalyptus* (henceforth "other angiosperms"), and conifers other than *Pinus* (henceforth "other conifers"). We chose these groupings since *Eucalyptus* and *Pinus* were the most commonly planted genera (FAO 2006a). The proportion of studies in each genus or type in our analysis is similar to the global distribution of genera and types globally (FAO 2001a). For instance, ~50% of sites in this analysis are *Pinus* plantations compared to ~45% of the afforested area globally (Table 1)(FAO 2001b, a). The majority of the original vegetation types in our database were grasslands or pastures (73%), followed by abandoned or degraded agricultural lands (25%), whereas only three sites (2%) corresponded to shrublands with incomplete canopy closure.

Across studies, the depth of the mineral soil varied greatly from 2.5 to 100 cm. There were only six studies with data on forest floor organic horizons, which together with inconsistent definitions of organic horizons, led us to restrict our analysis to mineral soils. We further restricted our analysis to the top 30cm of mineral soil since that depth increment contains the highest concentrations of soil organic matter and has the strongest reaction to afforestation (Jobbágy and Jackson 2000).

If a study reported C or N as a percent of soil mass, we converted the values to metric tons (C or N) ha^{-1} by multiplying the % carbon or nitrogen by 100, bulk density (g soil cm^{-3}), and sampling depth (cm). Some of the studies did not report soil bulk density. Initially, we attempted to estimate bulk density from soil texture, but this, too, was rarely reported. Therefore, where needed we estimated bulk density (g soil cm^{-3}) using equation 1 (Post and Kwon 2000):

(1) $$BD = \frac{100}{\frac{OM\%}{0.244} + \frac{100 - OM\%}{1.64}}$$

where OM% is the percent of soil organic matter, assuming that organic matter equals percent soil carbon divided by 0.58 (Mann 1986).

For all soil variables in this meta-analysis (soil pH, cations, C, and N) there were multiple methods used by different studies. Cations were measured predominately by flame atomic absorption spectroscopy (84%) with a smaller number (16%) by inductively coupled plasma mass spectrometry. Soil pH was measured with pH electrodes in deionized water (77%), 0.01M $CaCl_2$ (21%), and $BaCl_2$ (2%). Soil C and N were determined predominately by combustion (70%), with 28% of C analyses by Walkley-Black/dichromate digestion, 2% by loss on ingnition, and 23% of N analyses by Kjeldahl digestion. To compensate for potential methodological artifacts, we used the proportion response (response ratio of afforested value/control value described below) of variables for each site.

1.2.1 Meta-Analysis

Our goal was to determine the mean effect of afforestation on soil variables. We calculated the effect size of afforestation on a soil variable for a given site as a response ratio, $r = X^E / X^C$, where X^E is the mean value for a site of a given soil variable under afforestation and X^C is the mean value of the same site's control (Hedges et al. 1999, Gurevitch and Hedges 2001). To match the scale of pH (logarithmic) to the linear scales of all other variables in the meta-analysis, we transformed to hydrogen ion concentration values ($10^{-pH\ units}$) to calculate response ratios, yet we present the results of the meta-

analysis in pH units for ease of interpretation. The response ratio was then transformed by the natural logarithm to make the values linear, so that an increase in a variable due to afforestation would be proportional and on the same scale as a decrease.

Ideally, the meta-analysis of response ratios should be weighted by the sample size and variances for a study. However, for some studies we were unable to determine independent sample sizes and variances, and the data were frequently not normally distributed. To compensate for small sample sizes, variance, non-normality, and to include as many studies as possible, we used a non-parametric approach to statistical analyses, i.e., an unweighted meta-analysis (Gurevitch and Hedges 2001, Guo and Gifford 2002). Mean effect size (log response ratio) and 95% confidence intervals were generated by bootstrapping (10,000 iterations) in SAS (SAS Institute Inc., Cary, NC, USA) (Efron and Tibshirani 1993). A mean effect size was significantly different from zero if its 95% confidence interval did not overlap zero (Gurevitch and Hedges 2001). For ease of interpretation, we present the means of the variables for control and afforested rather than response ratios in some figures. This presentation is intended to provide a reasonable range of values on an absolute scale. However, the means alone of all sites in this study can mask the underlying effect size of afforestation due to variability in initial control values. To show the magnitude of the effect of afforestation while controlling for differences in initial control values, we also present the average response ratios (transformed to percent change due to afforestation) with 95% confidence intervals in square brackets. These percentages represent the average percent change for a given grass- or shrubland that has been afforested.

We also tested for correlations among the response ratios of the measured variables. Since several distributions were not Gaussian, we used the non-parametric Spearman's rank correlation coefficient. Correlation coefficients and tests of statistical significance were calculated using proc corr in SAS.

1.3 Results

1.3.1 Exchangeable Cations

Across diverse plantation types, we observed decreases in nutrient cations (Ca, K, Mg), increases in sodium (Na), or both concurrently with afforestation. Afforestation by *Eucalyptus*, *Pinus*, other conifers, and all vegetation types combined decreased soil Ca relative to controls by 37%, 31%, 16%, and 29% respectively (each analysis $p<0.05$, Figure 1, Table 3). Afforestation with other conifers decreased Mg concentration by 52% ($p<0.05$, Figure 1, Table 3). However, there was no significant effect of afforestation on soil Mg attributable to afforestation with *Eucalyptus*, *Pinus*, or other angiosperms. Afforestation with *Pinus* led to 23% lower concentrations of K (Figure 1, Table 3). Afforestation with *Eucalyptus*, other angiosperms, and *Pinus* raised soil Na relative to controls by 250%, 32%, and 81%, respectively (Figure 1, Table 3). Afforestation by other conifer genera did not induce a significant change in soil Na levels. If all afforestation types were combined, then soil Na concentration increased 71% relative to controls.

1.3.2 Soil pH and Base Saturation

Exchangeable cation concentrations and soil pH are closely linked, and plantations also typically increased acidity and lowered exchangeable base cation saturation. Afforestation with *Pinus*, other conifers, and all vegetation combined reduced

base saturation by 21%, 10%, and 17% respectively ($p<0.05$ for each, Figure 2, Table 3). There was no effect of *Eucalyptus* or other angiosperms on base saturation (Figure 2). Afforestation with *Eucalyptus* acidified soils versus controls from pH 6.0 to 5.3 (Figure 2, Table 3). *Pinus* plantations led to a moderate acidification from pH 5.7 to 5.4, other conifer plantations acidified soil from pH 4.6 to 4.4, and across all plantation types from 5.6 to 5.3 ($p<0.05$, Figure 2, Table 3).

Across all plantation types, there was a negative correlation (Spearman's ρ=-0.58, $p=0.006$) between the response ratios for hydrogen ion concentration (soil pH) and base saturation (Figure 3). We also found a negative correlation (Spearman's ρ=-0.56, $p<0.0001$) between response ratios for hydrogen ion concentration and calcium (Figure 3). Greater decreases in base saturation and calcium due to afforestation therefore correlate with greater decreases in pH.

1.3.3 Carbon and Nitrogen

Overall the effects of afforestation on soil organic C and N were the greatest for *Pinus* plantations. Afforestation with *Pinus* decreased soil C stocks (g m^{-2}) by 15% on average (Figure 4, Table 3). However, there was no significant change in soil C with afforestation for *Eucalyptus*, other conifers, or other angiosperms. Similarly, soil N decreased with afforestation in *Pinus* plantations and overall by 20% and 15%, respectively, but overall changes were driven exclusively by changes for *Pinus*. There was no significant change in soil N due to *Eucalyptus*, other angiosperms, or other conifers (Figure 4, Table 3). Soil C:N increased significantly by 5.7%, 11.6%, 5.9%, and

9.9% with afforestation by *Eucalyptus*, *Pinus*, other conifers, and all types combined, respectively (Figure 4, Table 3).

1.3.4 Afforestation Effects across Regions with High Rates of Afforestation

The consistent direction and magnitude of effects across many genera and regions suggest that these effects are fairly general and may provide a reasonable estimate for global effects of afforestation on soil nutrients. Based on this study and on United Nations Food and Agriculture Organization estimates of plantation area in regions with high rates of afforestation, we calculated the total amount of nutrients gained or lost from soils globally (Table 4). Given that much of the lost nutrient stock is likely stored in biomass and litter, these numbers represent large potential exports of harvested nutrients (and additions of Na). The largest losses of C and N from soils were in North and Central America with considerable losses also in China and Europe (Table 4). China and North and Central America lost the most Ca and China gained the most Na (Table 4).

1.4 Discussion

Our afforestation analysis revealed consistent effects on soil properties across a broad range of locations and tree genera. Depletion of exchangeable cations was observed in three of four plantation types. Increases in soil Na were also found across three plantation types (Figure 1). Consistent with the findings of Jackson et al. (2005), *Eucalyptus* and *Pinus* plantations significantly acidified soils (Figure 2); we also found that other conifers acidified soils (Figure 2). Soil C and N levels decreased, but only for *Pinus* plantations; however, afforestation with either *Eucalyptus* or *Pinus* significantly

raised the soil C:N (Figure 4). The fact that most of the significant differences were due to *Eucalyptus* or *Pinus* afforestation could be due either to the great availability of studies for those genera (and hence a greater power to detect differences), or because *Eucalyptus* or *Pinus* plantations are often not native to the region in which they are planted. The higher growth rates of these exotic plantations could lead to more drastic changes in soils than plantation using a native species of tree.

1.4.1 Exchangeable Cations and Sodium

Several potential mechanisms may explain the differences in exchangeable cations observed with afforestation (Figure 1): uptake outpacing rates of supply, increased leaching to groundwater, or decreases in mineral weathering. However, in the Argentine pampas, afforestation of grasslands with *Eucalyptus camaldulensis* was found to decrease mineral soil cations by redistribution from soil to biomass pools (Jobbágy and Jackson 2003). This redistribution of cations by *Eucalyptus* is attributable to increased cation uptake of plantations compared with the native grasses (Jobbágy and Jackson 2003). This mechanism likely explains our study's finding of the depletion of Ca, K, and Mg from soils across many different plantation genera (Figure 1). Additionally, Jobbágy and Jackson (2003) found decreased cation exchange capacity (CEC) with losses in cations; the lower CEC could indicate a reduced capacity of soils to store cations, which suggests that new inputs of cations (gypsum or lime fertilizer) might not always increase Ca stocks to pre-afforestation levels.

If we estimate the change in stocks of exchangeable soil cations (kg/ha) from the data in Figure 1 and the average bulk density and sampling depth from the dataset, then

the mean loss of Ca from soils due to afforestation with *Eucalyptus*, *Pinus*, and other conifers is 0.53, 1.25, and 0.34 Mg ha^{-1}, respectively. These soil losses are within the range of published values for total biomass Ca; for example, several *Eucalyptus* plantations in Australia had an estimated total biomass Ca of 0.32 Mg ha^{-1} and a *Pinus radiata* plantation in Spain was estimated at 0.33 Mg Ca ha^{-1} (Turner and Lambert 1986, Ouro et al. 2001). Additionally, the average amount of soil Mg lost due to afforestation with other conifers in this study was 0.27 Mg ha^{-1}, compared to an estimate of *Pinus radiata* Mg stocks of 0.68 Mg ha^{-1} (Ouro et al. 2001). The similarity in soil losses to total biomass content of Ca and Mg supports the hypothesis that uptake by plantations is a major driver of soil Ca and Mg loss (Richter et al. 1994) and that plantation management leaving as much residue in place as possible will minimize problems of soil fertility in the future.

The observed losses of exchangeable cations from mineral soils could decrease productivity of successive plantation rotations. Atmospheric inputs of Ca, K, and Mg are usually less than plant uptake, which is typically supplied through mineral weathering, mineralization, and leaching from plant biomass (Schlesinger 1997). Best practices of retaining logging residues and debarking harvested plantations on site could substantially reduce cation losses from afforestation. Residual parts of harvested trees with little commercial value (leaves, branches, and bark) contain the majority of Ca and Mg in forest biomass. Typically these residues are removed from the site or burned, leading to export or losses of cations through accelerated leaching (FAO 2002). Aboveground biomass in bark, leaves, twigs, and reproductive structures at Coweeta LTER contained

86% and 63% of the total biomass Ca and Mg (Day and Monk 1977). Retaining these residual components without mounding or burning (reducing leaching and erosion losses) could lead to lower long-term losses of soil Ca and Mg (Mendham et al. 2003a).

The observed increase in soil Na was likely caused by afforestation's effect on hydrology (Figure 1). Jobbágy and Jackson posited that this increased Na due to *Eucalyptus* afforestation could be caused by three mechanisms: enhanced capillary rise of water through soil due to drier soil under plantations, decreased leaching to deep groundwater, or water uptake by roots from deeper soil depths (Jobbágy and Jackson 2004b, a). Because sodium is not essential to plant biochemistry, plants exclude it while taking up water and other cations. (Marschner 1995, Schlesinger 1997, Jobbágy and Jackson 2004b). Jobbágy and Jackson (2004b) demonstrated that increased water uptake by *Eucalyptus* plantations with sodium exclusion led to soil and groundwater salinization.

1.4.2 Soil pH, Base Saturation, and the Soil Exchange Complex

Comparing soils from a similar climate, forest soils tend to be more acidic than grassland soils (Schlesinger 1997, Chapin et al. 2002). This difference in acidity can be generated through several mechanisms, including increased production of organic acids or generation of carbonic acid from higher rates of autotrophic respiration (Richter and Markewitz 1995). The increased acidity of forests may also be caused by increased uptake of cations by trees and consequent changes in the proportions of cations adsorbed to the soil exchange complex (Jobbágy and Jackson 2003, 2004b). The consistent effects on cations by afforestation in our analysis suggest that changes in the proportions of cations could be a major driver behind the higher acidity of forest soils (Figures 1 and 2).

For example, afforestation decreased Ca, Mg, and K in many different plantation types, and concurrently increased concentrations of Na and H^+ (Figures 1 and 3). Additionally, the correlation between decreased base saturation and Ca with increased H^+ suggests that the exchangeable cations (Ca, Mg, K) taken up by afforested plantations tend to be replaced on soil exchange sites by H^+. The consequence of this change is a soil exchangeable pool with a higher proportion of H and Na ions. The consistency of these effects in this study across broad geographic regions and differing tree plantation types suggests that relocation of cations could be a general mechanism driving the acidity of forests across many different ecosystems.

Although acidification was significant for *Eucalyptus*, *Pinus*, and other conifer plantations, the pH of the control soils (grassland or shrubland) also varied (Figure 2). This result suggests that the mechanism of acidification across plantation species is likely similar, but the actual impact of the change in pH depends on the conditions of the control site. The relationship between soil pH and soil fertility (e.g., cations) is not linear because of the logarithmic scale of pH; for example, soils with pH between ~5 and 8 have consistently high percentages of Ca in their exchangeable cation pool, but below pH 5 the Ca percentage drops precipitously (Brady and Weil 2002). In our study, afforestation with *Pinus* and other conifers lowered pH from 5.7 to 5.3 and 4.6 to 4.3. Although the changes are similar in number of pH units, the acidification in *Pinus* plantations probably has more implications for soil fertility, since the control soils for other conifers were already acidic (more than 10 times higher proton concentration) and

had less exchangeable bases to lose. For example, see the calcium losses in this study (Figure 1).

1.4.3 Soil Carbon and Nitrogen

We found a significant decrease in soil organic C and N with *Pinus* afforestation, but not with other species. This result agrees with the conclusions of Guo and Gifford (2002) who found afforestation by pines (but not broadleaf species) significantly reduced soil C. Since soil C and N are indices of soil fertility, the losses of C and N from soil under *Pinus* plantations may indicate a general loss in soil fertility (Brady and Weil 2002). However, unlike Guo and Gifford (2002), we did not find correlations between plantation age or depth of sampling and the log response ratio. Given that most of the sites were in their first rotation, observed soil responses to afforestation may not yet have come to equilibrium. Also, since afforested plantations are repeatedly harvested, they might not reach equilibrium in the same sense as a natural ecosystem recovering from disturbance.

The loss of soil C under a plantation with higher primary productivity seems counterintuitive; however, this loss could be due to differences in the distribution and decomposability of plantation biomass (Guo and Gifford 2002). Plantation tree roots are longer-lived and coarser than typical grass roots, and contribute less to soil organic material (Post and Kwon 2000, Guo and Gifford 2002). Additionally, plantations deposit more C as litter to the forest floor, but there was insufficient data available to evaluate how much C globally was stored as afforested forest floor material (Jobbágy and Jackson 2000, Post and Kwon 2000, Guo and Gifford 2002). A study in Australia found that

debris from a *Pinus radiata* plantation stored a large amount of C, but this only offsets 22% of the carbon lost from the mineral soils due to afforestation (Guo et al. 2006). Additionally, C in plantation forest floor material incorporates more slowly into soil organic matter than in native grass systems (Guo et al. 2006).

Carbon loss from soils as a result of *Pinus* afforestation influences potential rates of C sequestration. The average loss of soil C under pine plantations in this analysis was 4.1 Mg C per hectare; an average plantation with a 20-year rotation time can be assumed to contain ~75 Mg C on average per hectare (Vitousek 1991). The loss of soil C due to afforestation is therefore a modest 5.5% of C sequestered in vegetation. Harvesting of plantations usually results in additional losses of C from soils from increased rates of decomposition (Vitousek 1991). Though these losses of C from afforested soils are less than the sequestration potential in biomass, they are large enough to be considered in C budgets of these systems.

A potential method of reducing the impact of soil C and N loss is to retain logging residues on site. As mentioned above, logging residues are usually removed or burned before subsequent rotations are planted, which leads to a large loss of C and N (FAO 2002). Removing or burning residues from harvested plantations also decreases soil C and N contents; in Australia, for instance, burning logging residues led to a loss of 200-350 kg N per hectare (Merino and Edeso 1999, Mendham et al. 2003b). Conversely, retention of logging residues led to higher soil organic matter and N contents and higher rates of net N mineralization (Goncalves et al. 2000, FAO 2002). Retention also led to

increased productivity compared to burned sites in subsequent plantation rotations (Bouillet et al. 2000, Fan et al. 2000, Xu et al. 2000).

Soil C:N increased with both *Pinus* and *Eucalyptus* afforestation (Figure 4). Though the changes in C:N with *Eucalyptus* were not as large as for *Pinus*, the increase in C:N is potentially an indicator of lower soil organic matter quality (Brady and Weil 2002). Since *Pinus* plantations decreased both soil C and N contents, the increased C:N in *Pinus* plantations suggests that the depletion of N is more rapid (Figure 4). This more rapid decrease in N is likely due to increased plant uptake of N compared to native grasslands (Jobbágy and Jackson 2004b). Another possible implication of increased C:N ratios in these systems is increased microbial N immobilization (Brady and Weil 2002, Berthrong and Finzi 2006). Microbes immobilize more N in their biomass as C:N increases; as a consequence mineralization rates are lower, which leads to lower plant-available nitrogen and lower productivity.

1.4.4 Implications and Conclusions

This global study indicates that afforestation often leads to more acidic and nutrient-deficient mineral soils, but best management practices can help overcome some of these changes. Although these soil changes could impair the productivity of successive rotations; it is unclear how long it will take to see noticeable productivity declines. Turner and Lambert (1986) estimated that in Australia it would take ~320 years (4 rotations) before nutrient depletion (P and Ca) would impair productivity; however, this was estimated by total biomass nutrient stocks and total soil pools and not from actual measures of productivity over successive plantings (Turner and Lambert 1986). Estimates

of Chinese fir plantation yields show that annual biomass production by the third rotation drops by more than 50% (Zhang et al. 2004). In addition to nutrient depletion, plantation harvesting has been shown to compact soil which reduced regeneration of new seedlings by up to 51.5% (Balbuena et al. 2002).

With afforestation likely to continue as a useful mechanism for offsetting carbon dioxide emissions, management practices should be implemented to reduce soil impacts and improve sustainability. For instance, maintaining soil fertility could be accomplished through site and harvest management tools. Retention of logging slash, on site debarking and retention, and reduced burning of slash have been shown to reduce nutrient (Ca, Mg, N, etc.) export, loss of soil organic material, erosion losses, and soil compaction (Merino and Edeso 1999, Bouillet et al. 2000, Ouro et al. 2001, Mendham et al. 2003b, Merino et al. 2004). Depending on initial site conditions, combinations of these different management conservation practices could improve sustainability.

With demand rising for timber products, managers need either to harvest stocks from remaining native forests or to increase sustainable plantation forestry (ABARE and Jaako-Poyry 1999). Reducing soil degradation from afforestation and harvesting is important for productivity and reducing habitat loss. If managed sustainably, afforestation could simultaneously preserve remaining native forests and function as a long-term CO_2 sink.

1.5 Tables

Table 1: Studies included in this meta-analysis

Author(s)	Country	Plantation Type
(Adams et al. 2001)	New Zealand	Other Conifer
(Adams et al. 2001)	New Zealand	Pine
(Alfredsson et al. 1998)	New Zealand	Other Conifer
(Alfredsson et al. 1998)	New Zealand	Pine
(Alriksson and Olsson 1995)	Sweden	Other Conifer
(Barton et al. 1999)	Scotland	Pine
(Binkley and Resh 1999)	USA	Eucalyptus
(Binkley et al. 1989)	USA	Pine
(Burton et al. 2007)	Australia	Pine
(Chen et al. 2007)	China	Pine
(Chen et al. 2000)	New Zealand	Pine
(Condron and Newman 1998)	New Zealand	Other Conifer
(Condron and Newman 1998)	New Zealand	Pine
(Davis 1994)	New Zealand	Pine
(Davis 1995)	New Zealand	Pine
(Davis 2001)	New Zealand	Pine
(Davis and Lang 1991)	New Zealand	Pine
(Del Galdo et al. 2003)	Italy	Other Angiosperm
(Garbin et al. 2006)	Brazil	Pine
(Garg and Jain 1992)	India	Other Angiosperm
(Giddens et al. 1997)	New Zealand	Pine
(Gilmore and Boggess 1963)	USA	Pine
(Groenendijk et al. 2002)	New Zealand	Pine
(Guevara-Escobar et al. 2002)	New Zealand	Other Angiosperm
(Guo et al. 2007)	Australia	Pine
(Hawke and O'Connor 1993)	New Zealand	Pine
(Hofstede et al. 2002)	Ecuador	Other Angiosperm
(Hofstede et al. 2002)	Ecuador	Pine
(Huygens et al. 2005)	Chile	Pine
(Jain and Singh 1998)	India	Other Angiosperm
(Jobbágy and Jackson 2003)	Argentina	Eucalyptus
(Jug et al. 1999)	Germany	Other Angiosperm
(Lilienfein et al. 2000)	Brazil	Pine
(Lima et al. 2006)	Brazil	Eucalyptus
(Mao et al. 1992)	China	Eucalyptus
(Markewitz et al. 1998)	USA	Pine
(Martens et al. 2004)	USA	Other Angiosperm
(Menyailo et al. 2002)	Russia	Other Angiosperm
(Menyailo et al. 2002)	Russia	Other Conifer
(Menyailo et al. 2002)	Russia	Pine
(Merino et al. 2004)	Spain	Other Angiosperm
(Montagnini 2000)	Costa Rica	Other Angiosperm
(Musto 1992)	South Africa	Eucalyptus
(Musto 1992)	South Africa	Other Angiosperm
(Musto 1992)	South Africa	Pine
(Muys and Lust 1993)	Belgium	Other Angiosperm

Table 1 Continued: Studies included in this meta-analysis

Author(s)	Country	Plantation Type
(Nielsen et al. 1999)	Denmark	Other Conifer
(Noble et al. 1999)	Australia	Other Angiosperm
(Noble et al. 1999)	Australia	Pine
(Nosetto et al. 2006)	Argentina	Pine
(O'Connell et al. 2003)	Australia	Eucalyptus
(Ohta 1990)	Phillipines	Other Angiosperm
(Ohta 1990)	Phillipines	Pine
(Parfitt et al. 1997)	New Zealand	Pine
(Parfitt et al. 2003b)	New Zealand	Pine
(Payet et al. 2001)	South Africa	Pine
(Prosser et al. 1993)	Australia	Eucalyptus
(Quideau and Bockheim 1997)	USA	Pine
(Resh et al. 2002)	Puerto Rico	Eucalyptus
(Resh et al. 2002)	Puerto Rico	Other Angiosperm
(Reynolds et al. 1988)	UK	Other Conifer
(Rhoades and Binkley 1996)	USA	Eucalyptus
(Rhoades and Binkley 1996)	USA	Other Angiosperm
(Richter et al. 1994)	USA	Pine
(Ross et al. 1999)	New Zealand	Pine
(Ross et al. 2002)	New Zealand	Pine
(Saggar et al. 2001)	New Zealand	Pine
(Schipper and Sparling 2000)	New Zealand	Pine
(Scott et al. 2006)	New Zealand	Pine
(Sharrow and Ismail 2004)	USA	Other Conifer
(Singh et al. 1998)	India	Eucalyptus
(Singh et al. 1998)	India	Other Angiosperm
(Sparling et al. 2000)	New Zealand	Pine
(Vesterdal et al. 2002)	Denmark	Other Conifer
(Williams et al. 1977)	UK	Pine
(Wu et al. 2006b)	China	Pine
(Yeates and Saggar 1998)	New Zealand	Pine
(Yeates et al. 2000)	New Zealand	Pine
(Yuste et al. 2007)	USA	Pine
(Zhao et al. 2007)	China	Pine
(Zinn et al. 2002)	Brazil	Eucalyptus
(Zinn et al. 2002)	Brazil	Pine

Table 2: Number of studies in this meta-analysis by variable and afforestation type

Type	Analyses								
	Na	Ca	Mg	K	pH	BS%	C	N	C:N
Eucalyptus	8	30	10	10	16	3	26	16	16
Other Angiosperm	5	12	12	12	16	4	16	13	13
Pinus	33	46	46	42	68	28	71	61	61
Other Conifers	6	8	8	8	9	6	7	7	7
Overall	52	96	76	72	109	41	120	97	97

Table 3: Percent change due to Afforestation

Type	Analyses								
	Na	Ca	Mg	K	pH	BS%	C	N	C:N
Eucalyptus	250% [61, 674]	-37% [-25, -47]			-13% [-7.9, -18]				5.7% [.05, 11.3]
Other Angiosperm	32% [15, 50]								
Pinus	81% [42, 136]	-31% [-17, -43]		-23% [-2.1, -42]	-5.9% [-4.3 -7.5]	-21 [-2.9, -37]	-15% [-8.6, -21]	-20% [-12, -27]	11.6% [3.5, 20]
Other Conifers		-16% [-2.4, -29]	-52% [-27, -70]		-6.5% [-1.2, -12]	-10% [-4.5, -17]			5.9% [.18, 11]
Overall	71% [35, 120]	-29% [-20, -37]			-5.7% [-4.3 -7.5]	-17% [-2.3, -17]		-15% [-8.6, -21]	9.9% [4.2, 16]

Note: BS% = base saturation %. Values represent mean percent gain (positive values) or loss (negative values) generated by bootstrapping with 95% confidence interval in brackets.

