Soil C and N changes with afforestation of grasslands across gradients of precipitation and plantation age

Sean T. Berthrong,[1,5] Gervasio Piñeiro,[2] Esteban G. Jobbágy,[3] and Robert B. Jackson[1,4]

[1]*University Program in Ecology, Duke University, Campus Box 90338, Durham, North Carolina 27708 USA*
[2]*Laboratorio de Análisis Regional y Teledetección, IFEVA, Facultad de Agronomía, Universidad de Buenos Aires, CONICET, Buenos Aires, Argentina*
[3]*Grupo de Estudios Ambientales, IMASL, Universidad Nacional de San Luis and CONICET, Ejército de los Andes 950, San Luis 5700 Argentina*
[4]*Department of Biology and Nicholas School of the Environment, Duke University, Campus Box 90338, Durham, North Carolina 27708 USA*

Abstract. Afforestation, the conversion of unforested lands to forests, is a tool for sequestering anthropogenic carbon dioxide into plant biomass. However, in addition to altering biomass, afforestation can have substantial effects on soil organic carbon (SOC) pools, some of which have much longer turnover times than plant biomass. An increasing body of evidence suggests that the effect of afforestation on SOC may depend on mean annual precipitation (MAP). The goal of this study was to test how labile and bulk pools of SOC and total soil nitrogen (TN) change with afforestation across a rainfall gradient of 600–1500 mm in the Rio de la Plata grasslands of Argentina and Uruguay. The sites were all former grasslands planted with *Eucalyptus* spp. Overall, we found that afforestation increased (up to 1012 kg C·ha^{-1}·yr^{-1}) or decreased (as much as 1294 kg C·ha^{-1}·yr^{-1}) SOC pools in this region and that these changes were significantly related to MAP. Drier sites gained, and wetter sites lost, SOC and TN ($r^2 = 0.59$, $P = 0.003$; and $r^2 = 0.57$, $P = 0.004$, respectively). Labile C and N in microbial biomass and extractable soil pools followed similar patterns to bulk SOC and TN. Interestingly, drier sites gained more SOC and TN as plantations aged, while losses reversed as plantations aged in wet sites, suggesting that plantation age in addition to precipitation is a critical driver of changes in soil organic matter with afforestation. This new evidence implies that longer intervals between harvests for plantations could improve SOC storage, ameliorating the negative trends found in humid sites. Our results suggest that the value of afforestation as a carbon sequestration tool should be considered in the context of precipitation and age of the forest stand.

Key words: *afforestation; Argentina;* Eucalyptus *plantation; precipitation; soil nitrogen; soil organic carbon; Uruguay.*

Introduction

Afforestation, the conversion of treeless areas to forests, is a land use change that can 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). Globally, cultivars of evergreen species, such as *Eucalyptus* spp. and *Pinus* spp., with very high productivity relative to native trees, are the most frequently planted (Florence 1996, FAO 2006b). Currently, >140 Mha in total have been afforested on every continent except Antarctica, with 2.5 Mha afforested per year from 2000 through 2005 (FAO 2006a). The rapid expansion of plantation area high-

lights the need to study soil organic carbon (SOC) and soil nitrogen (TN) in these systems to understand the potential for long-term nutrient availability, sustainable productivity, and long-term carbon dioxide sequestration.

Forest plantations are usually harvested frequently, which, coupled with their high productivity, can deplete soil nutrients and alter soil chemistry and hydrology (Adams et al. 2001, Guo and Gifford 2002, Engel et al. 2005, Berthrong et al. 2009a, Wei et al. 2009). A global meta-analysis of the effect of afforestation on grassland soil properties showed that exchangeable cations (Ca, Mg, K) and N were lower in plantations than in adjacent native vegetation (Farley et al. 2008, Berthrong et al. 2009a). Additionally, soils have frequently 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. 2008). Afforestation of grasslands in our study system leads to lower stream flow and lower water tables (Jobbágy and Jackson 2004a, Farley et al. 2005,

Jobbágy and Jackson 2007, Farley et al. 2008). It has been shown for other regions that these soil alterations could lead to lower production in subsequent plantation rotations, though it is not clear if this result would hold true in the mesic grasslands of our study area (Zhang et al. 2004, Bi et al. 2007).

Interestingly, the direction and magnitude of afforestation's effect on SOC may depend on plantation age or mean annual precipitation (MAP). For instance, a global meta-analysis showed that plantations converted from pastures with >1200 mm MAP showed significant losses of soil C, while those at sites with <1200 mm MAP showed no change or slight gains in SOC (Guo and Gifford 2002). This effect was also found in grasslands undergoing woody plant encroachment in the southwest USA, where shrub-invaded former grasslands showed SOC losses above a MAP threshold of ~600 mm (Jackson et al. 2002). Age effects were found in afforested grasslands and pastures that lost SOC for 10 years after planting, but recovered SOC levels within 20–30 years (Davis and Condron 2002, Paul et al. 2002, de Koning et al. 2003). Plantation age in Ecuadorian grasslands had a similar effect on surface TN, with losses in TN observed in young plantations but gradual recovery observed after 15–20 years, although TN in soil layers deeper than 10 cm showed continued losses even 25 years after planting (Farley and Kelly 2004).