Table 4: Mean total significant losses or additions of nutrients with afforestation in surface soils across different regions of the world

Region or Country	Afforestation type	Area (10^3 ha)	Soil C lost (10^3 Tonnes)	Soil N Lost (10^3 Tonnes)	Soil Ca lost (10^3 Tonnes)	Soil Mg Lost (10^3 Tonnes)	Soil Na Added (10^3 Tonnes)
South Africa	*Eucalyptus*	566			302		354
	Pinus	724	2957	259	904		120
	Other Broadleaf	123					2
China	*Eucalyptus*	2397			1277		1498
	Pinus	10031	40971	3582	12521		1665
	Other Conifer	16160			5544	2392	
	Other Broadleaf	13304					186
South and South East Asia	*Eucalyptus*	4047			2156		2529
	Pinus	1734	1115	619	2164		288
	Other Conifer	273			94		
	Other Broadleaf	11104					155
Europe	*Pinus*	10945	44704	3908	13661		1817
	Other Conifer	9077			3114	1343	
	Other Broadleaf	3730					52

Table 4 Continued: Mean total significant losses or additions of nutrients with afforestation in surface soils across different regions of the world

Region or Country	Afforestation type	Area (10^3 ha)	Soil C lost (10^3 Tonnes)	Soil N Lost (10^3 Tonnes)	Soil Ca lost (10^3 Tonnes)	Soil Mg Lost (10^3 Tonnes)	Soil Na Added (10^3 Tonnes)
North and Central America	*Eucalyptus*	198			105		124
	Pinus	15440	63063	5513	19272		2563
	Other Conifer	88			30	13	
	Other Broadleaf	511					7
Australia and New Zealand	*Eucalyptus*	549			292		343
	Pinus	2602	10628	929	3248		432
	Other Conifer	163			56	24	
Brazil, Argentina, and Chile	*Eucalyptus*	3777			2012		2360
	Pinus	4253	17371	1519	5309		706
	Other Conifer	104			36	15	
	Other Broadleaf	585					8

Note: Estimated total area (ha) of afforested area for regions based on UN FAO data from voluntary country reports; not all countries in every region are represented (FAO 2006b, a). The estimates of area are conservative; only deliberately planted forests designated for harvest were counted. Losses or additions of C and nutrients were calculated from differences in means from Figures 1 and 4. Total C and N were measured up to 30cm of mineral soil. Total stocks for Ca, Mg, and Na were estimated using the average bulk density of soils (1.068 g/cm^3) and average sampling depth (0.14 m) in our database.

1.6 Figure Legends

Figure 1. Changes in soil exchangeable cations with afforestation. Significant increases due to afforestation ($p<0.05$) of a cation within a plantation type are indicated by the cation name and an up arrow (e.g. Na↑) and decreases by a down arrow (e.g. Ca↓). See Table 2 for number of studies used for each analysis, and Table 3 for means and 95% confidence intervals.

Figure 2. Changes in soil pH and base saturation with afforestation. An asterisk indicates a significant difference ($p<0.05$) between control and afforested for a given plantation type, and error bars represent standard error. Significance for pH was calculated based on $[H^+]$ but is presented here in pH units for ease of interpretation. See Table 2 for number of studies used for each analysis, and Table 3 for means and 95% confidence intervals.

Figure 3. Relationship between $[H^+]$, base saturation (BS%), and Ca. In the left panel, the response ratio of BS% is negative correlated with the response ratio of $[H^+]$ (Spearman's $\rho=-0.58$, $p=0.006$, $N=21$); 15 points are from *Pinus*, 1 *Eucalyptus*, 4 other conifers, and 1 other angiosperm. In the right panel, the response ratio of Ca is negatively correlated with the response ratio of $[H^+]$ (Spearman's $\rho=-0.56$, $p<0.0001$, $N=51$); 28 points are *Pinus*, 10 *Eucalyptus*, 4 other conifers, and 9 other angiosperms. Soils were analyzed up to 30cm deep with an average of 14cm.

Figure 4. Changes in soil carbon and nitrogen. An asterisk indicates a significant difference ($p<0.05$) between control and afforested for a given plantation type, and error bars represent standard error. See Table 2 for number of studies used for each analysis, and Table 3 for means and 95% confidence intervals.

1.7 Figures

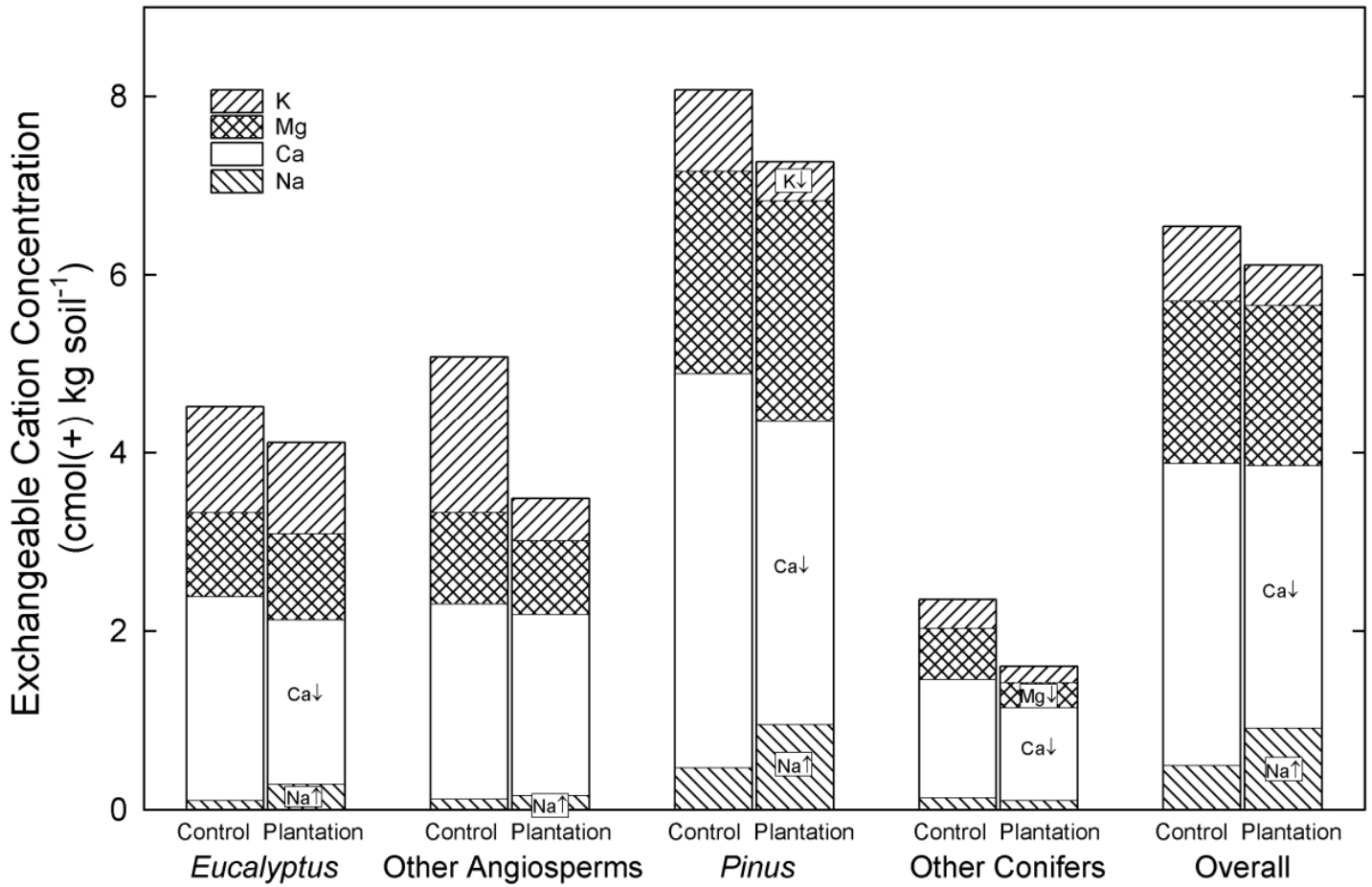

Figure 1: Changes in soil exchangeable cations with afforestation

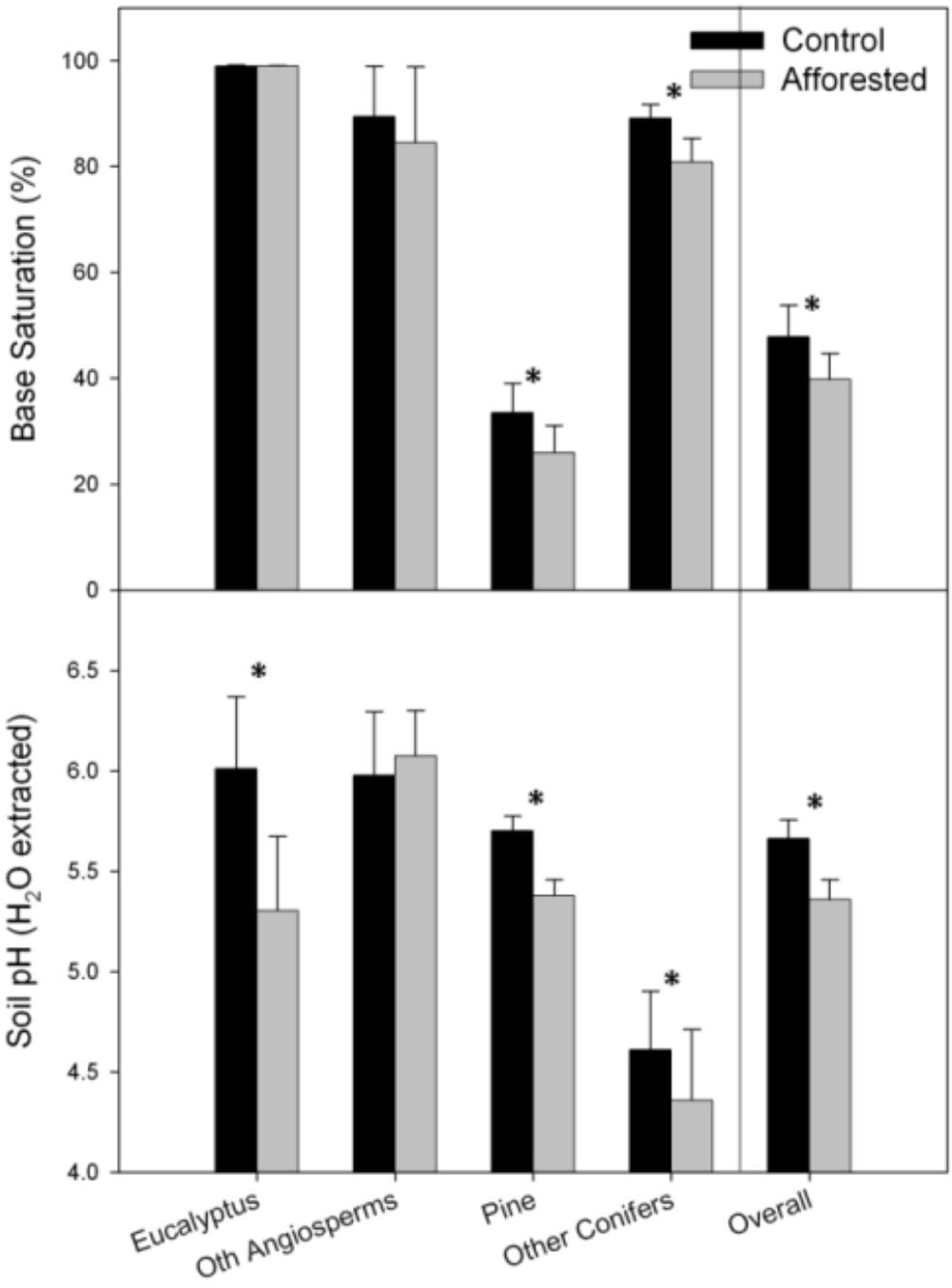

Figure 2: in soil pH and base saturation with afforestation

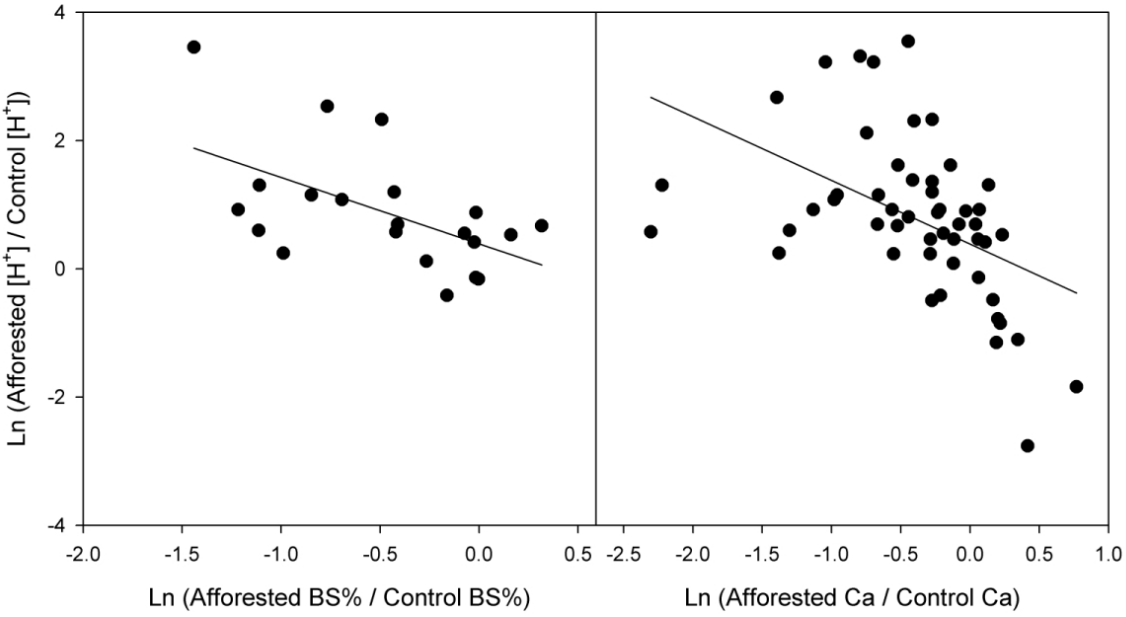

Figure 3: Relationship between [H^+], base saturation (BS%), and Ca

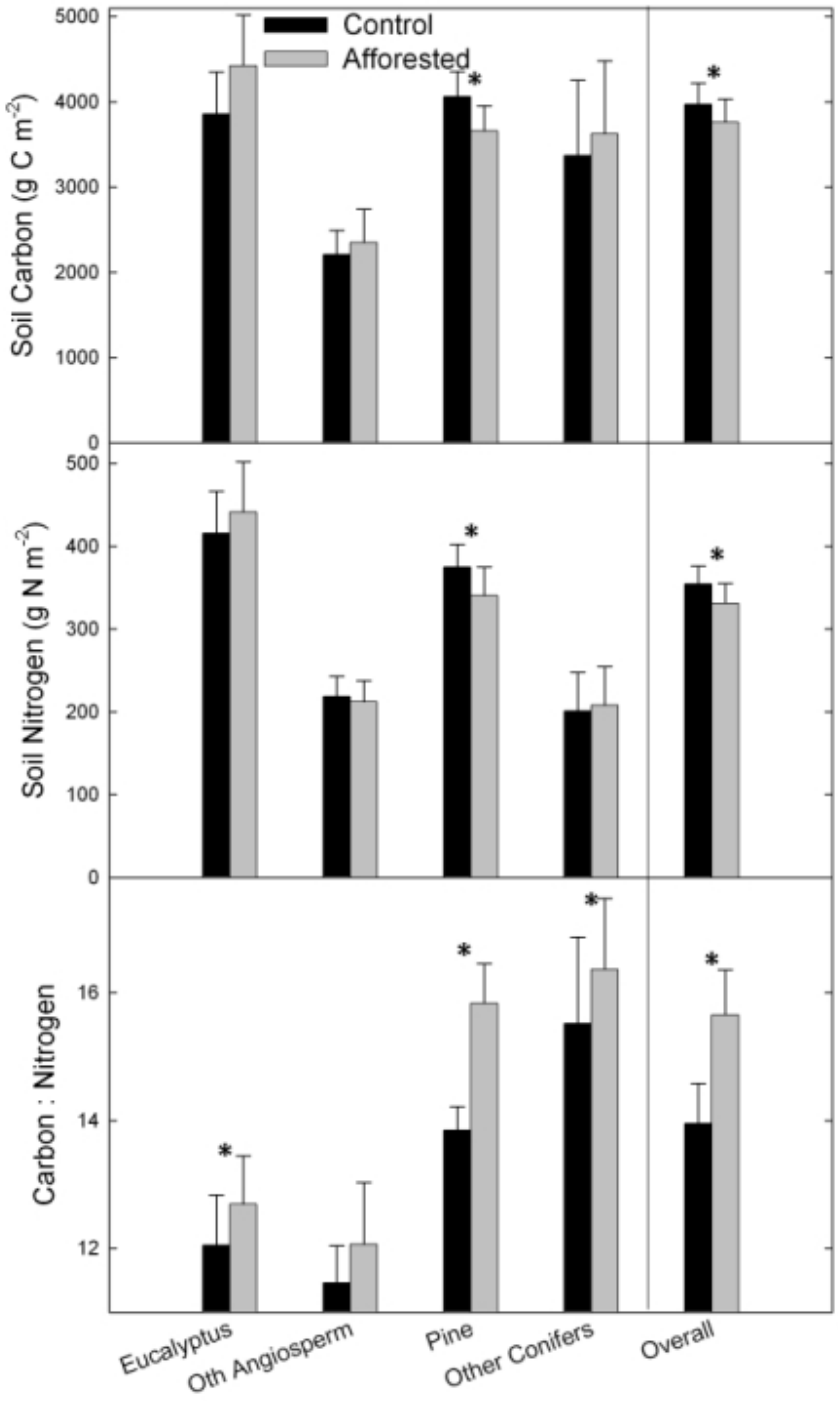

Figure 4: Changes in soil carbon and nitrogen

2. Afforestation alters soil functional gene composition and biogeochemical processes in grasslands of southern South America

2.1 Introduction

Although soil microbes mediate nearly all biogeochemical cycles in terrestrial ecosystems, we understand very little about how environmental changes affect these microbes and their functions (Jackson et al. 2002, Fitter et al. 2005, Martiny et al. 2006, Fierer et al. 2007a, Falkowski et al. 2008). Studying how environmental changes affect microbial functioning should help in predicting how biogeochemical cycles will respond to changing climate and land-use more broadly. Because of high levels of apparent redundancy for many biogeochemical gene families across microbial groups (9), small scale environmental effects that alter some microbial groups will not necessarily alter overall biogeochemical functioning (Fitter et al. 2005). In contrast, large scale environmental perturbations, such as land-use change, could drive major shifts in microbial populations that correspond to significant biogeochemical changes. The goal of this study was to examine how soil microbes and their associated biogeochemical functions responded to a land transformation, the conversion of native grasslands to forest plantations.

Many studies have examined the effects of changing land-use on soil microbial diversity; however, shifts in diversity will not necessarily alter the ability of soil microbes to perform biogeochemical functions. Functional gene microarrays that compare many orthologous gene sets controlling biogeochemical functions have recently emerged as a way to examine genes across a broad range of microorganisms and functions

simultaneously (Gentry et al. 2006, He et al. 2007). The Geochip microarray (version 2.0) simultaneously quantifies several thousand genes from diverse species and groups involved in soil C and N cycling (Wu et al. 2001, Wu et al. 2006a, He et al. 2007). This array allows for a detailed analysis of the biogeochemical gene profiles of soil microbes and is ideal for understanding how profile changes in response to environmental perturbations and experimentally imposed conditions.

Afforestation, defined by the Kyoto protocol as the establishment of tree plantations on land without forests for >50 years, is an ideal system to study the effects of land-use change on soil microbial functions. Such plantations already cover 140 million ha globally, with 2.8 million ha afforested annually; this rate is likely to increase as demand for wood products and the use of plantations as carbon offsets grows (FAO 2006a). Afforestation has many impacts on soil C and nutrient cycling that are likely linked to microbial activity and functions (Guo and Gifford 2002, Berthrong et al. 2009). Studying the impacts of this land-use change on soils should help improve predictions of plantation productivity and the current and future capacity of these systems to sequester carbon.

Plantation productivity and potential C sequestration are linked to microbial C and N cycling in soils. For instance, microbial decomposition of vegetation inputs to soils controls the plant available N supply (Schimel and Bennett 2004, Manzoni et al. 2008). Since N is usually the most common soil nutrient limiting plant growth, changes in soil N cycling will therefore often influence plant nutrient uptake and productivity (Schimel and Bennett 2004, Berthrong and Finzi 2006). In turn, alterations in nutrient uptake by plants

feed back into the quantity and quality of plant material supplied to microbial processes. Hence, changes in soil microbial functional genes and activity could alter the long-term carbon sequestration potential of plantations.

In this study we examined the effects of *Eucalyptus* afforestation on native grasslands in Uruguay. Because *Eucalyptus* plantations are common globally and tend to produce lower quality substrates for microbes than grasses do (Pozo et al. 1998, Wedderburn and Carter 1999), we hypothesized that these lower quality substrates (e.g. higher phenolic and other secondary compound content, higher C:N) would alter the functional gene composition of microbial communities and lead to altered rates of biogeochemical cycling, particularly pool sizes of soil carbon and nitrogen.

2.2 Methods

2.2.1 Site Description and Soil Sampling

Eucalyptus is the second most common genus used for afforestation globally and comprises ~40% of plantation area in southern South America and Brazil (FAO 2006b). Species of *Eucalyptus* are desirable for their robust growth in different climates and for their resistance to drought, pests, and low-nutrient soils; *Eucalyptus* also has the highest rate of growth of any commonly used afforestation genus in warm climates (Pryor 1976, Florence 1996, FAO 2006b). In turn, Uruguay has the fastest rate of afforestation increase in terms of percentage increase and second greatest in total area in South America, attributable in part to government subsidies (Geary 2001, FAO 2006b). The fast growth rate and proximity to paper mills in Uruguay gives these plantations one of the highest potential economic rates of return for forest products in the Western Hemisphere

(Cubbage et al. 2006). Uruguay is a particularly good location to study the effects of *Eucalyptus* plantations because it also has many replicated sites with similar planting techniques, diverse forest age classes, and soil substrates. Together with the historical absence of trees in the Rio de la Plata grasslands of Uruguay and Argentina, the region is an ideal one to study the effects of land-use change on soils (Jobbágy and Jackson 2004a, 2007).

We studied eight sites with adjacent plots of native grasslands and *Eucalyptus* plantations. The sites ranged from 50 to 200 m in elevation and were located near Minas, the capital of the Lavalleja department of Uruguay (34.37°S, 55.23°W). Mean annual precipitation is 1150 mm, and mean annual temperature is 16.5°C. Soils of the region are Udolls, Udalfs, and Udepts developed over granitic rock; soils at our sites were mostly Hapludalfs with typical soil depth of 30cm (Duran 1985, Farley et al. 2008a). Native grasslands in this region are a mix of C_3 and C_4 grasses that are frequently used for cattle and sheep grazing. *Eucalyptus* plantations in our study sites were not fertilized and were established between 1992 and 1996. Three of the plantations were in their second rotation and five were original plantings. Prior to afforestation, the plantation sites were native grasslands similar to their adjacent controls.

In September 2005, we sampled the top 10 cm of mineral soil using a 1.9-cm diameter soil core. At each adjacent grassland-*Eucalyptus* pair we collected five cores per transect along two 10 m transects in each vegetation (10 cores per vegetation, 20 cores per site). All cores for each vegetation type (10 cores) at each site were composited in a polyethylene bag and stored on ice for transport to the lab. Samples were homogenized

and sieved (2mm) to remove roots and rocks. Fresh soils were used for biogeochemical analyses; subsamples were stored at -80°C for DNA extraction.

2.2.2 Soil Biogeochemical Analyses and Statistics

Soil total extractable carbon and nitrogen, NH_4^+, NO_3^-, and microbial biomass C and N were determined as previously described (Berthrong and Finzi 2006). The methods were modified by using 2 g of field moist soil instead of 30 g. Total air-dried soil and litter C and N were determined by combustion in a Carlo-Erba Elemental Analyzer (CE Elantech, Lakewood, NJ USA). Air-dried soil and litter ^{13}C was determined on a Finnigan MAT Delta Plus XL continuous flow mass spectrometer systems; results are expressed as $\delta^{13}C‰$ versus the Pee Dee Belemnite (PDB) standard.

Leaf total phenolics were extracted from air-dried litter by placing 0.02g of ground leaf material in a centrifuge tube and adding 0.75ml of 70:30 acetone:water. The samples were placed in a sonicating bath for 10 minutes and then centrifuged at 10x gravity for 2 minutes. The supernatant was decanted and the process was repeated on the leaf material three times to ensure that all phenolics were extracted (Swain 1979). Concentration of total phenolics in the extracts, diluted 200x with double deionized water, were measured using the Folin method with a calibration curve of gallic acid solution (Swain and Hillis 1959).

We tested for significant differences in biogeochemical variables between grassland and plantation using paired t-tests in SAS (SAS institute, Cary, NC USA). All distributions were determined to be Gaussian before analysis. The level of replication was the individual site (one vegetation pair).

2.2.3 DNA Extraction and Whole-Community Genome Amplification

DNA was extracted from 10 g of soil using the "Powermax" kit (Mo Bio Laboratories, Carlsbad, CA, USA). The manufacturer's instructions were followed except for two modifications that reduced DNA shearing to produce high molecular weight DNA ideal for whole-community genome amplification. The first modification was to grind the soil in a mortar and pestle with liquid nitrogen instead of bead-beating the samples. The other modification was to place the samples in a 60°C incubator and rotate the tubes gently, 30 oscillations per minute, for one hour.

Extracted DNA was then concentrated by alcohol precipitation. One ml of DNA extract was combined with 0.8 ml ice-cold isopropanol and 0.1ml 3M NaCl; the mixture was inverted to mix and chilled at -20°C for 30 minutes to precipitate the DNA. The samples were then centrifuged at 10,000x g at 4°C for 10 minutes, the supernatant was removed and discarded, and the pellet containing the DNA was dried in a speed-vac. The pellet was then washed with 70% ethanol and centrifuged at 10,000x g; the supernatant was removed, and then the DNA pellet was dried by speed-vac and then resuspended in 50μl of deionized water.

To obtain sufficient genomic DNA and to dilute the effect of compounds that co-extract with soil DNA and inhibit microarray hybridization and signal detection, we performed a whole-community genome amplification by multiple stand displacement amplification (Wu et al. 2006a). An illustra GenomiPhi V1 DNA amplification kit (GE Healthcare Life Sciences, Piscataway, NJ, USA) was used as described in Wu (Wu et al. 2006a) as per the manufacturer's instructions, with several minor modifications. Briefly,

100ng of DNA was mixed with 9μl of sample buffer (containing random octamer primers) and denatured in a thermocycler at 95°C for 5 minutes. We then added to the sample 9μl of reaction buffer (salts and deoxynucleotide triphosphates), 1 μl enzyme mix (Phi29), 1μl single-stranded DNA binding protein (260ng/μl), and 1μl spermidine solution (1mM); the mixture was thoroughly mixed and incubated in a thermocycler at 30°C for 6 hours and then heated to 65°C for 10 minutes to stop the enzymatic reaction. All pipetting steps were performed in a sterile laminar flow hood to prevent the introduction of contaminant DNA prior to amplification. Non-template controls were amplified using sterile deionized water instead of sample DNA and were treated identically to the other samples throughout subsequent analyses.

2.2.4 Microarray Hybridization

The functional gene microarrays (FGA or Geochip 2.0) used in this study were constructed as previously described (He et al. 2005, Liebich et al. 2006, He et al. 2007). FGAs contained >27,000 gene- and group-specific oligonucleotide probes (50bp each) that target genes essential to carbon, nitrogen, sulfur, and metal transformations and biogeochemical cycling. Three criteria were used to ensure specificity of the probes: ≤90% sequence identity to non-specific targets, ≤20 bases of identical sequence stretches, and ≥-35kcal mol^{-1} binding free energy between probes and non target sequences (Liebich et al. 2006).

Amplified DNA was fluorescently labeled using Cy5 dye and the Klenow fragment (He et al. 2005). Briefly, 20μl of amplified DNA, 20μl of 2.5X random primers

(octamers, Invitrogen), and 0.3µl 10mM Spermidine were mixed and heated to 99.9°C for 5 minutes in a thermocycler, then immediately chilled on ice. To these samples we added 2.5µl dNTPs (5mM dATP, dGTP, dCTP, and 2.5mM TTP), 1µl 2.5mM Cy5-UTP, 2µl of 80U Klenow (Invitrogen), 0.7µl recombinant *E. coli* RecA protein (490ng/µl, Invitrogen), and 13.8µl sterile DI water. Samples were mixed by inverting, then centrifuged and incubated in a thermocycler at 37°C for 6 hours. Once Cy5 was added, all remaining steps were conducted in low-light to preserve the activity of the fluorescent dye. After incubation, the samples were cleaned using a QIAquick PCR purification kit following manufacturer's instructions (Qiagen, Valencia, CA, USA). We also used buffer PB for the QIAquick kit to avoid interference from the indicator dye in the standard PBI buffer. Cleaned and labeled samples were dried down in a speed vac.

Prehybridization solution containing 50% formamide, 5X SSC, 0.1% SDS, and 0.1mg/ml bovine serum albumin was heated to 50°C in a water bath. Microarrays were incubated in the 50°C prehybridization solution for 45 minutes, and then washed 3X with DI and 1X with isopropanol, and then dried with filtered compressed air. Microarrays, hybridization chambers, lifter coverslips, pipette tips, and 5X SSC were warmed to 60°C in a hybridization oven. For each sample, 38.4 µl of hybridization mix (final concentration: 50% formamide, 5X SSC, 0.10% SDS, 0.1µg/µl salmon sperm DNA, 0.0825 mM Spermidine) was added to the dried-down sample and mixed thoroughly. The solution was incubated at 95°C in a thermal cycler for 5 minutes, and then kept at 60°C while 1.6µl of 490ng/l RecA was added and mixed. On top of a 60°C heating block,

all 40μl of sample solution was pipetted onto the microarray at the edge of the lifter coverslip, and capillary action was used to cover the microarray surface with solution. The microarrays were sealed in water-tight hybridization chambers and incubated at 50°C in a water bath overnight. Microarrays were then washed in a bath of gently shaking 50°C solution of 1X and 0.1% SDS. Without allowing the microarrays to dry, we transferred them to a gently shaking bath of the same solution at room temperature. The microarrays were transferred to a gently shaking bath of 0.1X SSC and 0.1% SDS, and then through 4 baths of 0.1X SSC. Microarrays were then blown dry with filtered compressed air.

2.2.5 Microarray Processing, Image Analysis, and Statistics

Microarrays were scanned on a Scan Array 5000 (GSI, Moorpark, CA USA). We used 100% laser power and adjusted the photomultiplier tube (PMT) gain to maximize fluorescence while minimizing saturated probes. Microarray images were analyzed using Imagene 6.0 (Biodiscovery, El Segundo, CA USA). To avoid false positives, each poor-quality probe and any probes with a signal to noise ratio less than 2 was manually excluded. Signal to noise ratio was calculated as (signal intensity-background)/standard deviation. Each sample was run on three replicate arrays; only probes where there was a detectable hybridization signal on at least two out of three replicates were analyzed. To normalize values across arrays, each probe's signal intensity was divided by the mean signal intensity for its array. We then calculated the mean signal intensity across the replicate slides. For each probe, we divided the signal intensity of the plantation by its

paired grassland control and made the distribution symmetrical around 0 by taking the log base 2 (henceforth “log fold change”).

Distributions of log fold change for given genes of interest were generated in SAS (SAS Institute, Cary, NC USA). For each gene of interest, we used the Wilcoxon signed-rank test to test if afforestation increased or decreased a given gene type (e.g. non-zero log fold change). The signed rank test was chosen over a one sample t-test because the distributions of log fold change for most genes of interest were not Gaussian. We report the overall effect of treatment on a category of probes as the median, since all of the distributions were not normally distributed.

We tested if the composition of functional genes differed between treatments using multivariate approaches. We used a pairwise centroid linkage hierarchical clustering algorithm from the program Cluster (http://rana.lbl.gov) (Eisen et al. 1998) and visualized the clustering results using Treeview (http://rana.lbl.gov) (Eisen et al. 1998). Non-metric multidimensional scaling (PCOrd version 5) was used to create an ordination that represented the functional gene profile in two dimensions and to calculate correlations between ordination and biogeochemical variables.

2.3 Results

Across all probes detected with the microarrays, afforestation altered functional gene profiles in concert with biogeochemical pool sizes. Non-metric multidimensional scaling of all probes produced a two dimensional ordination with axes 1 and 2 representing 69% and 27%, respectively, of the variation in composition and relative abundance of genes from the eight sites. Seven of eight grasslands had very similar

functional gene profiles and grouped closely together in ordination space (Figure 5). Two plantations had similar profiles to the grasslands, while three other plantations separated from the grasslands along dimension 2 and three other plantations separated along dimension 1. Microbial biomass C, N, and total extractable N were positively correlated with dimension 1, indicating larger pools of these C and N forms in gene profiles associated with grasslands (Figure 5, Table 5). The C:N of microbial biomass and C:N of total extractable nutrients were negatively correlated with dimension 1, indicating narrower ratios in grassland profiles.

Afforestation altered the abundance and composition of carbon degradation genes in soils (Figure 6). Cluster analysis revealed several groups of carbon degradation probes (groups 1 and 3) with higher relative abundance in plantations (Figure 6). There were also several probes (group 2) that showed little difference in pattern between vegetation types (Figure 6). These unchanged probes suggest there are microbial strains whose functional genes are insensitive to at least some kinds of vegetation change. The differences in gene abundance and distribution across sites drove differences in functional gene profiles and led to the plantation sites separating from most of the grassland sites in ordination space (Figures 5, 6). Both groups that were specific to plantations and those that were common to both vegetation types shared functional genes for chitinases, cellulases, and laccases; however, some functional genes from different strains of the same species could be either restricted to one vegetation type or highly abundant in both, suggesting that functional gene profiles cannot be predicted solely by relative abundance of specific taxonomic groups. Likely the differences between

plantations and grasslands seen in the site clusters (Figure 6) and in groupings in ordination space are driven by groups 1 and 3 (specific to plantations) and by decreased abundance of functional genes as discussed below.

In addition to changing the profile of the functional genes present, afforestation led to an overall decrease in abundance of carbon degradation genes (Figure 7). The median decrease due to afforestation for carbon degradation probes was 0.021 (log-fold) or 1.5%, with a range from a 29.3% decrease to an 86.6% increase (Figure 7, $p=0.0194$). The most strongly affected subset of carbon degradation probes were chitinases, which decreased on average by 0.113 log fold (7.5%, $p=0.017$, Figure 7). The lower relative abundance of these probes contributed to the overall difference seen between grasslands and plantations in the functional gene profiles and clusters (Figures 5, 6).

Changes in carbon degradation genes were accompanied by changes in pool sizes of C in litter and soils. Eucalypt litter had 6 times higher phenolic concentrations and 13% more organic C than grassland litter had (Table 5). Total extractable C (soluble undigested organic carbon molecules) was 42.6 μg C g soil^{-1} in eucalypt soil compared to only 27.6 μg C g soil^{-1} in grasslands ($p<0.05$, Table 5); C in microbial biomass under eucalypts was 27% lower than in grasslands ($p<0.01$, Table 5). These changes, along with lower abundance of carbon degrading functional genes, suggest that the microbial community under eucalypts was less able to degrade the incoming eucalypt litter than grass litter, and that carbon in plantation soils is shifting from microbial biomass to TEC pools, which contain dissolved C that has not been incorporated into biomass (Figure 7, Table 5). This conclusion is further supported by the carbon stable isotope results: the

$\delta^{13}C$ of eucalypt soil is more negative (-22.3) than grassland soil (-20.3), which is more similar to the eucalypt litter $\delta^{13}C$ than grass litter, suggesting that after only ten years much of the eucalypt derived C has been incorporated and not been respired (Table 5).

Afforestation of grasslands also altered patterns and abundance of N cycle functional genes. Cluster analysis shows that 6 out of 8 profiles of grassland ammonification genes cluster together (Figure 8). Similar to profiles of carbon degradation genes, many ammonification genes were found in high abundance across both vegetation types (group 1, Figure 8), but some were found predominately in plantation soils (group 2, Figure 8). The average decrease in abundance for an ammonification gene due to afforestation was 10.7% ($p=0.0012$ Figure 9). Unlike ammonification genes, N fixation and nitrification genes did not show any distinct clustering patterns due to vegetation type (Figure 10). However, functional genes for N fixation decreased in average abundance by 7.85% with afforestation as assessed using log-fold change, which evaluates abundance across all related probes ($p<0.0001$, Figure 9). The decreases in relative abundance of functional genes in grasslands (both in C and N cycles) and the groups of probes that are distinct to plantations caused the differences observed in NMS ordination space between grasslands and plantations (Figure 5).

Changes in N cycle functional genes were accompanied by shifts in soil N pools. Microbial biomass N was 34.5% lower in eucalypt soil than in grassland soil (Table 5). Although the abundance of functional genes for ammonium production (ammonification and N fixation) was lower with afforestation, extractable ammonium in eucalypt soil was more than twice that of grassland soils (Table 5). Lower microbial biomass N with higher

ammonium and lower inputs from ammonification and N fixation could indicate lower microbial assimilation of N after afforestation. Lower assimilation of N by microbes is also supported by the wider C:N of microbial biomass in eucalypt soil, and potentially led to the wider C:N ratio of bulk soil under eucalypts (Table 5).

2.4 Discussion

The goal of this study was to assess how conversion from grassland to *Eucalyptus* plantation affected soil microbial community functional genes and how those changes related to C and N cycles in soils. We found decreased abundance and altered profile of C degrading functional genes due to afforestation (Figures 5-7). These changes were correlated with shifts of carbon from microbial biomass to less decomposed total extractable carbon under plantations (Table 5, Figure 5). We also found that afforestation led to decreases in abundance and shifted functional gene profiles of ammonification, nitrification, and N fixation genes, which correlated with decreased N in microbial biomass (Figure 8-6, Table 5). These changes in C and N functional genes and pools also widened the ratio of C to N in total extractable and bulk soil pools and decreased soil pH in plantations.

We found that *Eucalyptus* litter was higher in phenolic content and %C than grassland litter was, indicating greater inputs of organic acids to soils. The increased inputs of organic acids such as phenolic compounds are a major source of acidity in surface soils (Richter et al. 1994, Richter and Markewitz 1995). In addition, since *Eucalyptus* is evergreen and has no dormant season, its phenolic inputs contribute year round to soil acidity. The altered litter chemistry inputs from eucalyptus versus native

grasses are a likely reason for the decreased pH we found in surface soils at our sites and the differences in microbial gene structure and abundance (Jobbágy and Jackson 2003, 2004b).

Non-metric multidimensional scaling of all probes showed that seven of eight grasslands had similar assemblages of functional genes, but the plantation sites separated along both NMS axes showing no consistent grouping (Figure 5). A potential cause for the different profiles of the plantations could be differences in the profile of the sites prior to afforestation. Even small differences in the pre-plantation grasslands could lead to differing profiles after afforestation. This idea is supported by a recent study where soil microbial communities were transplanted from different soil types onto different plant litters but did not converge on the same composition when placed on the same litter substrate, which suggests that even very similar microbial communities can react very differently to new substrates (Strickland et al. 2009a). Our results suggest that soil microbes with different initial functional gene profiles can result in very different profiles in response to the same vegetation change.

In addition to shifts in overall functional gene structure, afforestation led to decreases in abundance of biogeochemically important genes in several important categories, independent of the taxonomic origin of the probes. Our analysis of specific categories of functional genes suggests that the taxonomic structure of the microbial community has less impact on soil biogeochemistry than the total abundance of functional genes in those categories. For example, in genes associated with carbon degradation and ammonification, vegetation types clustered in a similar pattern to the

NMS (Figures 5, 6, 8), but some taxa represented by multiple probes from different sources or strains (e.g. different culture collections or environmental isolates) appeared either as ubiquitous across vegetation types and specific to type depending on source of the probe (e.g. *Pleurotus*, *Bacillus*, and *Agrobacterium*).

The decreases in carbon degrading functional gene potential may have caused the overall decrease in carbon in the microbial biomass with afforestation. In addition, the lower capacity of the microbial community to degrade carbon compounds was correlated with an increased amount of carbon in the total extactable carbon pool. The total extractable carbon pool represents dissolved carbon substrates that could be respired or mineralized by microbes or eventually incorporated into more stable soil carbon pools. The accumulation of *Eucalyptus* carbon likely also led to the widening of the bulk soil C to N ratio, which indicates less labile material for soil microbial process and could reduce plant productivity in the long-term.

Changes in vegetation input from grass to *Eucalyptus* also led to a decrease in ammonification and nitrogen-fixation genes (Figure 9). Grassland and *Eucalyptu*s sites did not cluster together with probes of nitrogen fixation genes, which also indicates that the changes due to afforestation are not taxon specific (Figure 10). There are few plants that form symbiotic N fixing associations in native Pampas grasslands, but rhizosphere associated N fixing bacteria are common with grasses; the decrease in N fixing gene abundance is due either to under representation of N fixing soil bacteria on this microarray or to losses of these rhizosphere associated bacteria that are less common in forests (Graham et al. 1988, Elmerich et al. 1992, Baldani et al. 1997).

The lower inputs of mineral N from fixation and ammonification potentially caused the decrease in nitrogen in the soil microbial biomass (Table 5). The changing structure of carbon and nitrogen cycle functional genes also seems to have resulted in a widening of C:N in microbial biomass. This result could indicate a shift in the stoichiometry of the microbes present due to availability of nitrogen (Manzoni et al. 2008). Alternatively, since fungi have a higher C:N (10 to 15) than bacteria (3.5 to 7), plantations could be causing a shift towards more fungal biomass over bacterial (Paul 2007). However, there was no significant difference in the number of fungal and bacterial probes hybridized on the arrays between vegetation types, though a more definitive answer would require a biomass estimate from phospholipid fatty acid analysis.

The presence of a gene does not necessarily mean that it is being actively transcribed, providing some limitations in our ability to draw conclusions about the active function of microbes from these analyses. Analysis of mRNA would allow more direct connections to be drawn. However, recent research on environmental samples using both mRNA and genomic DNA microarrays has shown that the dominant species identified by mRNA arrays are also the most abundant in terms of genomic DNA (Bulow et al. 2008). This suggests that connections drawn between genomic DNA and biogeochemical cycles are reasonable. Nonetheless, future analyses could also incorporate mRNA transcripts, as methods to extract and analyze mRNA from soil continue to improve.

Microarrays are emerging as a valuable tool for evaluating ecological questions by analyzing biogeochemically relevant genes in a parallel manner. In agreement with our hypotheses, this study shows that afforestation of grasslands led to a decrease in

abundance of several important gene families important for soil C and N cycling. These functional gene changes and their associated shifts in biogeochemical cycles suggest that we can begin to predict changes in soil functions as these plantations continue to age. The changing microbial functions due to afforestation have led to a more acid soil and altered soil C and N pools (e.g. soil ^{13}C and soil C:N). In the long-term these biogeochemical shifts should have important implications for the productivity of successive rotations of plantations and alter the rate at which carbon is incorporated into soil.

2.5 Tables

Table 5: Soil biogeochemical pools

Soil or Litter Property	Native Grassland	Eucalypt Plantation
Litter %C	44 (0.38)	53 (0.23)***
Litter %N	0.55 (0.05)	0.68 (0.02)
Litter Phenolics (% of dry leaf mass)	1.1 (0.21)	6.6 (0.62)***
Litter $\delta^{13}C$ (‰ vs. PDB reference)	-17.18 (1.4)	-29.33 (0.13)**
Soil pH	4.55 (0.11)	4.21 (0.09)**
Soil Total Extractable C (μg C g $soil^{-1}$)	27.6 (1.96)	42.6 (4.84)*
Soil Total Extractable N (μg N g $soil^{-1}$)	12.3 (1.8)	8.9 (1.6)
Soil Total Extractable C:N	3.49 (0.39)	3.67 (0.33)
Soil Microbial Biomass C (μg C g $soil^{-1}$)	203.2 (29.0)	147.6 (14.6)**
Soil Microbial Biomass N (μg N g $soil^{-1}$)	41.2 (5.3)	27.0 (2.6)**
Soil Microbial Biomass C:N	4.89 (0.11)	5.50 (0.19)*
Soil Extractable NH_4^+ (μg N g $soil^{-1}$)	9.38 (2.1)	20.25 (3.9)*
Soil Extractable NO_3^- (μg N g $soil^{-1}$)	16.3 (4.6)	11.4 (2.2)
Bulk Soil %C	5.13 (0.5)	5.15 (0.6)
Bulk Soil %N	0.44 (0.05)	0.40 (0.04)
Soil C:N	11.95 (0.19)	12.93 (0.21)**
Bulk Soil $\delta^{13}C$ (per mil)	-20.3 (0.57)	-22.3 (0.35)*

Note: for all analyses N=8 paired sites. * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$.

2.6 Figure Legends

Figure 5. Biplot of non-metric multidimensional scaling analysis of all microarray probes (830 total). Dimensions 1 and 2 represent 69% and 27% of the variability in the original microarray dataset. Arrows represent correlations between ordination dimensions (functional gene profile) and biogeochemical pools. MB-C: microbial biomass carbon, MB-N: microbial biomass nitrogen, TEN: total extractable nitrogen, MB C:N: ratio of carbon to nitrogen in microbial biomass, TEC:TEN: ratio of total extractable carbon to total extractable nitrogen.

Figure 6. Cluster analysis of microarray data for probes related to carbon degradation genes. If a probe is white for a given site then its relative abundance was zero; higher relative abundance is indicated by increased shading. Site names are followed by either a "p" (for plantation and in italic bold) or a "g" (for grassland). On the y-axis, probes are identified by their target gene function followed by domain: A for Archaea, E for Eukaryotes, and B for Bacteria, and the species from which the probe was derived. Squares identify clusters of functional genes of interest.

Figure 7. Frequency distributions of carbon degradation and chitinase probes. Log fold change is the logarithm base 2 of the ratio of relative abundance of the plantation over that of the grassland for each probe. Note that positive values of log fold change indicate an increase in a given probe due to conversion to plantation from grassland and negative values indicate a decrease.

Figure 8. Cluster analysis of microarray data for probes related to ammonification genes. Please see legend of Figure 2 for description.

Figure 9. Frequency distributions of ammonification and N fixation probes. Log fold change is the natural logarithm of the ratio of relative abundance of the plantation over that of the grassland for each probe. Note that positive values of log fold change indicate an increase in a given probe due to conversion to plantation and negative values indicate a decrease.

Figure 10. Cluster analysis of microarray data for probes related to nitrogen fixation and ammonia oxidation genes. Please see legend of Figure 2 for description.