Smaller labile pools of SOC and TN in soil are also known to change with conversion to plantations and can display a faster response than total soil C and N. Afforestation of grasslands and croplands has led to larger proportional changes in labile pools, such as extractable and microbial C and N, than in total soil C and N pools (O'Connell et al. 2003, Macdonald et al. 2009). Changes in labile soil pools could have important implications for soil nutrient supply since these pools are good indicators of plant-available nutrients (Carter et al. 1999). The larger differences due to afforestation in labile pools may also indicate the future magnitude and direction of responses of larger total soil organic matter pools.

This study examined the effects of afforestation on pools of soil C and N across a gradient of sites varying in plantation age and annual precipitation within the temperate grasslands of southern South America. In particular, we studied how the relative distribution and amounts of SOC and TN differed between adjacent pairs of *Eucalyptus* plantations and native grassland vegetation and if those differences were related to precipitation or plantation age. We examined bulk amounts of SOC and TN, as well as their stoichiometric ratios. We also measured labile pools of SOC and TN, since these pools cycle more rapidly and should show a greater proportional response through time to changes in chemistry due to afforestation than bulk pools.

METHODS

Site description

The Rio de la Plata grasslands were treeless before European settlement, with the exception of a few riparian species (Soriano et al. 1991). The area today has extensive regions of agriculture and pasture and, increasingly, plantations (Soriano et al. 1991). In Uruguay, nearly all plantations are established on grasslands, and in Argentina roughly half are established on grasslands (Baldi et al. 2008, Tommasino 2010). Mean annual precipitation (MAP) in this region ranges from ~650 mm in the center of Argentina to 1600 mm in Uruguay and southern Brazil (New et al. 2002). This region has plantations that extend from dry areas, where precipitation limits growth for both plantation and grasslands, to wetter areas where precipitation greatly exceeds plant water needs (Nosetto et al. 2008).

Small-scale 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, wood lots, or shade, but more recently, commercial afforestation for multiple wood products has become common (Soriano et al. 1991, Geary 2001, Cubbage et al. 2006). *Eucalyptus* species in this region have been resistant to droughts and pests, displaying some of the highest growth rates documented in tree plantations (Pryor 1976, Florence 1996, FAO 2006b). The combination of growth in plantation area, high productivity, and more recently, accessible pulp mills, is making the Rio de la Plata grasslands an attractive and rapidly expanding region for forest product companies (Cubbage et al. 2006).

We identified 16 upland sites in the Rio de la Plata basin along a precipitation gradient where native grasslands and *Eucalyptus* spp. plantations occur directly adjacent to each other (Fig. 1; Appendix: Table A1). The plantation–grassland pairs were studied along a precipitation gradient from 650 mm MAP and 15°C mean annual temperature (MAT) in La Pampa province of Argentina to 1450 mm MAP and 18.5°C MAT in the Rivera department of Uruguay (FAO 1985, New et al. 2002). All grasslands were actively being grazed at the time of our sampling. There were several species of *Eucalyptus* planted across the sites (*E. globulus*, *E. grandis*, *E. camaldulensis*; Appendix: Table A1), each in the subgenus *Symphomyrtus*, with similar physiological and chemical profiles (Florence 1996). The *Eucalyptus* plantations we studied were initial plantings (i.e., first rotations) and ranged from 10 to 49 years of age.

Sampling and analytical methods

Soils were sampled in October and November of 2006. For each grassland, we collected five cores each along two replicate 10-m transects using a 1.9-cm corer from 0–10 cm and 10–20 cm depths of mineral soil. Transects were located 10–20 m from the edge of the paired adjacent plantation to avoid edge effects, but also to keep other soil factors similar to the plantation soils. We then repeated the process with two replicate 10-m transects of five cores each 10–20 m within the plantation. In order to reduce the effect of fine-scale soil heterogeneity, the soil cores within each replicate
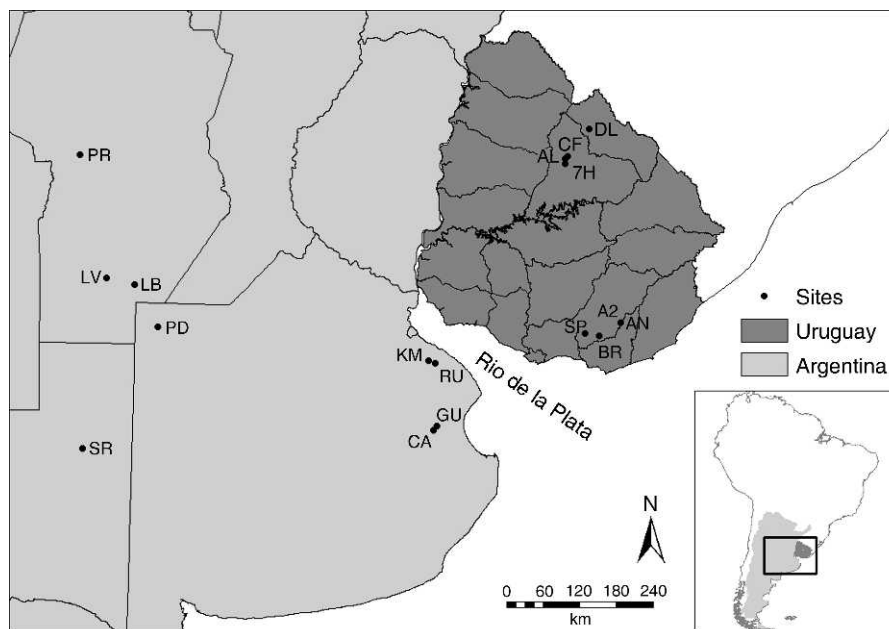
FIG. 1. Map of sites of adjacent pairs. Boundaries indicate the borders of provinces (Argentina) or departments (Uruguay). The inset shows the position of the study region in South America. Letters refer to site names: 7H, 7 Hermanos; AN, Anise 1; A2, Anise 2; AL, Almacén; BR, Barrios; CA, Castelli; CF, Confuso; DL, Don Luis; KM, Kilometer 55; LB, Laboulaye; LV, Levalle; PD, Piedritas; PR, Peron; RU, Ruta 36; SP, San Pedro; and SR, Santa Rosa. See *Methods: Site descriptions* for site descriptions.