2.7 Figures

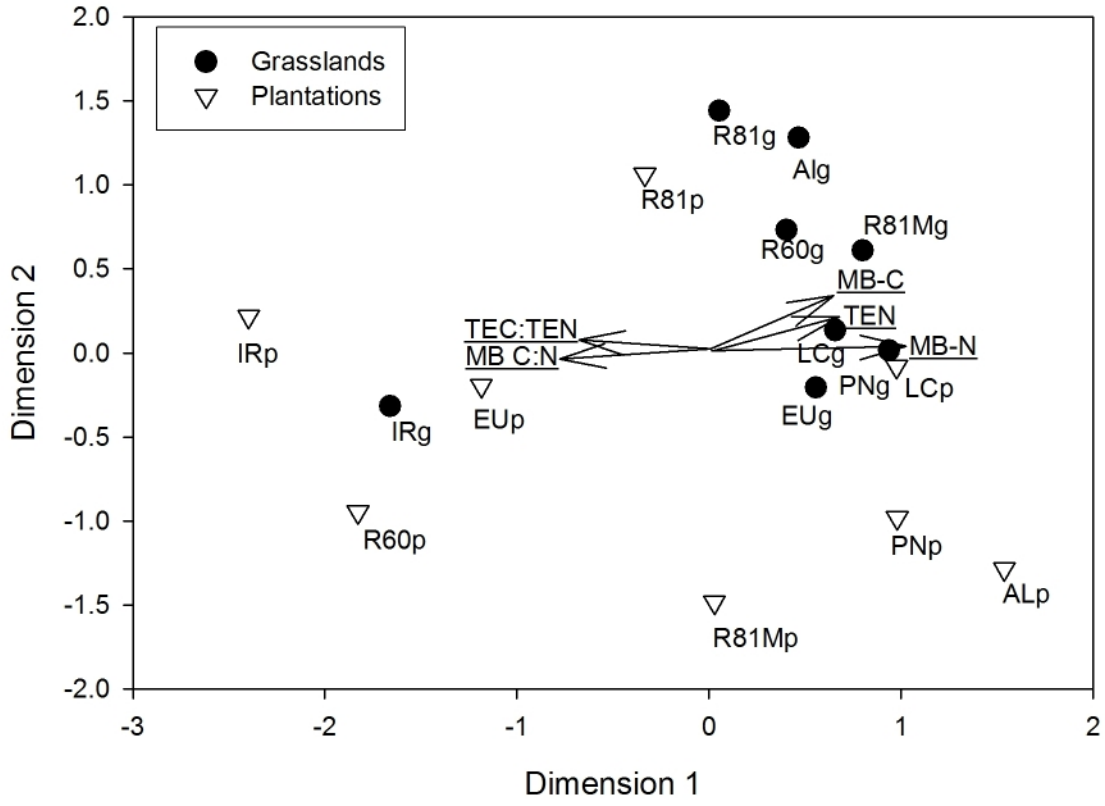

Figure 5: NMS ordination of functional gene profiles

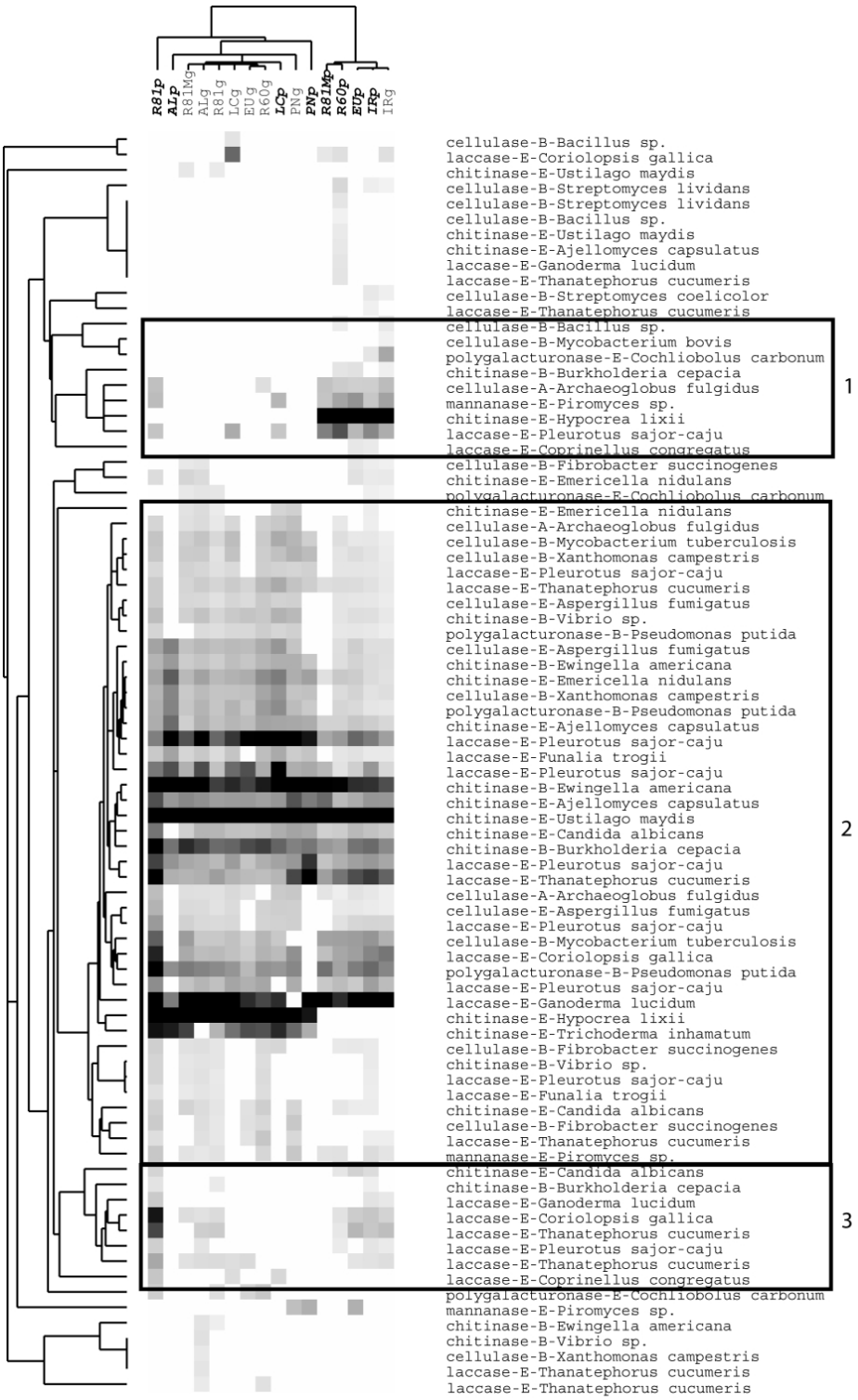

Figure 6: Clustering analysis of C degradation functional genes

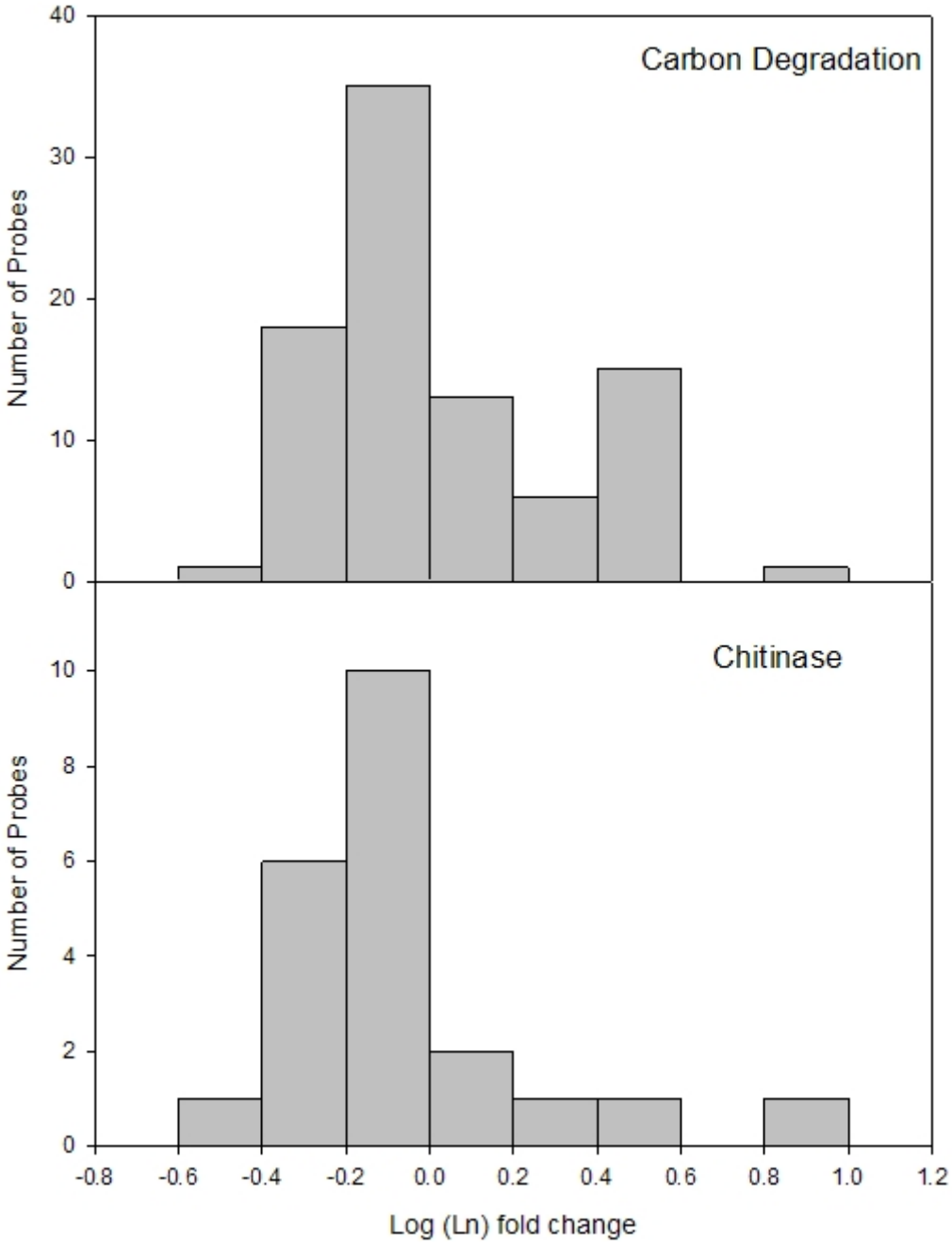

Figure 7: Frequency Distribution of C degradation and Chitinase probes

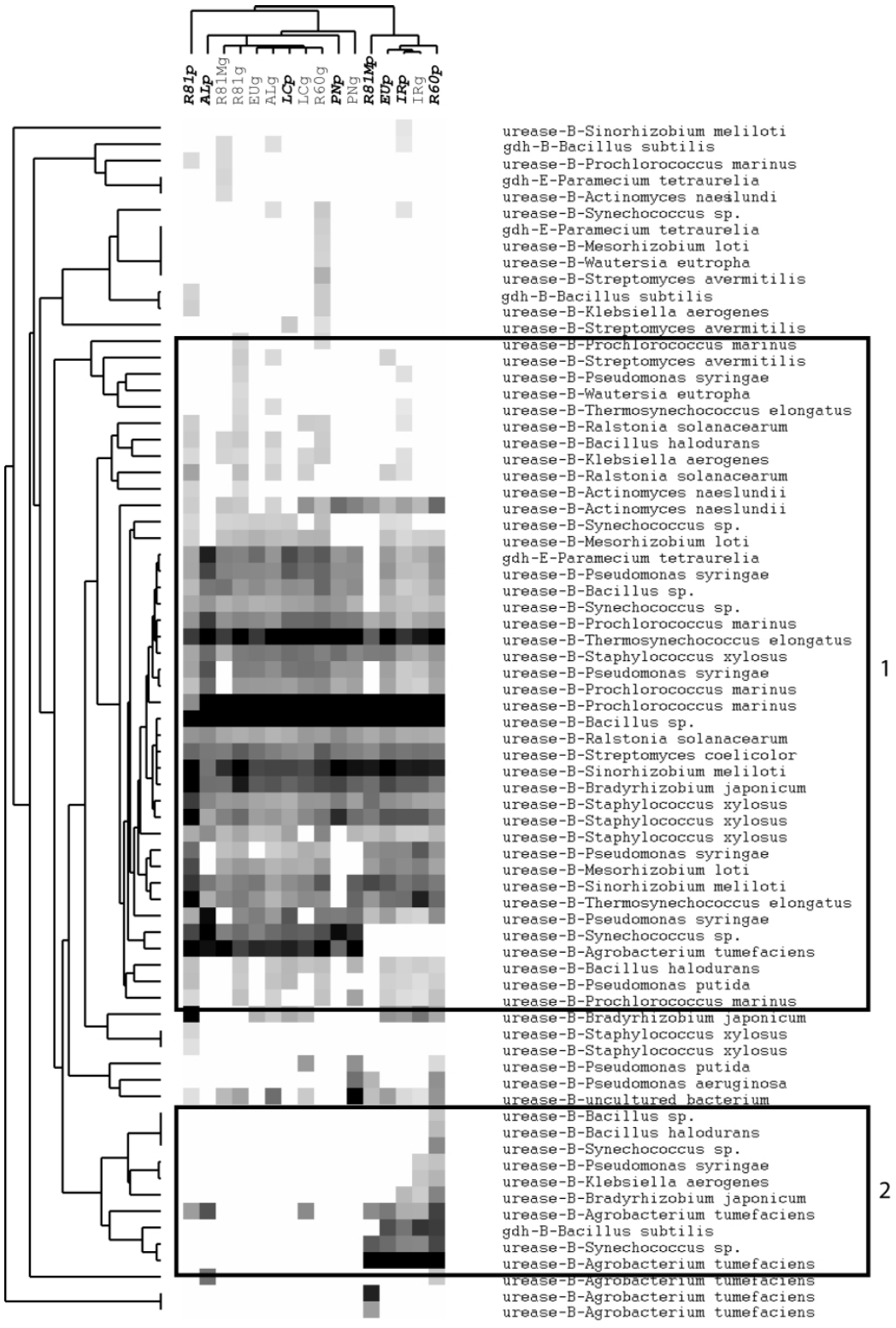

Figure 8: Cluster analysis of ammonification functional genes

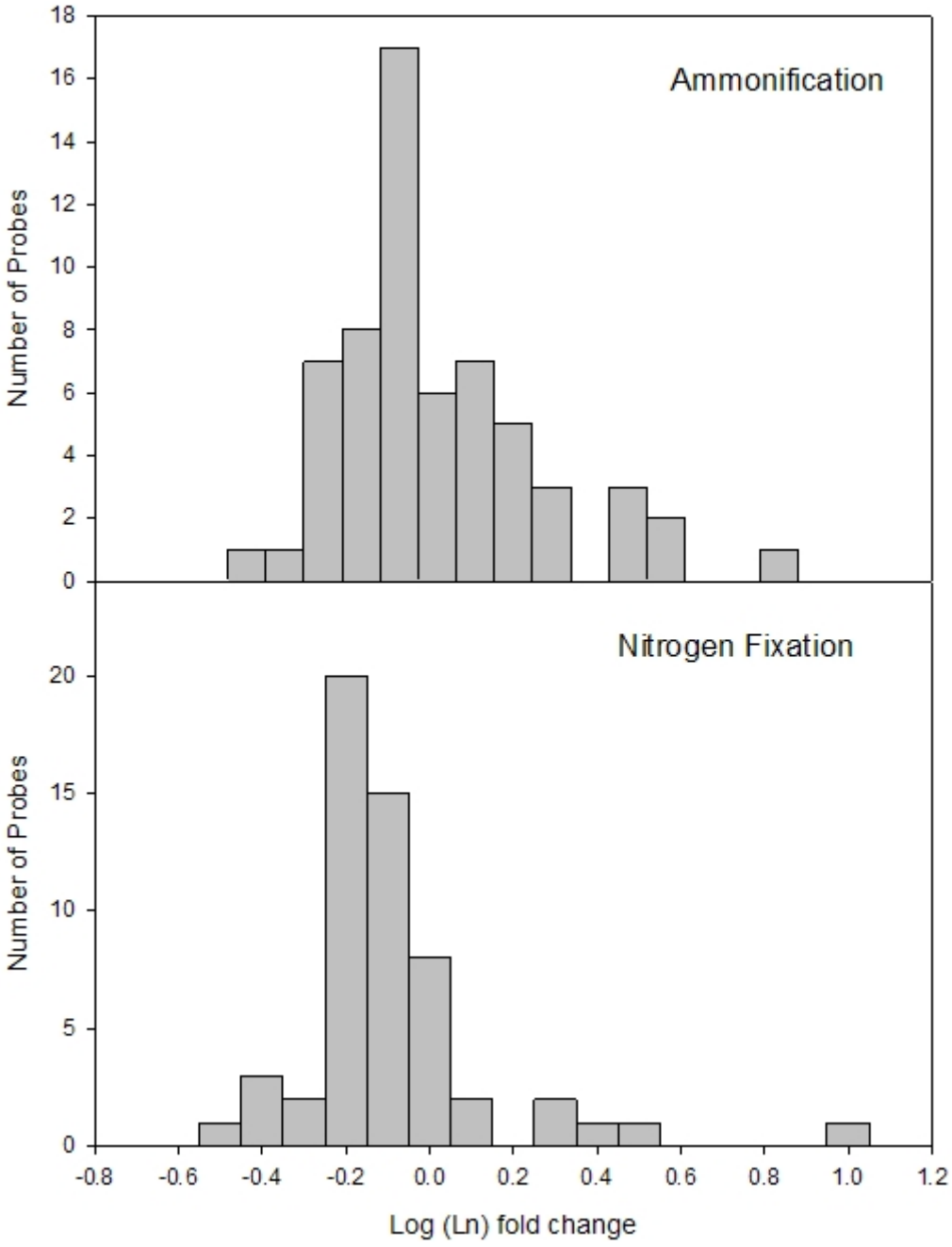

Figure 9: Frequency distribution of ammonification and N fixation functional genes

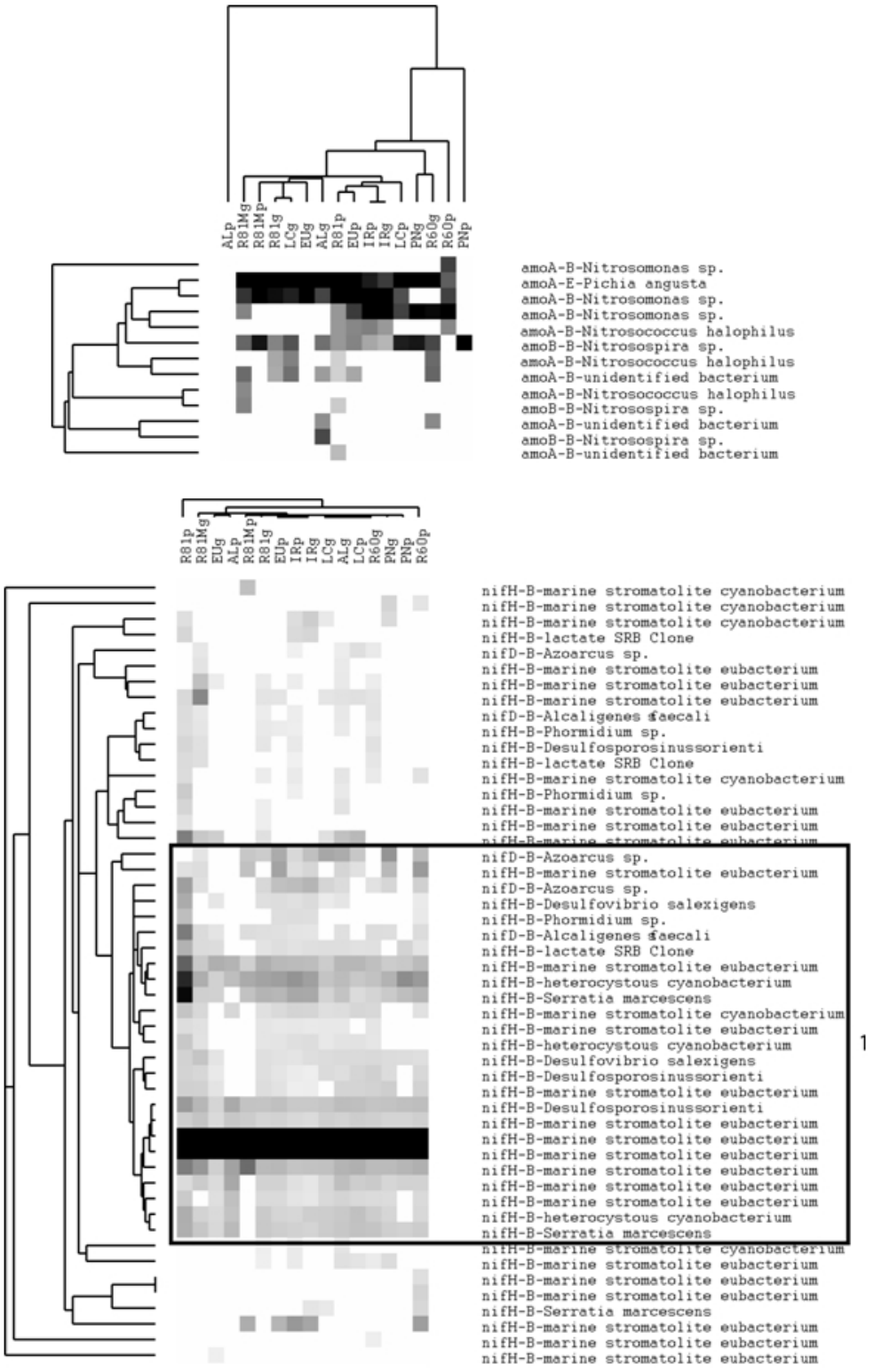

Figure 10: Cluster analysis of nitrification and N fixation functional genes

3. Controls on soil microbial diversity and decreases due to afforestation

3.1 Introduction

Soil microbes are one of the largest stores of biodiversity in the world, however, until the advent of modern molecular techniques of identification, the majority of soil microbes were unidentifiable (Torsvik et al. 1990, Pace 1997, Daniel 2005, Fierer et al. 2007a). It has been estimated that one gram of forest soil could contain up to 10,000 different microbial species, but roughly 90% of these organisms cannot be conventionally cultured (Torsvik et al. 1990, Daniel 2005). In total, more prokaryotic species inhabit a single soil sample than all known cultured prokaryotes combined (Daniel 2005). New DNA based techniques offer the opportunity to rapidly quantify the diversity of the whole soil microbial community independent of the limitations of culturing.

Molecular techniques have recently begun to reveal that microbes follow similar biogeography patterns to larger organisms. For instance, across a scale from centimeters to hundreds of meters in a salt marsh soil bacteria exhibited a positive power law relationship between number of taxa and area similar to plants; however, the bacterial relationship was flatter than the plants which indicates more diversity at smaller scales, but a slower accumulation of new taxa with increasing area (Horner-Devine et al. 2004). Additionally, a similar positive power law relationship has been found between microbial functional groups (groups of taxa that perform similar biogeochemical processes) and area (Zhou et al. 2008). This relationship was also considerably flatter than that observed

in plant or animal communities demonstrating that microbial functional groups have a slower turnover in space than other biotic communities.

In addition to the lack of knowledge of diversity of microbes in soils, there are only a few studies that have begun to address the factors that control the distribution of microbes in the environment. At a continental scale, it has been suggested that soil pH is the strongest control on soil microbial diversity with highest diversity found near neutral pH and declining diversity with either more acidic or alkaline soils (Fierer and Jackson 2006, Jones et al. 2009, Lauber et al. 2009). However, at such a broad continental scale soil pH is an integrator variable for many different soil factors, and not necessarily the only controlling factor on soil biodiversity at more local scales. Studies at a finer scale across a stream benthic mater pH gradient across the Hubbard brook experimental watershed showed that in addition to pH bacterial community diversity is related to the quality of the organic matter the communities develop on and amount and quality of inputs of C and N from surrounding streamwater (Fierer et al. 2007b).

The responses of microbial diversity to land-use change are also not well studied, though it offers an excellent opportunity to examine if changing inputs to microbial communities can change their diversity. In particular, afforestation, the conversion of previously treeless areas to forests (usually fast growing monocultures of timber species) drastically alters the quality and quantity of plant material inputs to soil microbes (Berthrong et al. In Press). Plantations of *Eucalyptus* are known to alter the hydrology, salinity, and organic matter content of formerly grassland soils (Jobbágy and Jackson 2003, 2004b), and are an excellent system to test how differing inputs of plant material

affect microbial diversity. *Eucalyptus* species are known to have very high growth rates and high levels of secondary compounds in their organic matter which is difficult for microbes to decompose (Pryor 1976, Chapuis-Lard et al. 2002).

In this paper, we study if converting from native grassland with high plant diversity to a monoculture of trees alters soil bacterial diversity. Afforestation should greatly alter the plant matter input quality and quantity supplied to microbial communities. We hypothesize that the lack of diversity in plant inputs combined with the increase in secondary compounds will lead to a decrease in the diversity of soil microbes. Changes in microbial diversity will also be related to changes in soil biogeochemical pools as the microbes that control inputs and exports of C and N adapt in response to the changing organic matter input.

3.2 Methods

3.2.1 Site Description and Sampling

This study was conducted pairs of adjacent native grasslands and *Eucalyptus globulus* plantations in the Lavalleja department of Uruguay. Uruguay is historically treeless, and mean annual precipitation is 1150 mm, and mean annual temperature is 16.5°C; soils of the region are Udolls, Udalfs, and Udepts developed over granitic rock (Duran 1985, Soriano et al. 1991). Soils at our sites were mostly Hapludalfs with typical soil depth of 30cm Mean annual precipitation is 1150 mm, and mean annual temperature is 16.5°C. Soils of the region are Udolls, Udalfs, and Udepts developed over granitic rock; soils at our sites were mostly Hapludalfs with typical soil depth of 30cm (Duran 1985, Soriano et al. 1991, Farley et al. 2008c). This region is ideal to study afforestation

since Uruguay has the second most area and fastest growth rate by area of *Eucalyptus* plantations in South America (Geary 2001, FAO 2006b). *Eucalyptus* is the second most common genus used for afforestation globally and comprises ~40% of plantation area in southern South America and Brazil (FAO 2006b). *Eucalyptus* are desirable for their robust growth in different climates and for their resistance to drought, pests, and low-nutrient soils; *Eucalyptus* also has the highest rate of growth of any commonly used afforestation genus in warm climates (Pryor 1976, Florence 1996, FAO 2006b). The fast growth rate and the recent construction of large paper mills on the Uruguay river makes plantations in this region some of the most valuable in the Western hemisphere (Cubbage et al. 2006).

We sampled eight adjacent pairs of native grasslands and *Eucalyptus* plantations. The sites ranged from 50 to 200 m in elevation and were located near Minas, the capital of the Lavalleja department of Uruguay (34.37°S, 55.23°W). Native grasslands in this region are a mix of C_3 and C_4 grasses that are frequently used for cattle and sheep grazing (Soriano et al. 1991). *Eucalyptus* plantations in our study sites were established between 1992 and 1996, and three of the plantations were in their second rotation and five were original plantings. Plantations in this region are not typically fertilized. At each adjacent grassland-*Eucalyptus* pair we collected five cores per transect along two 10 m transects in each vegetation (10 cores per vegetation, 20 cores per site). The cores for each vegetation type at each site were composited in a polyethylene bag and stored on ice for transport to the lab. Samples were homogenized and sieved (2mm) to remove roots and rocks. Fresh soils were used for biogeochemical analyses; subsamples were stored at -

80°C for DNA extraction. Biogeochemical pools characteristics were determined in Berthrong et al. (2009), and presented here in modified form (Table 6).

3.2.2 DNA extraction, PCR, and TRFLP

Soil DNA was extracted using Powermax kit as per manufacturer's instructions (MoBio Labs, Carlsbad, CA, USA). DNA yield and purity was measured with a nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Bacterial 16s rDNA was amplified using the primers 6-FAM labeled Bac8f (5'-AGAGTTTGATCCTGGCTCAG-3') and unlabelled primer Univ1492r (5'-GGTTACCTTGTTACGACTT-3') (Fierer and Jackson 2006). Each 25 µl PCR reaction contained 0.5 M of each primer, 1X Apex Taq master mix (Genesee Scientific), and ~15 ng template DNA. We ran 35 PCR cycles of 60 s at 94°C, 30 s at 50°C, and 60 s at 72°C. Six reactions were run per sample, and composited into two pools of 75 µl PCR product. Composited products were cleaned using Qiaquick PCR cleanup kits (Qiagen, Valencia, CA, USA). Product size was checked by agarose gel electrophoresis (1.5% agarose); all samples used produced a single band of ~1.5k bp.

Replicate products were then digested for six hours in a water bath with the restriction enzyme HaeIII and buffer as per manufacturer's instructions (Promega, Madison, WI, USA). Digested fragments were separated and quantified on a ABI 3700xl DNA sequencer with Genescan software (Applied Biosystems, Foster City, CA, USA). Only terminal fragments with 6-FAM incorporated with the primer Bact8f were quantified. Raw trflp profiles were standardized as described in Dunbar (2001) and Fierer and Jackson (2006). Only fragments between 50 and 700 basepairs with greater than 4%

of the total standardized fluorescence for a given sample were included in the analysis (Dunbar et al. 2001, Fierer and Jackson 2006). Different fragments were treated as operational taxonomic units (OTU) in subsequent analyses.

3.2.3 Statistical Analysis

We examined bacterial community composition using an ordination technique, non-metric multidimensional scaling (NMS). NMS was conducted on square root transformed relative fluorescence trflp data using the Sorenson distance metric in PCOrd (MJM software, Glenden Beach, OR, USA). Shannon diversity indices (H) were calculated based on OTUs, and linear regressions between H and soil variables were analyzed using proc reg in SAS (SAS institute, Cary, NC, USA). We tested for differences in diversity (H) and richness (S) between grassland and plantations using a paired t-test in SAS (SAS institute, Cary, NC, USA).

3.3 Results

Afforestation of grasslands decreased mean soil bacterial diversity and richness (Table 7). The mean Shannon diversity index (H) decreased from 2.86 in grasslands to 2.63 in plantations ($p<0.01$, Table 7). Ranges of H were fairly broad: grasslands ranged from 2.15 to 3.18 and plantations from 1.99 to 3.09 (Table 7). Afforestation also reduced overall OTU richness (S) from a mean of 27.6 in grasslands to 21.7 in plantations ($p<0.05$, Table 7). Richness ranged from 10 to 36 for grasslands and 8 to 30 for plantations.

Bacterial diversity and richness across vegetation types were correlated with several soil labile soil pools of C and N (Figure 11). Total extractable C was negatively

associated to both diversity and richness (Figure 11). Microbial biomass C and N pool sizes were positively associated with diversity and richness (Figure 11). Interestingly, other less labile pools, such as bulk soil C and N, C:N of different pools, and soil pH were not significantly related to the soil bacterial community (data not shown).

Despite the decrease in diversity and richness due to afforestation, the composition of soil bacterial OTUs did not differ between vegetation types (Figure 12). Bacterial community composition was examined by NMS, which resulted in a best configuration with two axes. The two axis solution explained 87% of the variability in the original data set with acceptable final stress value 8.73 (Figure 12). Across the two axes vegetation types did not separate well indicating similar soil bacterial community compositions (Figure 12). The lack of difference in bacterial community composition led to no associations with biogeochemical pools above R^2 of 0.20.

3.4 Discussion

Soil microbes are a large store of biodiversity, but we are only beginning to catalogue the true depth of that biodiversity. Additionally, we still do not have a very clear understanding of what controls the distribution of those soil microbes and how changing patterns of land-use and above-ground biodiversity will affect those distributions. The goal of this project was to examine how conversion from a diverse temperate grassland ecosystem to monocultures of *Eucalyptus globulus* affected soil bacterial diversity. In general our results matched our hypotheses that converting from the highly plant diverse grassland to *Eucalypt* plantations would result in reduced soil

bacterial diversity, and these differences would be driven by differences in the chemistry of new plant material derived from *Eucalyptus*.

Plant matter litter inputs from *Eucalyptus* were 20% higher in C percentage and six times higher in phenolic compounds, which was likely the driving cause behind the decreased diversity and richness seen in the soil bacterial community (Tables 6 and 7) (Swift et al. 1998). This finding agreed with several previous studies that found secondary plant compounds, such as phenolics, decreased microbial function and abundance (Souto et al. 2001, Castells et al. 2003, Nierop et al. 2006, Strickland et al. 2009a). This idea is also supported by the negative relationship between total extractable C and species diversity and richness. Total extractable carbon increased due to afforestation and likely contained a higher fraction of phenolic compounds leading to decreased diversity. Interestingly, these results contrast with studies that have found that addition of pure solutions of phenolic compounds can spur microbial respiration (Castells et al. 2003, Baptist et al. 2008). This is possibly due to the fact that the respiration responses were short term, and thus did not accurately describe the long-term effects of increased phenolic deposition on soil bacterial communities.

Afforestation also led to a loss of microbial abundance from soil as measured by losses in microbial biomass C and N (Table 6). The lower abundance of microbes could have been a driver of some of the species loss, but since there was no discernable change in the species composition it is difficult to test this conclusion (Figure 12). The lack of bacterial species composition shifts combined with the loss in diversity suggests that the larger and more diverse grassland bacterial community was pared down to a smaller and

less diversity community after afforestation as opposed to a shift in species and emergence of a new set of potentially dominant bacteria. This effect is supported by recent mesocosm experiments that show that microbial communities that developed on litter from one type of ecosystem do not perform biogeochemical functions as well when transplanted onto different litter types (Strickland et al. 2009a, Strickland et al. 2009b).

Interestingly, soil pH, which has been shown to be the best predictor of diversity on a continental scale, was not the best predictor of soil bacterial diversity in this system (Fierer and Jackson 2006, Jones et al. 2009, Lauber et al. 2009). Soil acidification was significant at our site, though much smaller in magnitude (0.2 pH units) than the gradient seen across the US, which likely lead to the lack of correlation (Fierer and Jackson 2006). At broader scales, soil pH integrates many different climatic and vegetation factors, thus is a good predictor across different ecosystems, but it has been suggested that local correlations are more closely tied to factors distinct to the particular study site (Hooper et al. 2000).

In contrast, the correlations between bacterial diversity with microbial biomass C and N and total extractable C observed in this study agreed with work on fine benthic organic matter from Hubbard Brook experimental watershed (Fierer et al. 2007b). Across a pH and organic matter gradient, available C and N and pH in stream water were found to be strongly correlated with bacterial community composition. These results suggest that as the scale of observation increases from local to watershed to continental, locally highly variable biogeochemical pools become poorer predictors of soil bacterial diversity.

In conclusion, soils are a vast store of biodiversity, and this study shows that losses of aboveground diversity (diverse grassland to plantation monoculture) are linked to below ground soil bacterial diversity losses. Though the diversity under plantations was still relatively high, it was significantly reduced from native grasslands, which suggests that accurate accounting for biodiversity loss due to afforestation should consider both losses of vegetation and soil microbes.

The primary mechanism for these changes was likely differences in plant matter input quality. These results also suggest that the bacterial community that developed under grasslands did not shift to a novel one under *Eucalyptus* but instead remained in a smaller less abundant form. This lack of a new community profile, though limited by the lack of phylogenetic specificity of TRFLPs, suggests that soil bacteria may not be entirely ubiquitous and instead display distinct biogeography. These results imply that the study of biodiversity loss due to land-use change should focus not only on above ground diversity loss but also soil bacteria.

3.5 Tables

Table 6: Soil biogeochemical properties

Soil or Litter Property	Native Grassland	Eucalypt Plantation
Litter %C	44 (0.38)	53 (0.23)***
Litter %N	0.55 (0.05)	0.68 (0.02)
Litter Phenolics (% of dry leaf mass)	1.1 (0.21)	6.6 (0.62)***
Litter $\delta^{13}C$ (‰ vs. PDB reference)	-17.18 (1.4)	-29.33 (0.13)**
Soil pH	4.55 (0.11)	4.21 (0.09)**
Soil Total Extractable C (μg C g $soil^{-1}$)	27.6 (1.96)	42.6 (4.84)*
Soil Total Extractable N (μg N g $soil^{-1}$)	12.3 (1.8)	8.9 (1.6)
Soil Total Extractable C:N	3.49 (0.39)	3.67 (0.33)
Soil Microbial Biomass C (μg C g $soil^{-1}$)	203.2 (29.0)	147.6 (14.6)**
Soil Microbial Biomass N (μg N g $soil^{-1}$)	41.2 (5.3)	27.0 (2.6)**
Soil Microbial Biomass C:N	4.89 (0.11)	5.50 (0.19)*
Soil Extractable NH_4^+ (μg N g $soil^{-1}$)	9.38 (2.1)	20.25 (3.9)*
Soil Extractable NO_3^- (μg N g $soil^{-1}$)	16.3 (4.6)	11.4 (2.2)
Bulk Soil %C	5.13 (0.5)	5.15 (0.6)
Bulk Soil %N	0.44 (0.05)	0.40 (0.04)
Soil C:N	11.95 (0.19)	12.93 (0.21)**
Bulk Soil $\delta^{13}C$ (per mil)	-20.3 (0.57)	-22.3 (0.35)*

Note: for all analyses N=8 paired sites. * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$. Values in parentheses are one standard error of the mean. (Adapted from Berthrong et al. in press)

Table 7: Soil bacterial community diversity and richness

	Diversity (H)			Richness (S)		
Vegetation	Mean	Min	Max	Mean	Min	Max
Grassland	2.86 (0.14)	2.15	3.18	27.6 (3.19)	10	36
Plantation	2.63 (0.14)**	1.99	3.09	21.7 (2.79)*	8	30

Note: Values in parentheses are standard errors. **=$p<0.01$ and *=$p<0.05$ for differences between means for different vegetation types.

3.6 Figure Legends

Figure 1. Relationship between soil bacterial diversity and biogeochemical pools. Total extractable C vs. H and S: r=-0.55 and -0.55 respectively (both $p<0.05$). Microbial biomass C vs. H and S: r=0.48 and 0.53 respectively (both $p<0.05$). Microbial biomass N vs. H and S: r=0.53 and 0.58 respectively (both $p<0.05$).

Figure 2. Nonmetric multidimensional scaling ordination results. Sites that are closer in ordination space have more similar patterns of species composition and abundance. The Sorenson distance metric was used for this ordination.

3.7 Figures

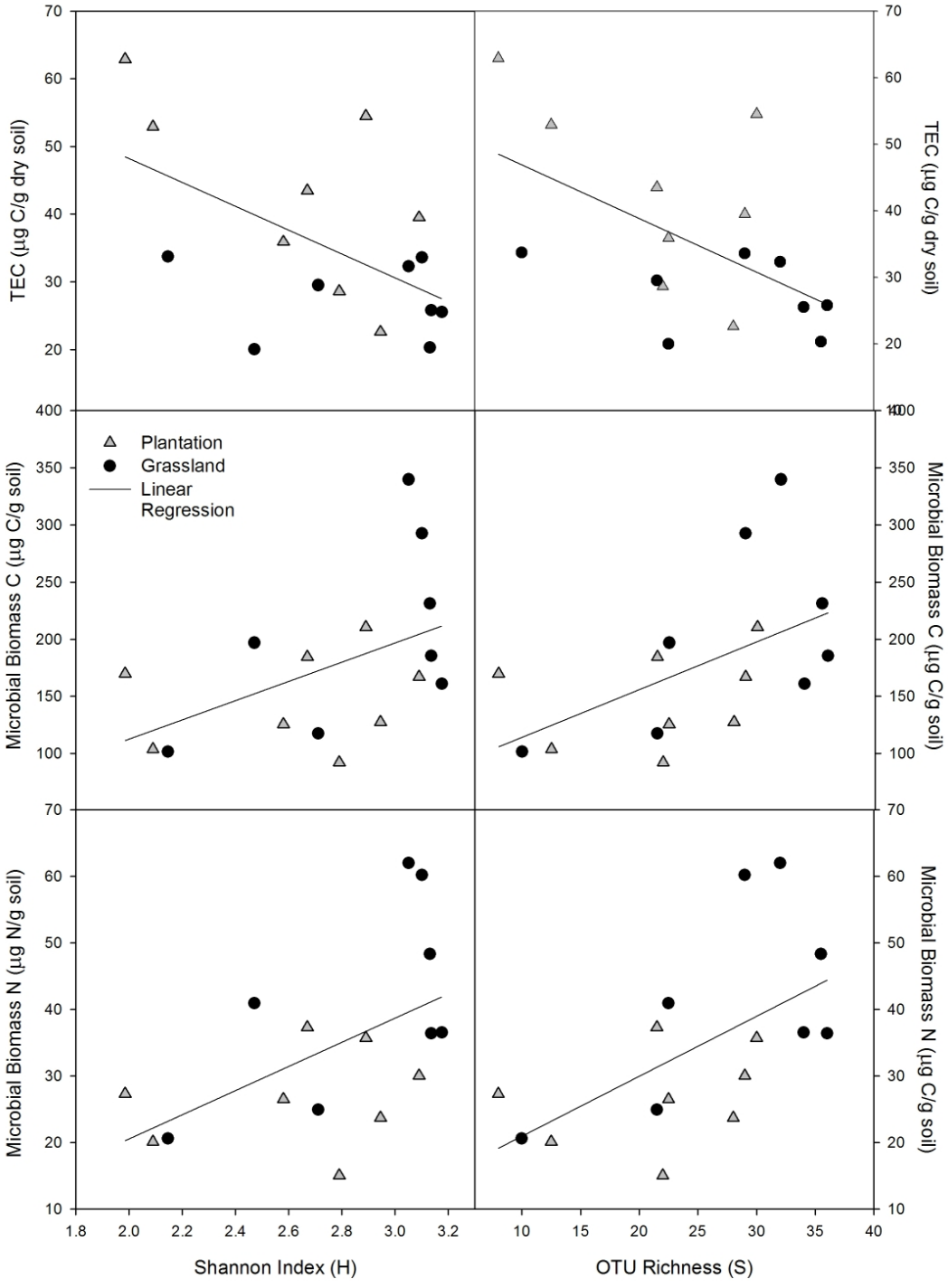

Figure 11: Relationship between soil bacterial diversity and biogeochemical pools

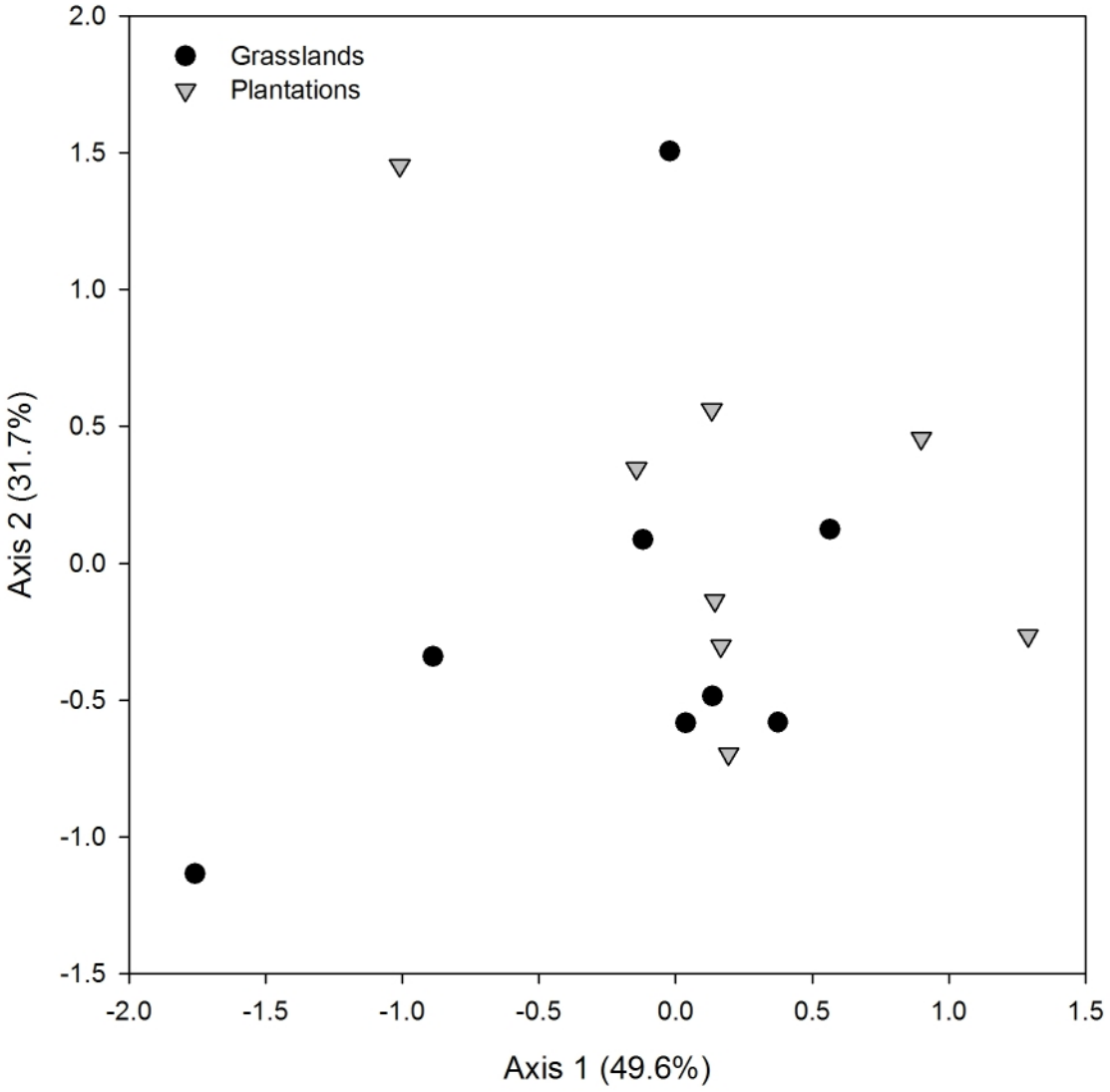

Figure 12: Non-metric multidimensional scaling ordination

4. Effects of afforestation on soil carbon and nitrogen across a precipitation and age gradient

4.1 Introduction

Soil organic matter is the largest terrestrial pool of carbon (Jobbágy and Jackson 2000) and could be a large part of the "missing sink" of carbon dioxide (Denman et al. 2007). Soil C is accumulated by land-use changes, such as reforestation of croplands or converting crops to pastures, but can also be lost by land-use changes such as the conversion of primary forests to plantation or crops (Vitousek 1991, Guo and Gifford 2002). Land-use change can also have significant impacts on soil nitrogen (N), the soil nutrient that most commonly limits terrestrial primary productivity (Schlesinger 1997, Farley and Kelly 2004, Marin-Spiotta et al. 2009, Wiesmeier et al. 2009). Availability of N could alter the primary productivity of ecosystems and hence the amount of carbon available to be converted to soil organic matter. Studying the interaction of land-use change with soil C and N is vital to better understand the effects of land-use change on soils.

Afforestation, the conversion of treeless areas to forests, is a land-use change that has been widely viewed as a potential mechanism to sequester carbon dioxide through accumulation of plant biomass (Vitousek 1991, Houghton et al. 1999, Wright et al. 2000, Hoffert et al. 2002, Jackson et al. 2002, Jackson and Schlesinger 2004). Evergreen species, such as *Eucalyptus* spp. and *Pinus* spp., with very high productivity are the most frequently planted genera (Florence 1996, FAO 2006b). Currently, 140 m ha globally have been afforested on every continent except Antarctica, with 2.5 m ha afforested per

year from 2000 through 2005 (FAO 2006a). The rapid growth of plantations suggests the need to study soil C and N in these systems to predict if they can continue to function as a long-term carbon dioxide sinks.

Afforested plantations are harvested frequently, which coupled with their high productivity can deplete soil carbon and nutrients and alter soil chemistry and hydrology (Adams et al. 2001, Guo and Gifford 2002, Ross et al. 2002, Engel et al. 2005, Berthrong et al. 2009). Exchangeable cations (Ca, Mg, K) and N were found to be lower in a global meta-analysis on the effect of afforestation on soils (Farley et al. 2008c, Berthrong et al. 2009). Additionally, soils have been shown to be more saline and acidic with afforestation (Williams et al. 1977, Alfredsson et al. 1998, Lilienfein et al. 2000, Jobbágy and Jackson 2004a, Nosetto et al. 2007, Farley et al. 2008c). Afforestation in our study system leads to lower streamflow and lower water tables (Jobbágy and Jackson 2004a, Farley et al. 2005, Jobbágy and Jackson 2007, Farley et al. 2008c). This alteration of soil chemistry can lead to lower soil fertility and less production in subsequent plantation rotations (Zhang et al. 2004, Bi et al. 2007).

Interestingly, the direction and magnitude of afforestation's effect on soils often depends on mean annual precipitation (MAP) and plantation age. MAP in Australia and New Zealand correlated with changes in soil C; plantations converted from pastures with 1200 mm or greater MAP showed significant losses of soil C while those with less than 1200 mm MAP showed no change or a slight gain in soil C (Guo and Gifford 2002). This effect was also found in grasslands undergoing woody plant encroachment in the southwest USA; sites with MAP greater than 600 mm that were invaded by shrubs lost

soil C while those with less than 600 mm MAP gained soil C (Jackson et al. 2002). Soil C was also affected by plantation age; plantations that were formerly grasslands were found to lose soil C for 10 years after planting, but soil C levels recovered by 20-30 years (Davis and Condron 2002, Paul et al. 2002). Plantation age in Ecuador had a similar effect on surface soil N with young plantations leading to losses from soil with gradual recovery after 15-20 years, although soil N in soils deeper than 10 cm showed continued losses for up to 25 years after planting (Farley and Kelly 2004).

Recent modeling studies suggest that changes in soil C and soil N due to afforestation are linked and controlled by different MAP (Halliday et al. 2003, Kirschbaum et al. 2008). Simulations based on Australian plantations indicate that higher MAP leads to higher leaching losses of N from plantation soils, which in turn leads to depletion of soil organic C (Kirschbaum et al. 2008). Other models suggest that the effect is more closely related to shifts in the C:N of vegetation and soil pools (Rastetter et al. 1992). However, there are few studies that examine these relationships in the field.

The goal of this study was to examine how soil C and N cycles were linked in their response to afforestation across a precipitation and age gradient. The gradients in this study provided a novel test of the effects of afforestation on soil C and N. We were able to test how pools of C and N react and interact with afforestation, and determine if model predictions that differences in N cycling control soil C loss or gain with afforestation were valid (Kirschbaum et al. 2008). Based on previous research, we hypothesized that changes in soil C would be associated with precipitation with xeric areas gaining C and more humid areas losing C, but those changes depended also on the

age of the plantation. If soil N does controlled losses and gain of soil C with afforestation, then it should also follow a similar pattern to soil C along the precipitation gradient. We also suspected that more labile pools of C and N would show a more dramatic effect due to afforestation than bulk soil pools since they are more closely tied to current plant inputs.