transect were composited in a polyethylene bag and stored on ice for transport to the laboratory, producing two replicate composite samples per land use type (four total per grassland–plantation paired site). We calculated soil bulk density for each composite transect sample of five cores using the total volume of the composited cores and the total mass of soil (corrected for oven-dried mass), and used it to scale results to g/m^2 (Culley 1993). Soil samples were homogenized and sieved (2-mm mesh) to remove large roots and rocks. A subsample was dried at 110°C to determine gravimetric water content. A litter sample was collected at each site adjacent to the core locations. There were no significant differences between vegetation types in soil bulk density, thus corrections to constant mass were not necessary (Davidson and Ackerman 1993). Initial carbon content of the grasslands varied, but there was also no significant relationship between soil or litter C and N pools and precipitation.

Soil total extractable C and N and microbial biomass C and N were determined as in Berthrong and Finzi (2006). Briefly, for each sample, two 10-g subsamples of field-moist soil were weighed into centrifuge tubes. The initial sample was extracted immediately with 30 mL of 0.5 mol/L K$_2$SO$_4$ and shaken for 1 h at 120 oscillations per minute. The sample was then centrifuged (5000× gravities) and filtered through a Whatman number 1 filter paper. For the second sample, we placed a cotton ball in the tube and added 2 mL of N-free chloroform and sealed and incubated the samples in the dark for 5 d. The chloroform cotton balls were then removed, and the

samples were allowed to ventilate in a fume hood for 1 h, after which they were extracted in the same manner as the first samples. Total C and N in the extracts were measured by combustion and gas analysis on a Shimadzu TOC-V with a TNM-1 module (Shimadzu Corporation, Kyoto, Japan). Total extractable C and N were determined from the initial samples, and microbial biomass was calculated as the difference in extractable C or N between fumigated and initial samples.

Total SOC and TN were determined on subsamples of soil after air-drying to a constant mass with a subsample oven dried at 105°C to determine gravimetric soil moisture content. Soils were ground finely with a ball mill, and total C and N were determined by combustion in a Carlo-Erba Elemental Analyzer (CE Elantech, Lakewood, New Jersey, USA). Application of 1 mL of 1 mol/L HCl to a small amount of soil showed no evidence of soil carbonates; this fact, plus the shallow sampling depth and neutral-to-acid pH of the soil, allowed us to consider total soil C to be equal to total organic C (Appendix: Table A1). Soil pH was measured by combining 2 g of air-dried soil with 5 mL of 0.01 mol/L CaCl$_2$. The slurry was swirled gently by hand and allowed to settle for 30 min. A potentiometric electrode was then used to measure the pH of the supernatant.

Statistical analysis

We measured the effect size of afforestation on biogeochemical pools as response ratios, $r = XE:XC$, where XE is the mean value for a site of a given soil variable under *Eucalyptus* and XC is the mean value of

the same site's paired control grassland (Gurevitch and Hedges 2001, Guo and Gifford 2002, Ainsworth and Long 2005). Given that each grassland and plantation pair was directly adjacent, this approach allowed us to compensate for any background differences in soils across the gradient by expressing the results relative to site-specific paired controls. To match the scale of pH (logarithmic) to the linear scales of the other biogeochemical pools, we converted 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 2008) to test if MAP, MAT, or plantation age were significant predictors for the effect of afforestation on biogeochemical pools. F tests were calculated for MAP, MAT, and plantation age, and were added to the overall model if the P value was less than the 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 ($r = 0.44$, $P < 0.05$, variance inflation factor = 1.24). This collinearity can inflate the amount of variance the model explains and distort 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 coefficients that minimize the variance inflation factor 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. Ridge regression does not alter the underlying ability of multiple regression to predict relationships between variables while controlling for covariates.