4.2 Methods

4.2.1 Site Description and Analytical Methods

The Rio de la Plata grasslands were historically treeless before European settlement, with the exception of a few riparian species (Soriano et al. 1991). The area has extensive areas of agriculture and animal pastures (Soriano et al. 1991). Mean annual precipitation (MAP) ranges from approximately 650 mm in the southwestern most area to 1600 mm in southern Brazil (New et al. 2002). This region is very well suited to studying the effects of varying precipitation since it has similar plantations throughout and extends from dry areas where precipitation is insufficient to meet plantation water demand to areas where precipitation greatly exceeds plantation water needs (Nosetto et al. 2008).

Afforestation has been common in the Rio de la Plata grasslands for at least 100 years. Historically, *Eucalyptus* plantations in this region were established for wind shelter or shade, but more recently afforestation has been motivated wood production (Soriano et al. 1991, Geary 2001, Cubbage et al. 2006). *Eucalyptus* species are desirable plantation trees in this region for their resistance to drought and pests, and they have the highest growth rate of any common afforestation genus in warm climates (Pryor 1976, Florence 1996, FAO 2006b). This region's combination of rapid growth in area of plantation and

accessible pulp mills makes plantations in this region some of the most valuable timber in North or South America (Cubbage et al. 2006). The high value and fast growth of plantations in this region make it vital area to study the impact of afforestation on soils.

We identified 17 sites with adjacent native grasslands and *Eucalyptus* spp. plantations in the Rio de la Plata basin. The plantation/grassland pairs are aligned along a precipitation gradient from 650 mm MAP in La Pampa province of Argentina to 1450mm MAP in the Rivera department of Uruguay. There were several different species of *Eucalyptus* planted across the sites (*E. globulus, E. grandis*, *E. camaldulensis*); however, all of the species are members of *Eucalyptus* subgenus *Symphomyrta* with similar physiological and chemical profiles (Florence 1996). The age of the *Eucalyptus* plantations ranged from 10 years to 49 years since first planting.

Soils were sampled in October and November of 2006 using a 1.9 cm-diameter soil corer. At each site and for each grassland or plantation, we collected five cores along two 10 m transects. Cores were taken from 0-10 cm and 10-20 cm depths of mineral soil. In order to reduce the effect of very fine scale soil heterogeneity, the cores within each transect were composited in a polyethylene bag and stored on ice for transport to the lab. Samples were homogenized and sieved (2mm) to remove large roots and rocks. We calculated soil bulk density for each core using the volume of the corer and the mass of dry soil, and used it to scale results to grams per m^2. There was no significant difference between vegetation types in bulk density. In addition to soil, at each site we collected leaf litter, which was air-dried and stored in polyethylene bags.

Soil total extractable carbon and nitrogen and microbial biomass C and N were determined as previously described (Berthrong and Finzi 2006). The methods were modified by using 10 g of field moist soil instead of 30 g. Total air-dried soil and litter C and N were determined by combustion in a Carlo-Erba Elemental Analyzer (CE Elantech, Lakewood, NJ USA). Soil pH was measured by combining 2 g of air dry soil with 5 ml of 0.01 M $CaCl_2$. The slurry was swirled gently by hand and allowed to settle for 30 minutes. A potentiometric electrode was then used to measure the pH of the supernatant.

4.2.2 Statistical Analysis

We measured the effect size of afforestation on biogeochemical pools as response ratios, $r = X_E / X_C$, where X_E is the mean value for a site of a given soil variable under *Eucalyptus* and X_C is the mean value of the same site's paired control grassland (Gurevitch and Hedges 2001, Guo and Gifford 2002, Ainsworth and Long 2005). To match the scale of pH (logarithmic) to the linear scales of the other biogeochemical pools, we transformed pH to hydrogen ion concentration values (10^{-pH}) to calculate response ratios. However, we discuss the hydrogen ion differences as pH values for ease of interpretation. The response ratio was then transformed by the natural logarithm to make the values linear, so that an increase in a variable due to afforestation would be proportional and on the same scale as a decrease.

We used stepwise regression by the method of least squares in SAS (SAS institute, Cary, NC USA) to test if MAP or plantation age were significant predictors for the effect of afforestation on biogeochemical pools. F-tests were calculated for both

plantation age and MAP and were added to the overall model if the p value was less than 0.15 (standard p-value for stepwise regression inclusion); however, if after adding another predictor the previously added predictor's p value rose above 0.15 controlling for other predictors in the model, then it was removed from the overall model.

Plantation age and MAP were weakly collinear ($r=0.44$, $p<0.05$). This collinearity can inflate the regression parameters if both predictors are included in the regression model. To compensate for the collinear predictors, we used ridge regression in SAS (Hoerl and Kennard 1970, 2000). This process calculates ridge coefficient that minimizes the inflation due to collinear predictors, and then recalculates regression parameters. The ridge corrected parameter estimates have a higher root mean square error for the model, but are generally better approximations of the true relationship between dependent and independent variables.

If neither MAP nor plantation age were significant predictors of the effect of afforestation for a given soil variable, we then tested if afforestation had a significant effect on that soil variable overall by using ANOVA in SAS (proc GLM, SAS Institute, Cary, NC, USA). We blocked paired analyses by site to better represent the paired nature of the sites. We used Tukey HSD for post-hoc comparisons of means across vegetation type.

4.3 Results

Changes in soil C with afforestation were negatively correlated with mean annual precipitation, while plantation age was positively correlated (Figure 13, Table 8). Sites with higher MAP generally lost C from pools with afforestation but gained C as

plantations aged (Figure 13). Sites with lower MAP generally increased their soil C pools compared to native grasslands. However, there was no relationship between MAP and the effect of afforestation on C pools at 10-20 cm (Figure 13, Table 8). In the upper 10 cm of soil, the effect of afforestation changed from a gain to a loss at ~1100 mm per year and roughly 20-25 years in age.

Consistent with our hypotheses, changes in soil N followed a similar pattern to soil C in response to afforestation at different precipitation levels (Figure 14, Table 9). Wetter sites generally lost soil N and drier sites gained N with gains increasing as plantations aged (Figure 14, Table 9). However, the slope of the relationship between total soil N and MAP was less steep (-0.0079, Table 9.) than that of total soil C and MAP (-0.0095, Table 8.) indicating that soil C change with afforestation responded more drastically to differences in MAP than soil N. The precipitation and plantation age where gains became losses with afforestation were similar to those seen with soil C at around 1100-1200 mm and 30 years (Figures 13, 14).

Litter C was not related to MAP or plantation age, though it was 10% higher in *Eucalyptus* than in grasslands ($p<0.001$, Table 11). This result is interesting given that litter C:N only trended higher in *Eucalyptus* compared to grasslands and litter N did not differ between vegetation types (Table 11). This corresponded with increase in soil C:N in all the but wettest sites (Figure 15). Additionally, soil C:N ratios were higher in plantations (12.2) than in grasslands (10.5; $p<0.01$). These changes suggest links between vegetation input and soil pool responses to afforestation.

Pools of microbial biomass C and total extractable C and N had a similar response to afforestation across the gradients compared to the responses of bulk soil C and N, with negative relationships with MAP and positive relationships with age in the top 10 cm of soil (Figures 13, 14). However, microbial biomass C was not associated with plantation age. Interestingly, the precipitation value where afforestation changed from a loss to a gain of extractable N (~1200 mm, Figure 14) was much higher than that of bulk soil N, and the age where extractable N began to accumulate with afforestation (~20 years, Figure 14) was earlier than bulk soil N (~30 years).

The effect of afforestation on relatively labile and quickly cycling C and N pools was not associated with either MAP or plantation age. However afforestation caused significant uniform differences across all the sites (Tables 11, 12). Microbial biomass N was 28% lower at 0-10 cm and 43% lower at 10-20 cm with afforestation compared to grasslands (Table 11, $p<0.01$). Microbial biomass C was 26% lower at 10-20 cm soil depth (Table 11, $p<0.01$), which was similar to the effect at 0-10 cm, though at that depth the effect was dependent on MAP (Figure 13). Soil pH decreased by 0.2 and 0.3 units with afforestation (Table 12, $p<0.05$).

4.4 Discussion

Afforestation is a tool for carbon sequestration, but its effects on soils indicate that it has some potential tradeoffs that must be weighed against the sequestration benefit (Vitousek 1991, Jackson et al. 2002, Nosetto et al. 2008). Several studies examined or modeled the effect of afforestation on soil C along gradients of precipitation or plantation age (Guo and Gifford 2002, Halliday et al. 2003, Jackson et al. 2005); however, to our

knowledge this is the first study to examine the effect of afforestation on both soil C and N incorporating a gradient of age and precipitation. Our study provides interesting support for the coupling of C and N cycling in response to land-use change across differing precipitation levels. Across both labile and bulk pools, we found that afforestation had a similar effect on biogeochemical storage of both elements.

Soil C has been shown to respond differently to afforestation for different precipitation levels, a response that a recent modeling study suggests is strongly tied to alterations in the nitrogen cycle (Kirschbaum et al. 2008). The model indicates that soils in xeric areas store C through increases in the soil C to N ratio, and at more humid areas increased decomposition and N losses due to leaching lead to C losses. Soil nitrogen data from our study support this theory; more xeric sites accumulated N with afforestation compared to grasslands while more humid sites lost N (Fig 2), and soil C:N ratios were higher with afforestation in most cases (Fig 3, Table 11). Additionally, soil microbial biomass C:N was less affected (i.e. lower C:N) in humid sites, which could lead to more mineralization of N and increased leaching of N. The increased N losses coupled with increased C:N led to more losses of C per unit N loss at more humid sites (Fig 3, Table 11).

Afforested soils initially lost C and N but recovered those elements with age and by 20-30 years began to gain C and N (Fig 1, 2). These results and the approximate age at which plantations begin to gain C and N are similar to findings in New Zealand, Australia, and Ecuador (Davis and Condron 2002, Paul et al. 2002, Farley and Kelly 2004). In contrast, modeling results suggested that soil C:N would take much longer to

respond to afforestation than pools of C and N did (Kirschbaum et al. 2008), but soil C:N ratio in this study increased in plantations of all ages (Table 12). The change in soil C:N could be due to the rapid response of C:N of total extractable and microbial biomass (Figure 15, Table 12); the increase in C:N of these quickly cycling pools could lead to soil microbes altering soil organic matter content faster than models would predict.

The relationship between the effect of afforestation on soil C and MAP in this study generally agrees with published observations. Similar to Guo and Gifford (2002), plantations at around 1200 mm MAP had no change in soil C with increasing amounts of soil C loss with increasing MAP. However, our results extend farther into drier areas and suggest the trend reverses in drier areas with increasing soil C storage with afforestation with decreasing MAP. Our results from drier areas are similar in slope to those of Jackson et al. (2002), though they found that areas invaded with woody plants tended to gain soil C with <500 mm MAP and lose C with >500 mm MAP compared to our results that suggest the change from gain to loss of soil C with afforestation is around 1000-1200 mm MAP. This difference could be due to the deeper rooting depth of *Eucalyptus* plantations versus grassland, which would allow plantations to access groundwater resources unavailable to the grasslands (Sharma et al. 1987, Le Maitre et al. 1999). This explanation could lead to relatively high productivity plantations growing on formerly less productive dry grasslands, hence a larger gain in C inputs with afforestation relative to small C inputs with native grasses.

The interaction between precipitation and plantation age and soil C can be visualized as a family of graphs (Figure 16). Though the applicability of this conceptual

diagram is limited to regions with similar rainfall patterns, it demonstrates how managers might consider plantation rotation length to maximize soil C and long-term productivity at a site. More humid sites that could potentially lose soil C with afforestation could use longer rotation times to counter balance the initial losses in soil C and N.

This study shows that soil C and N responses to afforestation are linked and depend on both the age and precipitation of the afforested region, which has important implications for the value of afforestation as a carbon sequestration tool. On the one hand, afforestation in drier regions could have added benefits due to additional sequestration of C in soil (Figure 13). However, this result should be treated with caution, since afforestation of drier areas can have deleterious effects on the hydrology of these regions (Jackson 2005). On the other hand, the C sequestration benefit of afforestation in more humid regions could be reduced by losses of soil C and N and reduced soil fertility and lower long-term productivity, though these effects could be potentially ameliorated with longer rotation times.

4.5 Tables

Table 8: Regression parameters of effect of afforestation on soil C pools predicted by plantation age and MAP

	Ordinary Least Squares			Ridge Regression (k=0.13)	
	Total Extract C	Micr. Biomass C	Total Soil C	Total Soil C	Total Extract C
<u>0-10 cm</u>					
MAP (cm year^{-1})	-0.010 (0.0047)	-0.019 (.0042)	-0.011 (0.0053)	-0.0098	-0.0095
Age (years)	0.025 (0.0092)	NS	0.017 (0.010)	0.016	0.023
Intercept	0.87 (0.86)	1.8 (0.45)	0.84 (0.76)	0.76	0.88
Model R^2	0.72	0.59	0.59		
Model p value	0.0003	0.0005	0.003		
<u>10-20 cm</u>					
MAP (cm year^{-1})	NS	NS	NS		
Age (years)	0.039 (0.0066)	NS	.024 (.0070)		
Intercept	-0.56 (0.19)	NS	-0.58 (0.20)		
Model R^2	0.71		0.46		
Model p value	<0.0001		0.0041		

Note: Values are estimates for regression parameters with standard errors in parentheses. The value of the ridge k was selected to reduce the variance inflation factor to 1. If the stepwise regression discarded either MAP or Age, then the resulting model only includes one predictor.

Table 9: Regression parameters of the effect of afforestation on soil N pools predicted by plantation age and MAP

	Ordinary Least Squares		Ridge Regression (k=0.13)
	Total Extract N	Total Soil N	Total Soil N
0-10 cm			
MAP (cm year^{-1})	-0.022 (0.0071)	-0.0087 (0.0045)	-0.0079
Age (years)	NS	0.014 (0.0088)	0.013
Intercept	2.7 (0.77)	0.56 (0.65)	0.49
Model R^2	0.41	0.57	
Model p value	0.0079	0.0042	
10-20 cm			
MAP (cm year^{-1})	NS	NS	
Age (years)	0.044 (0.013)	0.019 (0.0063)	
Intercept	-0.93 (0.37)	-0.53 (0.18)	
Model R^2	0.45	0.40	
Model p value	0.004	0.009	

Note: Values are estimates for regression parameters with standard errors in parentheses. The value of the ridge k was selected to reduce the variance inflation factor to 1. If the stepwise regression discarded either MAP or Age, then the resulting model only includes one predictor.

Table 10: Regression parameters of the effect of afforestation on soil pool C:N predicted by plantation age and MAP

	Ordinary Least Squares		Ridge Regression (k=0.013)
	Micr. Biomass C:N	Soil C:N	Micr. Biomass C:N
0-10 cm			
MAP (cm year^{-1})	-0.010 (0.0024)	NS	-0.0077
Age (years)	-0.014 (0.0047)	NS	-0.0096
Intercept	1.6 (0.34)	NS	1.28
Model R^2	0.59		
Model p value	0.0032		
10-20 cm			
MAP (cm year^{-1})	NS	-0.0031 (0.0012)	
Age (years)	0.014 (0.0065)	NS	
Intercept	-0.020 (0.19)	0.39 (0.13)	
Model R^2	0.25	0.33	
Model p value	0.050	0.021	

Note: Values are estimates for regression parameters with standard errors in parentheses. The value of the ridge k was selected to reduce the variance inflation factor to 1. If the stepwise regression discarded either MAP or Age, then the resulting model only includes one predictor.

Table 11: Mean values for C and N pools across vegetation types

Vegetation	Total Extractable C	Microbial Biomass C	Total C	Total Extractable N	Microbial Biomass N	Total N
0-10 cm						
Eucalyptus	14.1 (1.9)	21.9 (2.3)	3171 (329)	2.67 (0.45)	**4.00 (0.47)**	250 (24)
Grassland	7.32 (0.69)	28.0 (3.2)	2633 (270)	1.53 (0.36)	**5.58 (0.59)****	246 (24)
10-20 cm						
Eucalyptus	10.6 (1.7)	**12.0 (1.6)**	1897 (245)	1.57 (0.26)	**1.86 (0.25)**	171 (21)
Grassland	7.63 (1.4)	**16.3 (1.7)****	1761 (218)	1.10 (0.08)	**3.28(0.37)*****	171 (19)
Litter						
Eucalyptus			**50.1% (0.37)**			**1.21% (0.14)**
Grassland			**40.8%(0.83)*****			**1.20% (0.15)**

Note: values are g C m^{-2} or g N m^{-2} for soil, and %C and %N for litter. Variables in bold were not predicted by either plantation age or mean annual precipitation. Asterisks indicate significance level: *=p<0.05, **=p<0.01, ***=p<0.001.

Table 12: Mean values for pool C:N and soil pH

Vegetation	Total Extractable C:N	Microbial Biomass C:N	Total C:N	Soil pH
0-10 cm				
Eucalyptus	**6.77 (0.71)**	6.31 (0.47)	**12.2 (0.36)**	**4.66 (0.12)**
Grassland	**5.29 (0.36)**	5.15 (0.28)	**10.5 (0.19)****	**4.86 (0.13)****
10-20 cm				
Eucalyptus	**8.29 (0.72)**	6.99 (0.47)	10.6 (0.28)	**4.58 (0.15)**
Grassland	**7.07 (0.70)**	5.50 (0.27)	9.85 (0.23)	**4.89 (0.17)***
Litter				
Eucalyptus			**49.9% (5.7)**	
Grassland			**39.0% (3.63)**	

Note: Variables in bold were not predicted by either plantation age or mean annual precipitation. Asterisks indicate significance level: *=p<0.05, **=p<0.01, ***=p<0.001. Significance of pH was determined with soil $[H^+]$, but is presented in pH units for ease of interpretation.

4.6 Figure Legends

Figure 13. Association of soil carbon pools with MAP and plantation age. Y-axis is in units of ln response ratio (Ln (value in g C m^{-2} for *Ecalyputs*/value in g C m^{-2} for grassland)), namely, positive values indicate an increase in the pool due to afforestation, and negative values a decrease. Regressions were conducted separately for different soil depths, and only significant regression models are displayed. Regression parameter estimates are listed in Table 8.

Figure 14. Association of soil nitrogen pools with MAP and plantation age. Y-axis is in units of ln response ratio (Ln (value in g N m^{-2} for *Ecalyptus*/value in g N m^{-2} for grassland)), namely, positive values indicate an increase in the pool due to afforestation, and negative values a decrease. Regressions were conducted separately for different soil depths, and only significant regression models are displayed. Regression parameter estimates are listed in Table 9.

Figure 15. Association of C:N of soil pools with MAP and plantation age. Y-axis is in units of ln response ratio (Ln (value for *Ecalyputs*/value for grassland)), namely, positive values indicate an increase in the pool due to afforestation, and negative values a decrease. Regressions were conducted separately for different soil depths, and only significant regression models are displayed. Regression parameter estimates are listed in Table 10.

Figure 16. Family of linear regression models representing the relationship between effect of afforestation and plantation age at different precipitation levels. Based parameters estimated by ridge regression of 0-10 cm soil samples.

4.7 Figures

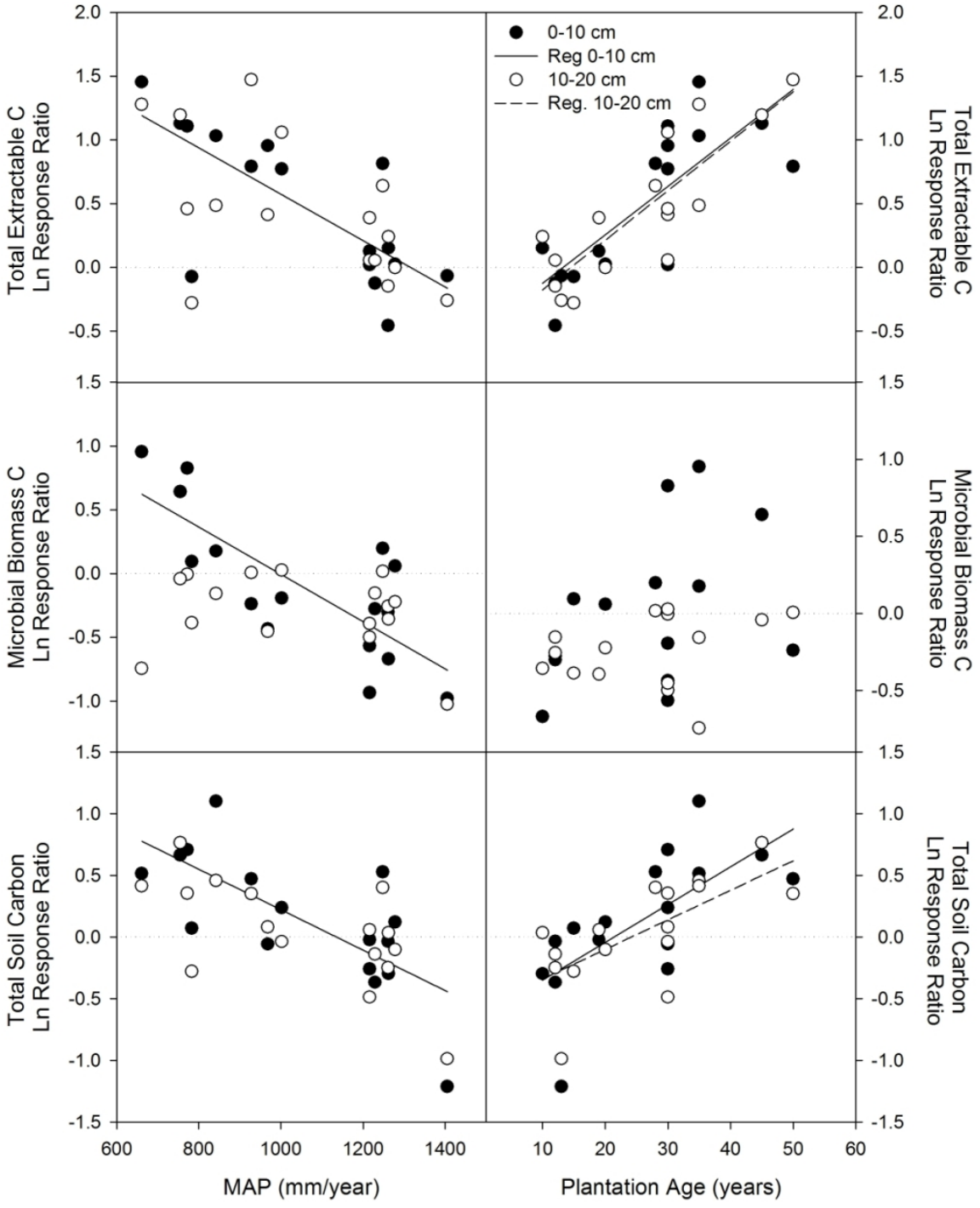

Figure 13: Association of soil C pools with MAP and plantation age

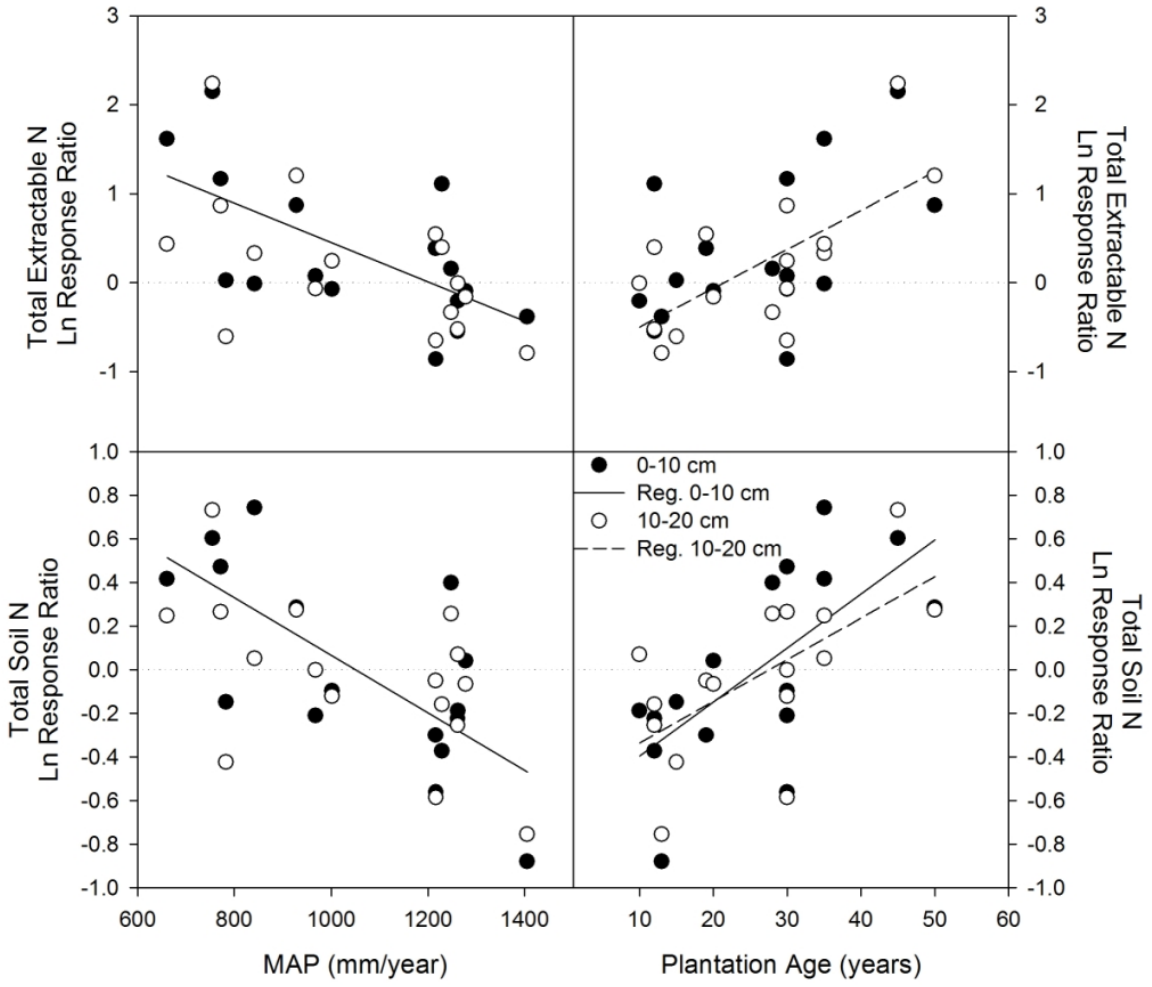

Figure 14: Association of soil N pools with MAP and plantation age

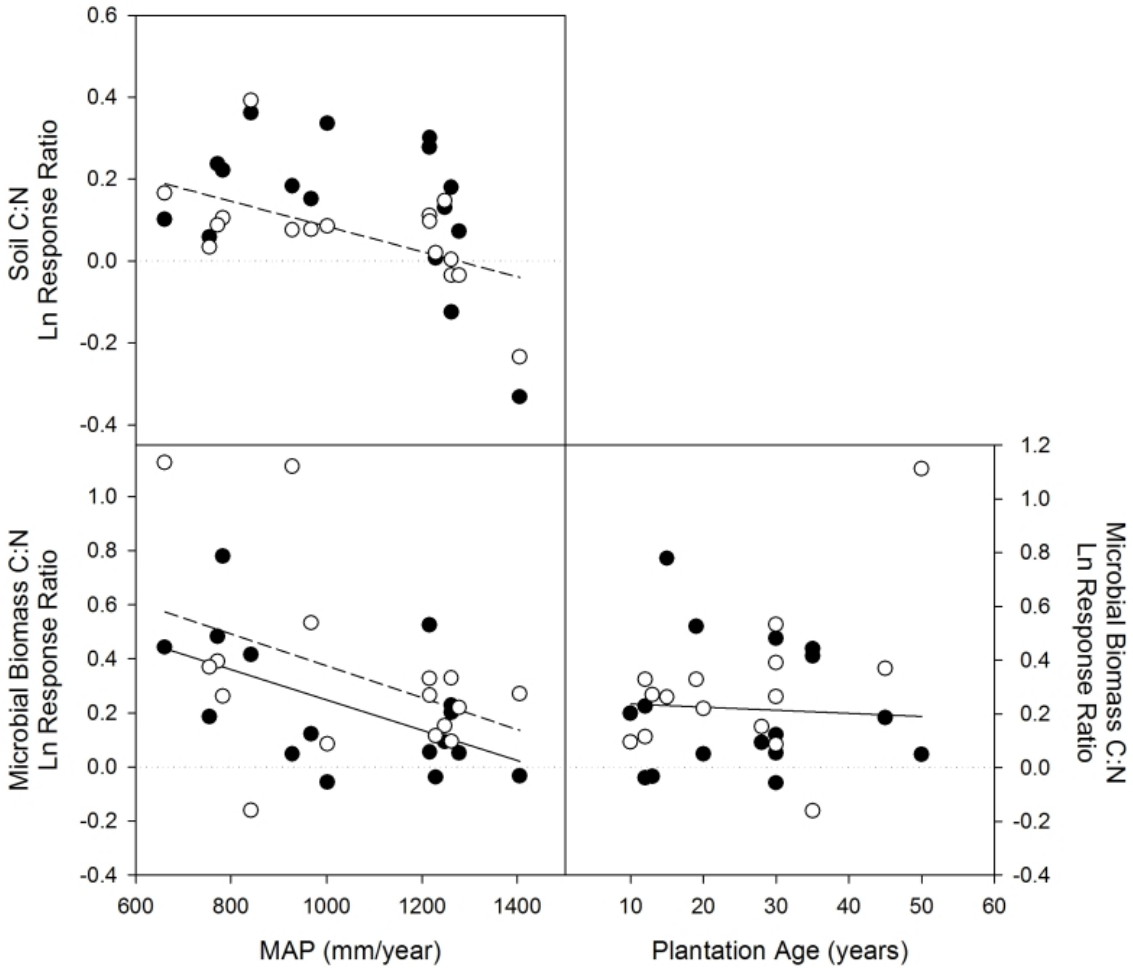

Figure 15: Association of C:N of soil pools with MAP and plantation age

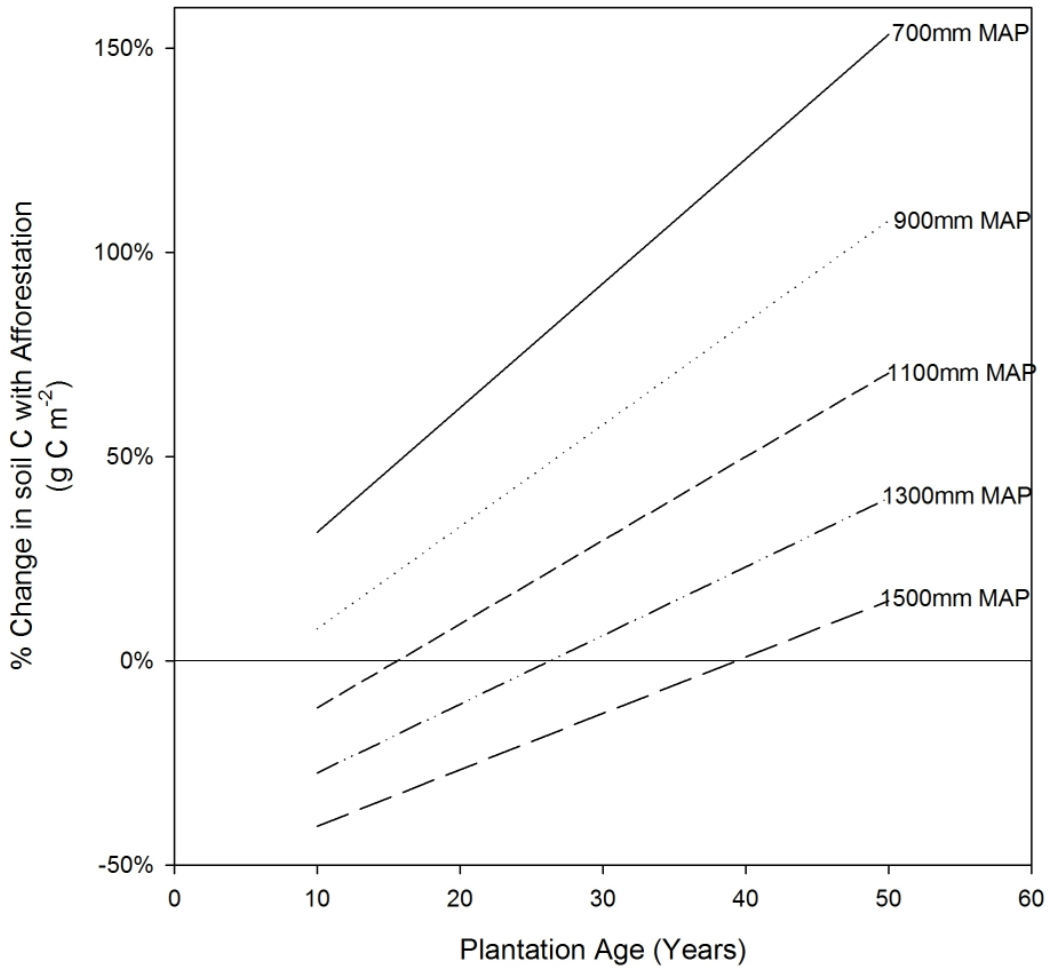

Figure 16: Family of linear regression models representing the relationship between effect of afforestation and plantation age at different precipitation levels

References

ABARE and Jaako-Poyry. 1999. Global outlook for plantations. Australian Bureau of Agricultural and Resource Economics, Canberra.

Adams, M. L., M. R. Davis, and K. J. Powell. 2001. Effects of grassland afforestation on exchangeable soil and soil solution aluminium. Australian Journal of Soil Research **39**:1003-1014.

Ainsworth, E. A. and S. P. Long. 2005. What have we learned form 15 years of free-air CO2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO2. New Phytologist **165**:351-371.

Alfredsson, H., L. M. Condron, M. Clarholm, and M. R. Davis. 1998. Changes in soil acidity and organic matter following the establishment of conifers on former grassland in New Zealand. Forest Ecology and Management **112**:245-252.

Alriksson, A. and M. T. Olsson. 1995. Soil Changes in Different Age Classes of Norway Spruce (Picea-Abies (L) Karst) on Afforested Farmland. Plant and Soil **169**:103-110.

Balbuena, R., P. Mac Donagh, J. Marquina, D. Jorajuria, A. Terminiello, and J. Claverie. 2002. Wheel traffic influence on Poplar regeneration and grass yield. Biosystems Engineering **81**:379-384.

Baldani, J. I., L. Caruso, V. L. D. Baldani, S. R. Goi, and J. Dobereiner. 1997. Recent advances in BNF with non-legume plants. Soil Biology & Biochemistry **29**:911-922.

Baptist, F., L. Zinger, J. C. Clement, C. Gallet, R. Guillemin, J. M. F. Martins, L. Sage, B. Shahnavaz, P. Choler, and R. Geremia. 2008. Tannin impacts on microbial diversity and the functioning of alpine soils: a multidisciplinary approach. Environmental Microbiology **10**:799-809.

Barton, D., S. M. Gammack, M. F. Billett, and M. S. Cresser. 1999. Sulphate adsorption and acidification of Calluna heathland and Scots pine forest podzol soils in north-east Scotland. Forest Ecology and Management **114**:151-164.

Berthrong, S. T. and A. C. Finzi. 2006. Amino acid cycling in three cold-temperate forests of the northeastern USA. Soil Biology & Biochemistry **38**:861-869.

Berthrong, S. T., R. B. Jackson, and E. G. Jobbágy. 2009. A global metaanalysis of soil exchangeable cations, pH, carbon, and nitrogen with afforestation. Ecological Applications **In press**.

Berthrong, S. T., C. W. Schadt, G. Pineiro, and R. B. Jackson. In Press. Afforestaiton alter the composition of functional genes in soil and biogeochemical processes in South American grasslands. Applied and Environmental Microbiology.

Bi, J., J. A. Blanco, B. Seely, J. P. Kimmins, Y. Ding, and C. Welham. 2007. Yield decline in Chinese-fir plantations: A simulation investigation with implications for model complexity. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **37**:1615-1630.

Binkley, D. and S. C. Resh. 1999. Rapid changes in soils following eucalyptus afforestation in Hawaii. Soil Science Society of America Journal **63**:222-225.

Binkley, D., D. Valentine, C. Wells, and U. Valentine. 1989. An Empirical-Analysis of the Factors Contributing to 20-Year Decrease in Soil-Ph in an Old-Field Plantation of Loblolly-Pine. Biogeochemistry **8**:39-54.

Bouillet, J. P., J. D. Nzila, J. P. Laclau, and J. Ranger. 2000. Effects of site management on *Eucalyptus* plantations in the equatorial zone, on the coastal plains of the Congo. Pages 11-20 *in* E. K. S. Nambiar, A. Tiarks, C. Cossalter, and J. Ranger, editors. Site management and productivity in tropical plantation forests: a progress report. Center for International Forestry Research, Desa Putera, Indonesia.

Brady, N. C. and R. R. Weil. 2002. The nature and properties of soils. 13th edition. Prentice Hall, Upper Saddle River, NJ.

Bulow, S. E., C. A. Francis, G. A. Jackson, and B. B. Ward. 2008. Sediment denitrifier community composition and *nirS* gene expression investigated with functional gene microarrays. Environmental Microbiology **10**:3057-3069.

Burton, J., C. R. Chen, Z. H. Xu, and H. Ghadiri. 2007. Gross nitrogen transformations in adjacent native and plantation forest's of subtropical Australia. Soil Biology & Biochemistry **39**:426-433.

Castells, E., J. Penuelas, and D. W. Valentine. 2003. Influence of the phenolic compound bearing species Ledum palustre on soil N cycling in a boreal hardwood forest. Plant and Soil **251**:155-166.

Chapin, F. S., P. Mattson, and H. Mooney. 2002. Principles of Terrestrial Ecosystem Ecology. Springer, New York.

Chapuis-Lard, L., D. Contour-Ansel, and F. Bernhard-Reversat. 2002. High-performance liquid chromatography of water-soluble phenolics in leaf litter of three Eucalyptus hybrids (Congo). Plant Science **163**:217-222.

Chen, C. R., L. M. Condron, M. R. Davis, and R. R. Sherlock. 2000. Effects of afforestation on phosphorus dynamics and biological properties in a New Zealand grassland soil. Plant and Soil **220**:151-163.

Chen, L. D., J. Gong, B. J. Fu, Z. L. Huang, Y. L. Huang, and L. D. Gui. 2007. Effect of land use conversion on soil organic carbon sequestration in the loess hilly area, loess plateau of China. Ecological Research **22**:641-648.

Condron, L. M. and R. H. Newman. 1998. Chemical nature of soil organic matter under grassland and recently established forest. European Journal of Soil Science **49**:597-603.

Cubbage, F., P. MacDonagh, J. S. Júnior, R. Rubilar, P. Donoso, A. Ferreira, V. Hoeflich, V. M. Olmos, G. Ferreira, G. Balmelli, J. Siry, M. N. Báez, and J. Alvarez. 2006. Timber Investment Returns for Selected Plantations and Native Forests in South America and the Southern United States. New Forests **33**:237-255.

Daniel, R. 2005. The metagenomics of soil. Nature Reviews Microbiology **3**:470-478.

Davis, M. 1994. Topsoil properties under tussock grassland and adjoining pine forest in Otago, New Zealand. New Zealand Journal of Agricultural Research **37**:465-469.

Davis, M. 1995. Influence of radiata pine sedlings on chmical properties of some New Zealand montane grassland soils. Plant and Soil **176**:255-262.

Davis, M. 2001. Soil properties under pine forest and pasture at two hill country sites in Canterbury. New Zealand Journal of forestry Science **31**:3-17.

Davis, M. and M. H. Lang. 1991. Increased nutrient availability in topsoils under conifers in the South Island high country. New Zealand Journal of forestry Science **2**:165-179.

Davis, M. R. and L. M. Condron. 2002. Impact of grassland afforestation on soil carbon in New Zealand: a review of paired-site studies. Australian Journal of Soil Research **40**:675-690.

Day, F. P. and C. D. Monk. 1977. Seasonal Nutrient Dynamics in Vegetation on a Southern Appalachian Watershed. American Journal of Botany **64**:1126-1139.

Del Galdo, I., J. Six, A. Peressotti, and M. F. Cotrufo. 2003. Assessing the impact of land-use change on soil C sequestration in agricultural soils by means of organic matter fractionation and stable C isotopes. Global Change Biology **9**:1204-1213.

Denman, K. L., G. Brasseur, A. Chidthaisong, P. Ciais, P. M. Cox, R. E. Dickinson, D. Hauglustaine, C. Heinze, E. Holland, D. Jacob, U. Lohmann, S. Ramachandran,

P. L. d. S. Dias, S. C. Wofsy, and X. Zhang. 2007. Couplings Between Changes in the Climate System and Biogeochemistry.*in* S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M.Tignor, and H. L. Miller, editors. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, New York, NY.

Dunbar, J., L. O. Ticknor, and C. R. Kuske. 2001. Phylogenetic specificity and reproducibility and new method for analysis of terminal restriction fragment profiles of 16S rRNA genes from bacterial communities. Applied and Environmental Microbiology **67**:190-197.

Duran, A. 1985. Los Suelos del Uruguay. Editorial Agropecuaria Hemisferio Sur S.R.L., Montevideo.

Efron, B. and R. J. Tibshirani. 1993. An introduction to the bootstrap. Chapman & Hall, New York.

Eisen, M. B., P. T. Spellman, P. O. Brown, and D. Botstein. 1998. Cluster analysis and display of genome-wide expression patterns. Proceedings of the National Academy of Sciences of the United States of America **95**:14863-14868.

Elmerich, C., W. Zimmer, and C. Vielle. 1992. Associative Nitrogen-fixing Bacteria. Pages 212-258 *in* G. Stacey, R. H. Burris, and H. J. Evans, editors. Biological nitrogen fixation. Chapman & Hall, New York.

Engel, V., E. G. Jobbágy, M. Stieglitz, M. Williams, and R. B. Jackson. 2005. Hydrological consequences of eucalyptus afforestation in the argentine pampas. Water Resources Research **41**.

Falkowski, P. G., T. Fenchel, and E. F. Delong. 2008. The microbial engines that drive Earth's biogeochemical cycles. Science **320**:1034-1039.

Fan, S., C. Yang, Z. He, Z. He, S. Lin, S. Lu, J. Ying, and X. Yang. 2000. Effects of site management in chinese fir (*Cunninghamia lanceolata*) plantations in Fujian province, China. Pages 3-10 *in* E. K. S. Nambiar, A. Tiarks, C. Cossalter, and J. Ranger, editors. Site management and productivity in tropical plantation forests: a progress report. Center for International Forestry Research, Bogor, Indonesia.

FAO. 2001a. Future Production from Forest Plantations. Report based on the work of C. Brown. UN FAO, Rome.

FAO. 2001b. Global Forest Resource Assessment 2000. United Nations Food and Agriculture Organization, New York City.

FAO. 2002. Forest plantation productivity. Report based on the work of W.J. Libby and C. Palmberg-Lerche., UN FAO, Rome.

FAO. 2006a. Global Forest Resource Assessment 2005. United Nations Food and Agriculture Organization, New York City.

FAO. 2006b. Global planted forests thematic study: results and analysis, by A. Del Lungo, J. Ball and J. Carle. 38, Rome (also at www.fao.org/foresty/site/10368/en).

Farley, K. A., E. G. Jobbágy, and R. B. Jackson. 2005. Effects of afforestation on water yield: a global synthesis with implications for policy. Global Change Biology **11**:1565-1576.

Farley, K. A. and E. F. Kelly. 2004. Effects of afforestation of a paramo grassland on soil nutrient status. Forest Ecology and Management **195**:281-290.

Farley, K. A., G. Pineiro, S. M. Palmer, E. G. Jobbágy, and R. B. Jackson. 2008a. Stream acidifcation and base cation losses with grassland afforestation. Water Resources Research **44**:Article Number W00A03.

Farley, K. A., G. Pineiro, S. M. Palmer, E. G. Jobbágy, and R. B. Jackson. 2008b. Stream acidifcation and base cation losses with grassland afforestation. Water Resources Research **In Press**.

Farley, K. A., G. Pineiro, S. M. Palmer, E. G. Jobbágy, and R. B. Jackson. 2008c. Stream acidification and base cation losses with grassland afforestation. Water Resources Research **44**:Article Number W00A03.

Fierer, N., M. Breitbart, J. Nulton, P. Salamon, C. Lozupone, R. Jones, M. Robeson, R. A. Edwards, B. Felts, S. Rayhawk, R. Knight, F. Rohwer, and R. B. Jackson. 2007a. Metagenomic and small-subunit rRNA analyses reveal the genetic diversity of bacteria, archaea, fungi, and viruses in soil. Applied and Environmental Microbiology **73**:7059-7066.

Fierer, N. and R. B. Jackson. 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences of the United States of America **103**:626-631.

Fierer, N., J. L. Morse, S. T. Berthrong, E. S. Bernhardt, and R. B. Jackson. 2007b. Environmental controls on the landscape-scale biogeography of stream bacterial communities. Ecology **88**:2162-2173.

Fitter, A. H., C. A. Gilligan, K. Hollingworth, A. Kleczkowski, R. M. Twyman, J. W. Pitchford, and N. S. B. Programme. 2005. Biodiversity and ecosystem function in soil. Functional Ecology **19**:369-377.

Florence, R. G. 1996. Ecology and Silviculture of Eucalypt Forests. CSIRO, Collingwood, Australia.

Garbin, M. L., R. B. Zandavalli, and L. R. Dillenburg. 2006. Soil patches of inorganic nitrogen in subtropical Brazilian plant communities with *Araucaria angustifolia*. Plant and Soil **286**:323-337.

Garg, V. and R. Jain. 1992. Influence of fuelwood trees on sodic soils. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **22**:729-735.

Geary, T. F. 2001. Afforestation in Uruguay - Study of a changing landscape. Journal of Forestry **99**:35-39.

Gentry, T. J., G. S. Wickham, C. W. Schadt, Z. He, and J. Zhou. 2006. Microarray applications in microbial ecology research. Microbial Ecology **52**:159-175.

Giddens, K. M., R. L. Parfitt, and H. J. Percival. 1997. Comparison of some soil properties under Pinus radiata and improved pasture. New Zealand Journal of Agricultural Research **40**:409-416.

Gilmore, A. R. and W. R. Boggess. 1963. Effects of past agricultural practices on the survival and growth of planted trees. Soil Science Society Proceedings **27**:98-102.

Goncalves, J. P., M. I. P. Serrano, K. C. F. S. Mendes, and J. L. Gava. 2000. Effects of site management in a *Eucalyptus grandis* plantation in the humid tropics: Sao Paulo, Brazil. Pages 3-10 *in* E. K. S. Nambiar, A. Tiarks, C. Cossalter, and J. Ranger, editors. Site management and productivity in tropical plantation forests: a progress report. Center for International Forestry Research, Bogor, Indonesia.

Graham, P. H., J. Bale, D. Baker, M. Fried, J. Roskoski, K. T. Mackay, and E. Craswell. 1988. The Contribution of Biological Nitrogen-Fixation to Plant-Production - an Overview of the Symposium and Its Implications. Plant and Soil **108**:1-6.

Groenendijk, F. M., L. M. Condron, and W. C. Rijkse. 2002. Effects of afforestation on organic carbon, nitrogen and sulfur concentrations in New Zealand hill country soils. Geoderma **108**:91-100.

Guevara-Escobar, A., P. D. Kemp, A. D. Mackay, and J. Hodgson. 2002. Soil properties of a widely spaced, planted poplar (Populus deltoides)-pasture system in a hill environment. Australian Journal of Soil Research **40**:873-886.

Guo, L. B., E. Bek, and R. M. Gifford. 2006. Woody debris in a 16-year old Pinus radiata plantation in Australia: Mass, carbon and nitrogen stocks, and turnover. Forest Ecology and Management **228**:145-151.

Guo, L. B. and R. M. Gifford. 2002. Soil carbon stocks and land use change: a meta analysis. Global Change Biology **8**:345-360.

Guo, L. B. B., M. B. Wang, and R. M. Gifford. 2007. The change of soil carbon stocks and fine root dynamics after land use change from a native pasture to a pine plantation. Plant and Soil **299**:251-262.

Gurevitch, J. and L. V. Hedges. 2001. Meta-analysis: Combining the results fo independent experiments. Pages 347-369 *in* S. M. Scheiner and J. Gurevitch, editors. Design and analysis of ecological experiments. Oxford University Press, New York.

Halliday, J. C., K. R. Tate, R. E. McMurtrie, and N. A. Scott. 2003. Mechanisms for changes in soil carbon storage with pasture to Pinus radiata land-use change. Global Change Biology **9**:1294-1308.

Hawke, M. F. and M. B. O'Connor. 1993. Soil pH and nutrient levels at Tikitere agroforestry research area. Australian Journal of Soil Research **40**:873-886.

He, Z. L., T. J. Gentry, C. W. Schadt, L. Y. Wu, J. Liebich, S. C. Chong, Z. J. Huang, W. M. Wu, B. H. Gu, P. Jardine, C. Criddle, and J. Zhou. 2007. GeoChip: a comprehensive microarray for investigating biogeochemical, ecological and environmental processes. Isme Journal **1**:67-77.

He, Z. L., L. Y. Wu, X. Y. Li, M. W. Fields, and J. Z. Zhou. 2005. Empirical establishment of oligonucleotide probe design criteria. Applied and Environmental Microbiology **71**:3753-3760.

Hedges, L. V., J. Gurevitch, and P. S. Curtis. 1999. The meta-analysis of response ratios in experimental ecology. Ecology **80**:1150-1156.

Hoerl, A. E. and R. W. Kennard. 1970. Ridge Regression - Biased Estimation for Nonorthogonal Problems. Technometrics **12**:55-&.

Hoerl, A. E. and R. W. Kennard. 2000. Ridge regression: Biased estimation for nonorthogonal problems. Technometrics **42**:80-86.