If MAP, MAT, and plantation age were not a significant predictor of the effect of afforestation for a given soil variable, we then tested if afforestation had a significant effect on that soil variable across all sites by using ANOVA in SAS (proc GLM; SAS Institute 2008). We blocked paired analyses by site to better represent the paired nature of the sites. We used Tukey's hsd for post hoc comparisons of means across vegetation type.

Results

Changes in soil organic carbon (SOC) with afforestation were negatively related to mean annual precipitation, but positively correlated with plantation age ($P < 0.05$ for each; Fig. 2, Table 1). Afforested sites with lower MAP generally gained SOC, whereas sites with higher MAP generally lost SOC (Fig. 2). Plantations also tended to lose carbon compared to grasslands until ~24 years of age (95% CI = 21.1–27.7 yr), after which they tended to gain increasing amounts of SOC (Fig. 2). In the upper 10 cm of soil, the effect of afforestation on soil carbon changed from a gain to a loss at ~1150 mm/yr (95% CI = 1093–1189 mm/yr; Fig. 2). In contrast to results for the top 10 cm of soil, soils at 10–20 cm depth showed no relationship between MAP and the effect of afforestation on C pools (Fig. 2, Table 1). MAT was not a significant predictor of changes in SOC when controlling for MAP and plantation age.

Changes in total soil N and total extractable C and N mirrored those of SOC with afforestation (Figs. 2 and 3, Table 2). Wetter sites generally lost TN and drier sites gained TN, with gains increasing as plantations aged (Fig. 3, Table 2). However, the slope of the relationship between total soil N and MAP was a less steep (slope = −0.0079; Table 2) than that of total SOC and MAP (slope = −0.0095; Table 1), albeit not significantly so (95% CI of slope for SOC = −0.022 to −0.0005 and TN = −0.018 to −0.00094). The general increase in soil C:N also suggests that SOC or soil C:N responded more to differences in MAP than soil N did (Figs. 2–4, Tables 3 and 4). The transition points between gains and losses with afforestation in TN were 1050 mm/yr (95% CI = 956–1145 mm/yr) and 26 years (95% CI = 20.9–30.9 yr; Figs. 1 and 2). The transition point for extractable N (1110 mm/yr, 95% CI = 1003–1224 mm/yr) was higher than that for TN, though the difference was not significant (CIs overlapping). Microbial biomass C was not significantly associated with plantation age, and MAT was not a significant predictor of any of the above variables when controlling for MAP and plantation age.

Tissue chemistry differed significantly in the transition from grasslands to plantations, with *Eucalyptus* litter having higher carbon concentrations. *Eucalyptus* litter averaged 50.1% ± 0.37% C (mean ± SE) compared to 40.8% ± 0.83% C for native grasses ($P < 0.001$; Appendix: Table A1). Litter N was not significantly different for *Eucalyptus* (1.21% ± 0.14% N) and grasses (1.20% ± 0.15% N; Appendix: Table A1). Litter C to N ratios for *Eucalyptus* averaged 52.2 ± 5.85, which was higher than grass litter at 38.5 ± 3.43), though not significantly so (Appendix: Table A1). Litter total C and N concentrations showed no relationship to precipitation, MAT, or plantation age.

The effect of afforestation on some labile and quickly cycling C and N pools was not associated with either MAP or plantation age, but differed significantly across all sites (Tables 4, 5). Microbial biomass N was 28% lower at 0–10 cm and 43% lower at 10–20 cm in Eucalypt sites than in adjacent grasslands ($P < 0.01$; Table 4). Microbial biomass C was 26% lower at 10–20 cm soil depth ($P < 0.01$; Table 4). The average effect on microbial biomass C was similar at 0–10 cm to that of
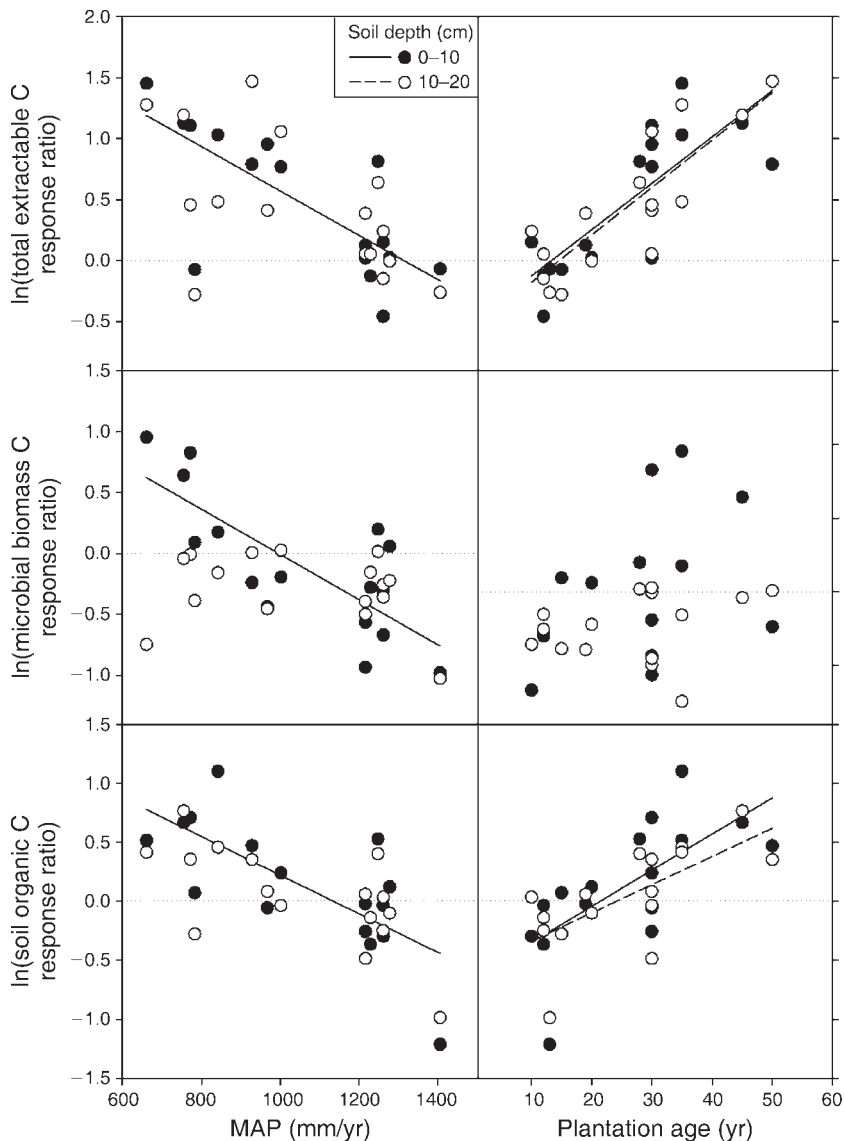
FIG. 2.   Association of soil carbon pools with mean annual precipitation (MAP) and plantation age. The *y*-axis gives the ln-transformed response ratio, ln(value for *Eucalyptus* : value for grassland); positive values indicate an increase in the pool due to afforestation, and negative values indicate a decrease. Regressions were conducted separately for different soil depths, and only significant regression models are displayed. Regression parameter estimates are listed in Table 1.

10–20 cm, but at 0–10 cm microbial biomass C was significantly related to MAP (Fig. 2). Soil pH decreased by 0.2 and 0.3 units with afforestation ($P < 0.05$; Table 5).

Soil C:N ratios were usually higher in plantations than grasslands, but the difference among land uses decreased with increasing MAP (Table 5, Fig. 4). Therefore, in wet areas afforestation did not substantially change C:N ratios of microbial biomass, total soil C:N, or extractable organic matter. Conversely, aboveground litter C:N ratios were always higher in *Eucalyptus* plantations and showed no evidence of changing across the precipitation gradient (Table 5).

DISCUSSION

To understand its full value as a tool for carbon sequestration, afforestation of grasslands should be considered in the context of its effects on soil properties (Vitousek 1991, Jackson et al. 2005, Nosetto et al. 2008). While previous studies have examined or modeled the effect of afforestation on soil organic carbon (SOC) along gradients of precipitation or plantation age (Guo and Gifford 2002, Halliday et al. 2003, Jackson et al. 2005), this study has combined both variables across a network of afforestation–grassland pairs. We examined bulk and labile pools of carbon and nitrogen and found

TABLE 1. Regression parameters of the response ratio of afforestation, ln(afforested : control), on soil C pools predicted by plantation age (age) and mean annual precipitation (MAP).

Soil depth and regression parameter	Ordinary least squares			Ridge regression ($k = 0.13$)	
	Total extractable C	Microbial biomass C	Total SOC	Total extractable C	Total SOC
0–10 cm					
MAP (cm/yr)	−0.010 (0.0047)	−0.019 (0.0042)	−0.011 (0.0053)	−0.0095	−0.0098
Age (yr)	0.025 (0.0092)	NS	0.017 (0.010)	0.023	0.016
Intercept	0.87 (0.86)	1.8 (0.45)	0.84 (0.76)	0.88	0.76
Model R^2	0.72	0.59	0.59	...	...
Model P value	0.0003	0.0005	0.003	...	...
10–20 cm					
MAP (cm/yr)	NS	NS	NS	...	...
Age (yr)	0.039 (0.0066)	NS	0.024 (.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	...	...

Notes: Values are estimates for regression parameters, with standard errors in parentheses, based on stepwise regression. If both MAP and age were included in the model, parameter estimates were modified by ridge regression. MAP was calculated in cm/yr for ease of interpretation. The value of the ridge k was selected to reduce the variance inflation factor to 1. Ellipses indicate data not applicable; and NS, not significant.

that the shifts in SOC, TN, and C:N imposed by tree plantations varied with MAP and plantation age. Our results suggest similar patterns of gains and losses of SOC and TN depending on MAP and plantation age, but also some variation in how tightly SOC and N were

stoichiometrically coupled in response to vegetation change under different environmental conditions.