Hoffert, M. I., K. Caldeira, G. Benford, D. R. Criswell, C. Green, H. Herzog, A. K. Jain, H. S. Kheshgi, K. S. Lackner, J. S. Lewis, H. D. Lightfoot, W. Manheimer, J. C. Mankins, M. E. Mauel, L. J. Perkins, M. E. Schlesinger, T. Volk, and T. M. L.

Wigley. 2002. Advanced technology paths to global climate stability: Energy for a greenhouse planet. Science **298**:981-987.

Hofstede, R. G. M., J. P. Groenendijk, R. Coppus, J. C. Fehse, and J. Sevink. 2002. Impact of pine plantations on soils and vegetation in the Ecuadorian High Andes. Mountain Research and Development **22**:159-167.

Hooper, D. U., D. E. Bignell, V. K. Brown, L. Brussaard, J. M. Dangerfield, D. H. Wall, D. A. Wardle, D. C. Coleman, K. E. Giller, P. Lavelle, W. H. Van der Putten, P. C. De Ruiter, J. Rusek, W. L. Silver, J. M. Tiedje, and V. Wolters. 2000. Interactions between aboveground and belowground biodiversity in terrestrial ecosystems: Patterns, mechanisms, and feedbacks. Bioscience **50**:1049-1061.

Horner-Devine, M. C., M. Lage, J. B. Hughes, and B. J. M. Bohannan. 2004. A taxa-area relationship for bacteria. Nature **432**:750-753.

Houghton, R. A. and J. L. Hackler. 1999. Emissions of carbon from forestry and land-use change in tropical Asia. Global Change Biology **5**:481-492.

Houghton, R. A., J. L. Hackler, and K. T. Lawrence. 1999. The US carbon budget: Contributions from land-use change. Science **285**:574-578.

Huygens, D., P. Boeckx, O. Van Cleemput, C. Oyarzun, and R. Godoy. 2005. Aggregate and soil organic carbon dynamics in South Chilean Andisols. Biogeosciences **2**:159-174.

Jackson, R. B., J. L. Banner, E. G. Jobbágy, W. T. Pockman, and D. H. Wall. 2002. Ecosystem carbon loss with woody plant invasion of grasslands. Nature **418**:623-626.

Jackson, R. B., E. G. Jobbágy, R. Avissar, S. B. Roy, D. J. Barrett, C. W. Cook, K. A. Farley, D. C. le Maitre, B. A. McCarl, and B. C. Murray. 2005. Trading water for carbon with biological sequestration. Science **310**:1944-1947.

Jackson, R. B. and W. H. Schlesinger. 2004. Curbing the US carbon deficit. Proceedings of the National Academy of Sciences of the United States of America **101**:15827-15829.

Jain, R. K. and B. Singh. 1998. Biomass production and soil amelioration in a high density Terminalia arjuna plantation on sodic soils. Biomass & Bioenergy **15**:187-192.

Jobbágy, E. G. and R. B. Jackson. 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecological Applications **10**:423-436.

Jobbágy, E. G. and R. B. Jackson. 2003. Patterns and mechanisms of soil acidification in the conversion of grasslands to forests. Biogeochemistry **64**:205-229.

Jobbágy, E. G. and R. B. Jackson. 2004a. Groundwater use and salinization with grassland afforestation. Global Change Biology **10**:1299-1312.

Jobbágy, E. G. and R. B. Jackson. 2004b. The uplift of soil nutrients by plants: Biogeochemical consequences across scales. Ecology **85**:2380-2389.

Jobbágy, E. G. and R. B. Jackson. 2007. Groundwater and soil chemical changes under phreatophytic tree plantations. Journal of Geophysical Research-Biogeosciences **112**:G02013, doi:02010.01029/02006JG000246.

Jones, R. T., M. S. Robeson, C. L. Lauber, M. Hamady, R. Knight, and N. Fierer. 2009. A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. Isme Journal **3**:442-453.

Jug, A., F. Makeschin, K. E. Rehfuess, and C. Hofmann-Schielle. 1999. Short-rotation plantations of balsam poplars, aspen and willows on former arable land in the Federal Republic of Germany. III. Soil ecological effects. Forest Ecology and Management **121**:85-99.

Kirschbaum, M. U. F., L. Bin Guo, and R. M. Gifford. 2008. Why does rainfall affect the trend in soil carbon after converting pastures to forests? A possible explanation based on nitrogen dynamics. Forest Ecology and Management **255**:2990-3000.

Lal, R. 2008. Carbon sequestration. Philosophical Transactions of the Royal Society B-Biological Sciences **363**:815-830.

Lauber, C. L., M. Hamady, R. Knight, and N. Fierer. 2009. Pyrosequencing-Based Assessment of Soil pH as a Predictor of Soil Bacterial Community Structure at the Continental Scale. Applied and Environmental Microbiology **75**:5111-5120.

Le Maitre, D. C., D. F. Scott, and C. Colvin. 1999. A review of information on interactions between vegetation and groundwater. Water Sa **25**:137-152.

Liebich, J., C. W. Schadt, S. C. Chong, Z. L. He, S. K. Rhee, and J. Z. Zhou. 2006. Improvement of oligonucleotide probe design criteria for functional gene microarrays in environmental applications. Applied and Environmental Microbiology **72**:1688-1691.

Lilienfein, J., W. Wilcke, M. A. Ayarza, L. Vilela, S. D. Lima, and W. Zech. 2000. Soil acidification in Pinus caribaea forests on Brazilian savanna Oxisols. Forest Ecology and Management **128**:145-157.

Lima, A. M. N., I. R. Silva, J. C. L. Neves, R. F. Novais, N. F. Barros, E. S. Mendonca, T. J. Smyth, M. S. Moreira, and F. P. Leite. 2006. Soil organic carbon dynamics following afforestation of degraded pastures with eucalyptus in southeastern Brazil. Forest Ecology and Management **235**:219-231.

Mann, L. K. 1986. Changes in Soil Carbon Storage after Cultivation. Soil Science **142**:279-288.

Manzoni, S., R. B. Jackson, J. A. Trofymow, and A. Porporato. 2008. The global stoichiometry of litter nitrogen mineralization. Science **321**:684-686.

Mao, D. M., Y. W. Min, L. L. Yu, R. Martens, and H. Insam. 1992. Effect of Afforestation on Microbial Biomass and Activity in Soils of Tropical China. Soil Biology & Biochemistry **24**:865-872.

Marin-Spiotta, E., W. L. Silver, C. W. Swanston, and R. Ostertag. 2009. Soil organic matter dynamics during 80 years of reforestation of tropical pastures. Global Change Biology **15**:1584-1597.

Markewitz, D., D. D. Richter, H. L. Allen, and J. B. Urrego. 1998. Three decades of observed soil acidification in the calhoun experimental forest: Has acid rain made a difference? Soil Science Society of America Journal **62**:1428-1439.

Marschner, H. 1995. Mineral nutrition of higher plants. 2nd edition. Academic Press, New York.

Martens, D. A., T. E. Reedy, and D. T. Lewis. 2004. Soil organic carbon content and composition of 130-year crop, pasture and forest land-use managements. Global Change Biology **10**:65-78.

Martiny, J. B. H., B. J. M. Bohannan, J. H. Brown, R. K. Colwell, J. A. Fuhrman, J. L. Green, M. C. Horner-Devine, M. Kane, J. A. Krumins, C. R. Kuske, P. J. Morin, S. Naeem, L. Ovreas, A. L. Reysenbach, V. H. Smith, and J. T. Staley. 2006. Microbial biogeography: putting microorganisms on the map. Nature Reviews Microbiology **4**:102-112.

McCarl, B. A. and U. A. Schneider. 2001. Climate change - Greenhouse gas mitigation in US agriculture and forestry. Science **294**:2481-2482.

Mendham, D. S., A. M. O'Connell, and T. S. Grove. 2003a. Change in soil carbon after land clearing or afforestation in highly weathered lateritic and sandy soils of south-western Australia. Agriculture Ecosystems & Environment **95**:143-156.

Mendham, D. S., A. M. O'Connell, T. S. Grove, and S. J. Rance. 2003b. Residue management effects on soil carbon and nutrient contents and growth of second rotation eucalypts. Forest Ecology and Management **181**:357-372.

Menyailo, O. V., B. A. Hungate, and W. Zech. 2002. Tree species mediated soil chemical changes in a Siberian artificial afforestation experiment - Tree species and soil chemistry. Plant and Soil **242**:171-182.

Merino, A. and J. M. Edeso. 1999. Soil fertility rehabilitation in young Pinus radiata D. Don. plantations from northern Spain after intensive site preparation. Forest Ecology and Management **116**:83-91.

Merino, A., A. Fernandez-Lopez, F. Solla-Gullon, and J. M. Edeso. 2004. Soil changes and tree growth in intensively managed Pinus radiata in northern Spain. Forest Ecology and Management **196**:393-404.

Montagnini, F. 2000. Accumulation in above-ground biomass and soil storage of mineral nutrients in pure and mixed plantations in a humid tropical lowland. Forest Ecology and Management **134**:257-270.

Musto, J. W. 1992. Impacts of plantation forestry on various soil types. Pages 38-51 Institute for Commercial Forestry Research Annual Research Report. ICFR, Scottsville, South Africa.

Muys, B. and N. Lust. 1993. Ecological changes following afforestation with different tree species on a sandy loam soil in Flanders, Belgium.*in* C. Watkins, editor. Ecological effects of Afforestation. CAB international, Wallingford, UK.

New, M., D. Lister, M. Hulme, and I. Makin. 2002. A high-resolution data set of surface climate over global land areas. Climate Research **21**:1-25.

Nielsen, K. E., U. L. Ladekarl, and P. Nornberg. 1999. Dynamic soil processes on heathland due to changes in vegetation to oak and Sitka spruce. Forest Ecology and Management **114**:107-116.

Nierop, K. G. J., J. M. Verstraten, A. Tietema, J. W. Westerveld, and P. E. Wartenbergh. 2006. Short- and long-term tannin induced carbon, nitrogen and phosphorus dynamics in Corsican pine litter. Biogeochemistry **79**:275-296.

Noble, A. D., I. P. Little, and P. J. Randall. 1999. The influence of Pinus radiata, Quercus suber, and improved pasture on soil chemical properties. Australian Journal of Soil Research **37**:509-526.

Nosetto, M. D., E. G. Jobbagy, and J. M. Paruelo. 2006. Carbon sequestration in semi-arid rangelands: Comparison of Pinus ponderosa plantations and grazing exclusion in NW Patagonia. Journal of Arid Environments **67**:142-156.

Nosetto, M. D., E. G. Jobbágy, T. Toth, and C. M. Di Bella. 2007. The effects of tree establishment on water and salt dynamics in naturally salt-affected grasslands. Oecologia **152**:695-705.

Nosetto, M. D., E. G. Jobbágy, T. Toth, and R. B. Jackson. 2008. Regional patterns and controls of ecosystem salinization with grassland afforestation along a rainfall gradient. Global Biogeochemical Cycles **22**:-.

O'Connell, A. M., T. S. Grove, D. S. Mendham, and S. J. Rance. 2003. Changes in soil N status and N supply rates in agricultural land afforested with eucalypts in south-western Australia. Soil Biology & Biochemistry **35**:1527-1536.

Ohta, S. 1990. Initial Soil Changes Associated with Afforestation with Acacia-Auriculiformis and Pinus-Kesiya on Denuded Grasslands of the Pantabangan Area, Central Luzon, the Philippines. Soil Science and Plant Nutrition **36**:633-643.

Ouro, G., P. Perez-Batallon, and A. Merino. 2001. Effects of sylvicultural practices on nutrient status in a Pinus radiata plantation: Nutrient export by tree removal and nutrient dynamics in decomposing logging residues. Annals of Forest Science **58**:411-422.

Pacala, S. and R. Socolow. 2004. Stabilization wedges: Solving the climate problem for the next 50 years with current technologies. Science **305**:968-972.

Pace, N. R. 1997. A molecular view of microbial diversity and the biosphere. Science **276**:734-740.

Parfitt, R. L., H. J. Percival, R. A. Dahlgren, and L. F. Hill. 1997. Soil and solution chemistry under pasture and radiata pine in New Zealand. Plant and Soil **191**:279-290.

Parfitt, R. L., D. J. Ross, and L. F. Hill. 2003a. Soil nitrogen mineralisation changes rapidly when pine is planted into herbicide-treated pasture - the first two years of growth. Australian Journal of Soil Research **41**:459-469.

Parfitt, R. L., N. A. Scott, D. J. Ross, G. J. Salt, and K. R. Tate. 2003b. Land-use change effects on soil C and N transformations in soils of high N status: comparisons under indigenous forest, pasture and pine plantation. Biogeochemistry **66**:203-221.

Paul, E. A. 2007. Soil microbiology, ecology, and biochemistry. 3rd Edition edition. Academic Press, New York.

Paul, K. I., P. J. Polglase, J. G. Nyakuengama, and P. K. Khanna. 2002. Change in soil carbon following afforestation. Forest Ecology and Management **168**:241-257.

Payet, C., M. C. Scholes, and K. Balkwill. 2001. Some effects of the cultivation of pine on the chemistry of ultramafic soils. South African Journal of Science **97**:603-608.

Post, W. M. and K. C. Kwon. 2000. Soil carbon sequestration and land-use change: processes and potential. Global Change Biology **6**:317-327.

Pozo, J., A. Basaguren, A. Elosegui, J. Molinero, E. Fabre, and E. Chauvet. 1998. Afforestation with *Eucalyptus globulus* and leaf litter decomposition in streams of northern Spain. Hydrobiologia **374**:101-109.

Prosser, I. P., K. J. Hailes, M. D. Melville, R. P. Avery, and C. J. Slade. 1993. A Comparison of Soil Acidification and Aluminum under Eucalyptus Forest and Unimproved Pasture. Australian Journal of Soil Research **31**:245-254.

Pryor, L. D. 1976. The Biology of Eucalypts. Edward Arnold, London.

Quideau, S. A. and J. G. Bockheim. 1997. Biogeochemical cycling following planting to red pine on a sandy prairie soil. Journal of Environmental Quality **26**:1167-1175.

Rastetter, E. B., R. B. McKane, G. R. Shaver, and J. M. Melillo. 1992. Changes in C-storage by terrestrial ecosystems - How C-N interactions restrict responses to CO2 and temperature. Water Air and Soil Pollution **64**:327-344.

Resh, S. C., D. Binkley, and J. A. Parrotta. 2002. Greater soil carbon sequestration under nitrogen-fixing trees compared with Eucalyptus species. Ecosystems **5**:217-231.

Reynolds, B., C. Neal, M. Hornung, S. Hughes, and P. A. Stevens. 1988. Impact of Afforestation on the Soil Solution Chemistry of Stagnopodzols in Mid-Wales. Water Air and Soil Pollution **38**:55-70.

Rhoades, C. and D. Binkley. 1996. Factors influencing decline in soil pH in Hawaiian Eucalyptus and Albizia plantations. Forest Ecology and Management **80**:47-56.

Richter, D. D. and D. Markewitz. 1995. How Deep Is Soil - Soil, The Zone Of The Earths Crust That Is Biologically-Active, Is Much Deeper Than Has Been Thought By Many Ecologists. Bioscience **45**:600-609.

Richter, D. D., D. Markewitz, C. G. Wells, H. L. Allen, R. April, P. R. Heine, and B. Urrego. 1994. Soil Chemical-Change during 3 Decades in an Old-Field Loblolly-Pine (Pinus-Taeda L) Ecosystem. Ecology **75**:1463-1473.

Ross, D. J., K. R. Tate, N. A. Scott, and C. W. Feltham. 1999. Land-use change: effects on soil carbon, nitrogen and phosphorus pools and fluxes in three adjacent ecosystems. Soil Biology & Biochemistry **31**:803-813.

Ross, D. J., K. R. Tate, N. A. Scott, R. H. Wilde, N. J. Rodda, and J. A. Townsend. 2002. Afforestation of pastures with Pinus radiata influences soil carbon and nitrogen pools and mineralisation and microbial properties. Australian Journal of Soil Research **40**:1303-1318.

Saggar, S., C. B. Hedley, and G. J. Salt. 2001. Soil microbial biomass, metabolic quotient, and carbon and nitrogen mineralisation in 25-year-old Pinus radiata agroforestry regimes. Australian Journal of Soil Research **39**:491-504.

Schimel, J. and J. Bennett. 2004. Nitrogen mineralization: Challenges of a changing paradigm. Ecology **85**:591-602.

Schipper, L. and G. Sparling. 2000. Performance of soil condition indicators across taxonomic groups and land uses. Soil Science Society of America Journal **64**:300-311.

Schlesinger, W. H. 1997. Biogeochemistry: an analysis of global change. 2nd edition. Academic Press, Boston.

Scott, N. A., K. R. Tate, D. J. Ross, and A. Parshotam. 2006. Processes influencing soil carbon storage following afforestation of pasture with Pinus radiata at different stocking densities in New Zealand. Australian Journal of Soil Research **44**:85-96.

Sharma, M. L., R. J. W. Barron, and M. S. Fernie. 1987. Areal distribution of infiltration parameters and some soil physical properties in lateritic catchments. Journal of Hydrology **94**:109-127.

Sharrow, S. H. and S. Ismail. 2004. Carbon and nitrogen storage in agroforests, tree plantations, and pastures in western Oregon, USA. Agroforestry Systems **60**:123-130.

Singh, G., H. Singh, and P. P. Bhojvaid. 1998. Amelioration of sodic soils by trees for wheat and oat production. Land Degradation & Development **9**:453-462.

Soriano, A., R. J. C. Leon, O. E. Sala, R. S. Lavado, V. A. Deregibus, M. A. Cauhepe, O. A. Scaglia, C. A. Velaquez, and J. H. Lemcoff. 1991. Rio de la Plata Grasslands.

Pages 367-407 *in* R. T. Coupland, editor. Ecosystems of the world 8A: Natural grasslands. Elsevier, Amsterdam.

Souto, X. C., J. C. Bolano, L. Gonzalez, and M. J. Reigosa. 2001. Allelopathic effects of tree species on some soil microbial populations and herbaceous plants. Biologia Plantarum **44**:269-275.

Sparling, G. P., T. G. Shepherd, and L. A. Schipper. 2000. Topsoil characteristics of three contrasting New Zealand soils under four long-term land uses. New Zealand Journal of Agricultural Research **43**:569-583.

Strickland, M. S., C. Lauber, N. Fierer, and M. A. Bradford. 2009a. Testing the functional significance of microbial community composition. Ecology **90**:441-451.

Strickland, M. S., E. Osburn, C. Lauber, N. Fierer, and M. A. Bradford. 2009b. Litter quality is in the eye of the beholder: initial decomposition rates as a function of inoculum characteristics. Functional Ecology **23**:627-636.

Swain, T. 1979. Tannins and Lignins. Pages 657-682 *in* G. A. Rosenthal and D. H. Jansen, editors. Herbivores: Their interaction with secondary plant metabolites. Academic Press, New York.

Swain, T. and W. E. Hillis. 1959. The phenolic consitituents of *Prunus domestica* I.--The quantitative analysis of phenolic constituents. Journal of the Science of Food and Agriculture **10**:63-68.

Swift, M. J., O. Andren, L. Brussaard, M. Briones, M. M. Couteaux, K. Ekschmitt, A. Kjoller, P. Loiseau, and P. Smith. 1998. Global change, soil biodiversity, and nitrogen cycling in terrestrial ecosystems: three case studies. Global Change Biology **4**:729-743.

Torsvik, V., J. Goksoyr, and F. L. Daae. 1990. High Diversity In Dna Of Soil Bacteria. Applied and Environmental Microbiology **56**:782-787.

Turner, J. and M. J. Lambert. 1986. Effects of Forest Harvesting Nutrient Removals on Soil Nutrient Reserves. Oecologia **70**:140-148.

Vesterdal, L., E. Ritter, and P. Gundersen. 2002. Change in soil organic carbon following afforestation of former arable land. Forest Ecology and Management **169**:137-147.

Vitousek, P. M. 1991. Can Planted Forests Counteract Increasing Atmospheric Carbon-Dioxide. Journal of Environmental Quality **20**:348-354.

Wedderburn, M. E. and J. Carter. 1999. Litter decomposition by four functional tree types for use in silvopastoral systems. Soil Biology & Biochemistry **31**:455-461.

Wiesmeier, M., D. P. Dick, C. Rumpel, R. S. D. Dalmolin, A. Hilscher, and H. Knicker. 2009. Depletion of soil organic carbon and nitrogen under Pinus taeda plantations in Southern Brazilian grasslands (Campos). European Journal of Soil Science **60**:347-359.

Williams, B. L., J. M. Cooper, and D. G. Pyatt. 1977. Effects of Afforestation with Pinus-Contorta on Nutrient Content, Acidity and Exchangeable Cations in Peat. Forestry **51**:29-35.

Wright, J. A., A. DiNicola, and E. Gaitan. 2000. Latin American forest plantations - Opportunities for carbon sequestration, economic development, and financial returns. Journal of Forestry **98**:20-23.

Wu, L. Y., X. Liu, C. W. Schadt, and J. Z. Zhou. 2006a. Microarray-based analysis of subnanogram quantities of microbial community DNAs by using whole-community genome amplification. Applied and Environmental Microbiology **72**:4931-4941.

Wu, L. Y., D. K. Thompson, G. S. Li, R. A. Hurt, J. M. Tiedje, and J. Z. Zhou. 2001. Development and evaluation of functional gene arrays for detection of selected genes in the environment. Applied and Environmental Microbiology **67**:5780-5790.

Wu, Y. Q., G. H. Liu, B. J. Fu, Z. F. Liu, and H. F. Hu. 2006b. Comparing soil CO2 emission in pine plantation and oak shrub: Dynamics and correlations. Ecological Research **21**:840-848.

Xu, D. P., Z. J. Yang, B. Dell, and M. Gong. 2000. Effects of site management in *Eucalyptus urophylla* plantations in Guangdong province, China. Pages 3-10 *in* E. K. S. Nambiar, A. Tiarks, C. Cossalter, and J. Ranger, editors. Site management and productivity in tropical plantation forests: a progress report. Center for International Forestry Research, Bogor, Indonesia.

Yeates, G. W., M. F. Hawke, and W. C. Rijkse. 2000. Changes in soil fauna and soil conditions under Pinus radiata agroforestry regimes during a 25-year tree rotation. Biology and Fertility of Soils **31**:391-406.

Yeates, G. W. and S. Saggar. 1998. Comparison of soil microbial properties and fauna under tussock-grassland and pine plantation. Journal of the Royal Society of New Zealand **28**:523-535.

Yuste, J. C., D. D. Baldocchi, A. Gershenson, A. Goldstein, L. Misson, and S. Wong. 2007. Microbial soil respiration and its dependency on carbon inputs, soil temperature and moisture. Global Change Biology **13**:2018-2035.

Zhang, X. Q., M. U. F. Kirschbaum, Z. H. Hou, and Z. H. Guo. 2004. Carbon stock changes in successive rotations of Chinese fir (Cunninghamia lanceolata (lamb) hook) plantations. Forest Ecology and Management **202**:131-147.

Zhao, Q., D. H. Zeng, D. K. Lee, X. Y. He, Z. P. Fan, and Y. H. Jin. 2007. Effects of Pinus sylvestris var. mongolica afforestation on soil phosphorus status of the Keerqin Sandy Lands in China. Journal of Arid Environments **69**:569-582.

Zhou, J. Z., S. Kang, C. W. Schadt, and C. T. Garten. 2008. Spatial scaling of functional gene diversity across various microbial taxa. Proceedings of the National Academy of Sciences of the United States of America **105**:7768-7773.

Zinn, Y. L., D. V. S. Resck, and J. E. da Silva. 2002. Soil organic carbon as affected by afforestation with Eucalyptus and Pinus in the Cerrado region of Brazil. Forest Ecology and Management **166**:285-294.

Biography

Sean T. Berthrong was born on February 25th, 1980 in Toronto, Ontario and graduated *cum laude* with a dual Bachelor's degree in Biology and Classical Civilization from Boston University in 2001 and went on to earn a Master's degree in Biology from Boston University in 2003 working with Adrien Finzi.

Sean came to Duke University in 2003 to work at the free-air carbon dioxide enrichment site (FACE) as an associate in research. In 2004 he began his Ph.D. with Rob Jackson. Over the course of his graduate work, Sean received several awards and fellowships including a graduate research fellowship from the US Department of Energy, a fellowship from the Duke University Biology Department, and a Dissertation Enhancement Grant from the National Science Foundation. Several funding agencies provided grants for his doctoral work including the Keever endowment, the Tinker foundation, and Sigma Xi. He was also cited for the best student oral presentation at the 6th International Symposium on Ecosystem Behavior in 2009 in Helsinki, Finland, and as an Oosting Fellow for outstanding Ph.D. research in the field of ecology in 2008.

While a graduate student, Sean has had several professional accomplishments. These included publishing papers in *Applied and Environmental Microbiology*, *Ecological Applications*, *Ecology*, *Soil Biology and Biochemistry*, and *Science*. In addition, his work has been featured in *Scientific American*. In collaboration with fellow graduate students, Jenifer Morse and Liz Sudduth, he designed and taught a course, "Restoration Ecology," at Elon Univeristy in Elon, NC. Sean will begin a postdoctoral fellowship at Cornell University in the Fall of 2009.