SOC responds differently to afforestation for different precipitation levels, a response that a recent modeling study suggests is tied to alterations in the nitrogen cycle
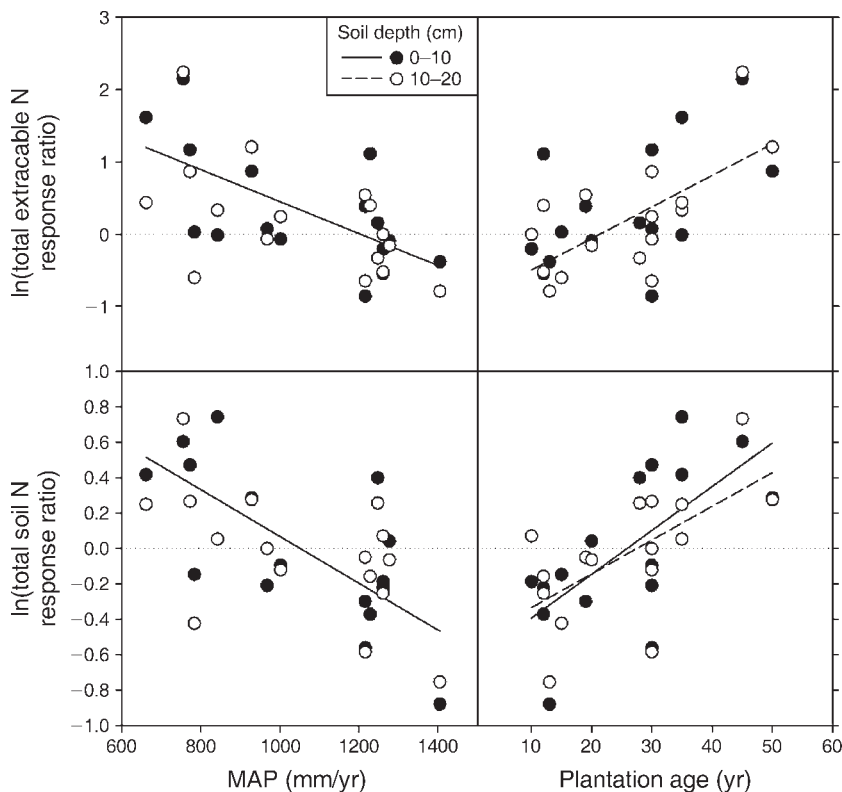

FIG. 3. Association of soil nitrogen pools with mean annual precipitation (MAP) and plantation age. The *y*-axis gives the ln-transformed response ratio, ln(value for *Eucalyptus* : value for grassland); positive values indicate an increase in the pool due to afforestation, and negative values indicate a decrease. Regressions were conducted separately for different soil depths, and only significant regression models are displayed. Regression parameter estimates are listed in Table 2.

TABLE 2. Regression parameters of response ratios of afforestation on soil N pools predicted by plantation age and MAP.

Soil depth and regression parameter	Ordinary least squares		Ridge regression ($k = 0.13$) Total soil N
	Total extractable N	Total soil N	
0–10 cm			
MAP (cm/yr)	−0.022 (0.0071)	−0.0087 (0.0045)	−0.0079
Age (yr)	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/yr)	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	⋯

Notes: Values are estimates for regression parameters, with standard errors in parentheses, based on stepwise regression. If both MAP and age were included in the model, parameter estimates were modified by ridge regression. The value of the ridge k was selected to reduce the variance inflation factor to 1. MAP was calculated in cm/yr for ease of interpretation. Ellipses indicate that data were not applicable; and NS indicates not significant.

(Kirschbaum et al. 2008). Their model indicates that soils in xeric regions store C through increases in the soil C:N, while in more humid areas, increased decomposition and N losses through leaching lead to C losses. Soil nitrogen data from our study support this explanation:

More xeric sites accumulated N with afforestation compared to grasslands, whereas more humid sites lost N (Fig. 3); soil C:N was also higher with afforestation in most cases (Fig. 4, Table 4). Additionally, soil microbial biomass C:N was less affected (i.e., lower C:N) in humid
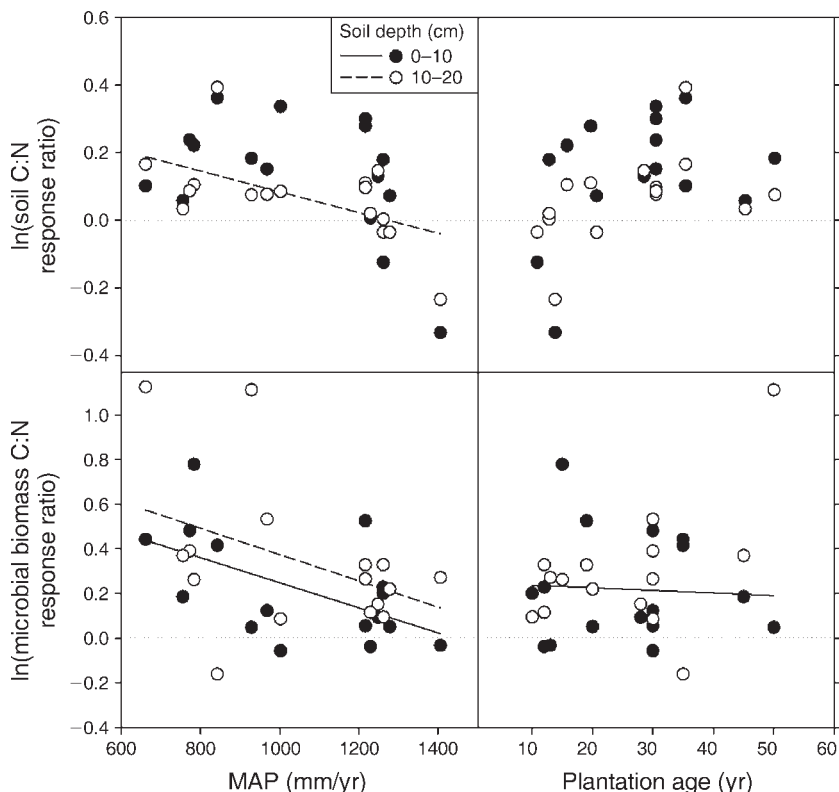

Fig. 4. Association of C:N of soil pools with mean annual precipitation (MAP) and plantation age. The *y*-axis gives the ln-transformed response ratio, ln(value for *Eucalyptus*: value for grassland); positive values indicate an increase in the pool due to afforestation, and negative values indicate a decrease. Regressions were conducted separately for different soil depths, and only significant regression models are displayed. Regression parameter estimates are listed in Table 3.

TABLE 3. Regression parameters of response ratios of afforestation on soil pool C:N predicted by plantation age and MAP.

Soil depth and regression parameter	Ordinary least squares		Ridge regression ($k = 0.013$) microbial biomass C:N
	Microbial biomass C:N	Soil C:N	
0–10 cm			
MAP (cm/yr)	−0.010 (0.0024)	NS	−0.0077
Age (yr)	−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/yr)	NS	−0.0031 (0.0012)	⋯
Age (yr)	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	⋯

Notes: Values are estimates for regression parameters, with standard errors in parentheses, based on stepwise regression. If both MAP and age were included in the model, parameter estimates were modified by ridge regression. The value of the ridge k was selected to reduce the variance inflation factor to 1. MAP was calculated in cm/yr for ease of interpretation. Ellipses indicate data not applicable; and NS, not significant.

sites and was higher in xeric sites, which also suggests support for the model in labile pools (Fig. 4, Table 4).

The patterns in SOC that we observed along the precipitation gradient may also be partially explained by the interaction between moisture and litter chemistry driving decomposition rates (Aerts 1997, Wang et al. 2004). Litter with higher amounts of secondary compounds, such as lignin and polyphenols produced by *Eucalyptus* (Corbeels et al. 2003, Berthrong et al. 2009b), will decompose more slowly than grass litter, which typically has lower phenolic and lignin concentrations compared to *Eucalyptus* growing in the same climatic zones (Henriksen and Breland 1999, Jalota et al. 2006, Kirschbaum et al. 2008). This effect, however, will likely be magnified at the drier end of the precipitation gradient where climatic effects on litter decomposition may play a stronger role in SOC storage (Fig. 2). In contrast, in more humid environments, litter decomposes more quickly, and the slowing of decomposition by secondary compounds is less important than in drier climates (Meentemeyer 1978).

Afforested soils initially lost C and N at wetter sites, but generally recovered those elements with age if the plantations were left to grow longer than 20–30 years

(Figs. 2 and 3). Our results match those reported for New Zealand, Australia, and Ecuador (Davis and Condron 2002, Paul et al. 2002, Farley and Kelly 2004). Lower net primary productivity in recently established plantations compared to established grasslands together with high biomass retention in tree stems could reduce net litter inputs into the soil for several years during plantation establishment, explaining this multi-decadal but transient effect on SOC pools. 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), yet soil C:N ratio in our study increased in plantations of all ages (Table 5). This change could be due to the rapid response of C:N of total extractable and microbial biomass (Fig. 4, Table 5). The differences in these quickly cycling pool ratios could lead to soil microbes altering soil organic matter content faster than models would predict.

The changing effects of afforestation on SOC across our MAP gradient were generally consistent with previously published observations. Like Guo and Gifford (2002), we found neutral effects on SOC at ~1150 mm/yr and net SOC losses in wetter regions. By

TABLE 4. Mean values (with standard errors in parentheses) for microbial biomass (MB) C and N.

Soil depth and vegetation	Microbial biomass C (g C/m^2)	Microbial biomass N (g N/m^2)
0–10 cm		
Eucalyptus	⋯	4.00 (0.47)
Grassland	⋯	5.58 (0.59)**
10–20 cm		
Eucalyptus	12.0 (1.6)	1.86 (0.25)
Grassland	16.3 (1.7)**	3.28 (0.37)***

Notes: MB was not related to MAP or plantation age at certain depths, so blocked ANOVA was conducted to test for differences across all pairs between grassland and plantations for these pools. Ellipses indicate that the data were analyzed differently; please see Fig. 2. Asterisks indicate significance difference level for the comparison between land uses within each soil layer.

** $P < 0.01$; *** $P < 0.001$.

TABLE 5. Mean values for pool C:N and soil pH.

Soil depth and vegetation	Total extractable C:N	Total C:N	Soil pH
0–10 cm			
Eucalyptus	6.77 (0.71)	12.2 (0.36)	4.66 (0.12)
Grassland	5.29 (0.36)	10.5 (0.19)**	4.86 (0.13)**
10–20 cm			
Eucalyptus	8.29 (0.72)	. . .	4.58 (0.15)
Grassland	7.07 (0.70)	. . .	4.89 (0.17)*

Notes: Total extractable (TE) C:N, total C:N, and soil pH were not related to MAP or plantation age at some depths, so blocked ANOVA was conducted to test for differences across all pairs between grassland and plantations for these pools. Significance of pH was calculated with soil [H^+] but is presented in pH units for ease of interpretation. Ellipses indicate that the data were analyzed differently; please see Fig. 4. Asterisks indicate significance level for the comparison between land uses within each soil layer.

* $P < 0.05$; ** $P < 0.01$.

extending into drier areas, we found net SOC gains of as much as 150% at 700 mm/yr. Our sites showed a similar slope of response of net SOC change to MAP to those reported by Jackson et al. (2002) for grasslands that were invaded with woody plants, but with a wetter crossover point (neutral effects on SOC observed at ~600 mm/yr in that study). The difference could be due to the deeper rooting depth of *Eucalyptus* plantations vs. grasses, which would allow plantations to access groundwater resources unavailable to the grasslands (Sharma et al. 1987, Le Maitre et al. 1999, Jackson et al. 2002, Jobbágy and Jackson 2003, 2004b). This explanation, in addition to changes in C:N, 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 SOC can be visualized as a family of graphs (Fig. 5). Though the applicability of this conceptual diagram is limited to grassland regions with similar rainfall amounts, it demonstrates how managers might consider plantation rotation length to maximize SOC and long-term productivity at a site. Relatively humid sites that could potentially lose SOC with afforestation could be managed for longer rotation times to counterbalance the initial losses in SOC and N.

There are several issues to consider about the generality of our results. First, this study concentrated on afforestation of grassland soils with no post-glacial history of forests. Ecosystems other than temperate grasslands, e.g., crops, primary forests, or secondary forests, might display different patterns of soil responses to afforestation (Guo and Gifford 2002). Second, we limited our analysis to the most active pools of C and N in the top 20 cm of soil. Soil C responses to afforestation have been found to be similar to depths of 50 cm globally, but a deeper sampling scheme might lead to different results (Guo and Gifford 2002). Similarly, afforestation of grasslands may have important impacts on deeper soil C and N pools on much longer time scales (Jobbágy and Jackson 2000). Third, more labile pools of

C and N, such as extractable C and N pools and microbial biomass C and N, can change more in short time frames than bulk soil pools do. This temporal variability might mask some significant relationships between afforestation and these pools in this study; however, the general agreement in direction and magnitude of the relationships of labile pools and bulk pools of C and N in this study suggests an important link between labile and bulk soil pools.

In conclusion, these results suggest that soil costs or benefits of afforestation depend on the ecological context and management decisions. On the one hand, afforestation in drier regions could have added benefits attributable to additional C sequestration and N availability in soil (Fig. 2). However, this result should be considered carefully against data showing that
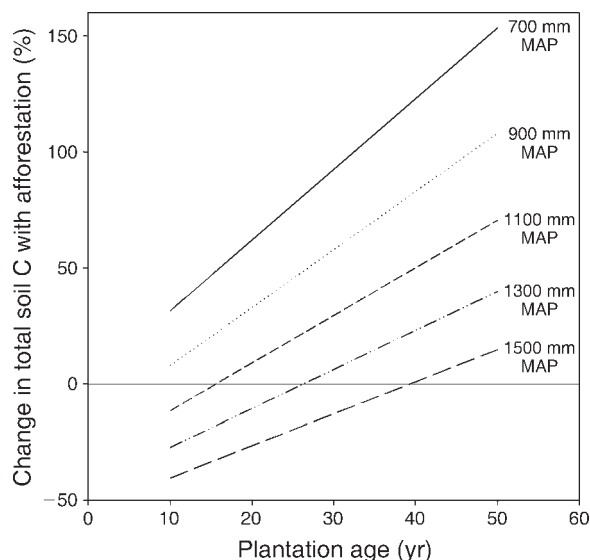
FIG. 5. Family of linear regression models representing the relationship between the effect of afforestation and plantation age at different mean annual precipitation (MAP) levels. Regression parameters were estimated by ridge regression of 0–10 cm soil samples.

afforestation of drier areas can decrease water availability relatively more than in wetter regions (Farley et al. 2005, Jackson et al. 2005). The C sequestration benefit of afforestation in more humid regions could, in turn, be reduced somewhat by losses of SOC and N, reduced soil fertility, and lower long-term productivity. These results will help facilitate informed plantation management decisions and more accurate estimations of C sequestration potentials of afforestation.

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SUPPLEMENTAL MATERIAL

Appendix

A table showing site information on climate, soil, and plantation history (*Ecological Archives* A022-004-A1).