

# *Pinus ponderosa* alters nitrogen dynamics and diminishes the climate footprint in natural ecosystems of Patagonia

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## Summary

**1.** Understanding climate effects on plant–soil interactions in terrestrial ecosystems remains challenging due to the fact that floristic composition covaries with climate, particularly along rainfall gradients. It is difficult to separate effects of precipitation *per se* from those mediated indirectly through changes in species composition. As such, afforestation (the intentional planting of woody species) in terrestrial ecosystems provides an ecological opportunity to assess the relative importance of climate and vegetation controls on ecosystem processes.

**2.** We investigated the impacts of 35 years of afforestation on ecosystem N dynamics, in ecosystems ranging from arid shrub-steppe to closed-canopy forest in Patagonia, Argentina. Sites of natural vegetation and adjacent sites planted with a single exotic species, *Pinus ponderosa*, were identified in five precipitation regimes along a continuous gradient of 250–2200 mm mean annual precipitation (MAP). We evaluated C and N parameters of vegetation and soil, as well as natural abundance of <sup>13</sup>C and <sup>15</sup>N in leaves, roots, ectomycorrhizae (EcM) and soils.

**3.** In natural vegetation, most leaf traits (%N, C:N ratios, leaf mass per area,  $\delta^{15}\text{N}$  values) demonstrated strong significant relationships with MAP, while these relationships were nearly absent in afforested sites. In addition, the EcM of native southern beech and pine trees were significantly enriched in <sup>15</sup>N relative to leaves at all sites where they were present. While soil C and N pools in both vegetation types increased with MAP, overall pool sizes were significantly reduced in afforested sites.

**4. Synthesis.** Observed relationships between leaf traits and precipitation in natural vegetation may be driven largely by shifts in species composition and plant–soil interactions, rather than direct effects of precipitation. Our results suggest that a change in the species composition of the dominant vegetation is sufficient to alter C and N cycling independently of climate constraints: pine afforestation homogenized N dynamics across sites spanning an order of magnitude of MAP. These results highlight the important control of ectomycorrhizal associations in affecting C and N dynamics. Additionally, they serve to demonstrate that altering natural species composition alone is sufficient to cause large, detectable impacts on N turnover independently of direct climate effects.

**Key-words:** Argentina, carbon cycling,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , ectomycorrhizae, *Nothofagus* spp., plant–soil (belowground) interactions, rainfall gradient, stable isotopes, temperate forest

## Introduction

Understanding interacting controls on biogeochemical cycles is key to evaluating how human impact will affect terrestrial ecosystem processes and their responses to global changes (Finzi *et al.* 2011). While climate and vegetation are major

controls on carbon (C) and nitrogen (N) cycling in terrestrial ecosystems, the relative importance of these controls is often obscured by the fact that dominant life form, species composition and net primary productivity covary along gradients of precipitation in natural ecosystems (Bertiller, Elissalde & Rostagno 1995; Schulze *et al.* 1996; Mazzarino *et al.* 1998; Austin & Sala 2002; McCulley *et al.* 2005; Bertiller *et al.* 2006; McCulley, Burke & Lauenroth 2009). Isolating the effects of

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variation in water availability and vegetation, respectively, on ecosystem processes has proven challenging thus far.

At the ecosystem scale, mean annual precipitation (MAP) demonstrates clear, strong relationships with primary productivity along natural gradients (Sala *et al.* 1988; Paruelo *et al.* 1998; Austin 2002), although how precipitation directly controls C turnover in low rainfall ecosystems (Austin 2011) and modulates N cycling (Austin & Vitousek 1998; Schuur & Matson 2001; McCulley, Burke & Lauenroth 2009) depends on the indirect effects of plant species' composition (Vinton & Burke 1997; Diehl *et al.* 2003; Eviner & Chapin 2003; Bertiller *et al.* 2006). It has been hypothesized that increasing soil C and plant N demand with increasing precipitation may tend to immobilize and retain N in soil and plant biomass, driving N conservation in wetter ecosystems (Austin & Sala 2002; McCulley, Burke & Lauenroth 2009). In arid and semi-arid ecosystems, on the other hand, standing pools of inorganic N, and asynchrony between N availability and biotic demand, may result in 'leaky' N cycling (Austin & Sala 1999; Walvoord *et al.* 2003; Austin *et al.* 2004). The relative importance of vegetation and climate in affecting ecosystem N dynamics is still unclear.

Plant species directly influence N cycling via leaf and root litter chemistry impacts on decomposition rates and consequent N availability (Hobbie 1992; Eviner & Chapin 2003; Freschet *et al.* 2013). In addition, differential plant strategies to access N stemming from interactions with other organisms, including absorption of organic N (McKane *et al.* 2002) or symbiosis with mycorrhizae, may also mediate plant influences on N cycling (Chapman *et al.* 2006). Ectomycorrhizal fungi, which transfer N to plants in exchange for C, play an important role in plant N uptake, and have been shown to be capable of attacking structural polymers and mobilizing otherwise inaccessible nutrients (Smith & Read 1997; Read & Perez-Moreno 2003). Ectomycorrhizae (EcM) may thus facilitate greater access by plants to N physically, via the great soil volume their hyphae explore, as well as biochemically, through decomposition and access to N stored in recalcitrant organic material. Plants which produce low-quality, recalcitrant litter and form associations with ectomycorrhizal fungi may be relatively conservative with respect to N cycling (Chapman *et al.* 2006; Orwin *et al.* 2011).

Afforestation, broadly defined as the practice of planting trees in areas without native woody vegetation, represents an important form of land use change globally, and in South America. As of 2010, planted forests accounted for 264 million hectares worldwide, with roughly 1.4 million hectares in Argentina, and this form of land use is growing steadily (FAO 2010). Because of their fast growth, introduced species, particularly *Pinus* spp. and *Eucalyptus* spp., are often preferred for plantations in South America (Sedjo 1999). In northwestern Patagonia, ponderosa pine plantations constitute the majority of afforested lands (Schlichter & Laclau 1998; Laclau 2003; Licata *et al.* 2008). Estimates from nearly a decade ago put the amount of land planted with *Pinus ponderosa* in the region at 70 000 hectares, growing at a rate of approximately 5000 hectares per year (Laclau 2003).

Beyond production purposes, afforestation has been proposed as a cost-effective method of sequestering atmospheric C in regions around the globe (Wright, DiNicola & Gaitan 2000; Lal 2008), although there is some debate as to the feasibility of this as a large-scale measure (Jackson *et al.* 2005; Bonan 2008). Specifically in South America, afforestation of arid and semi-arid lands in Patagonia has been proposed as a mechanism to recover C losses and establish C sinks on degraded lands (Nosetto, Jobbágy & Paruelo 2006).

While the impacts of afforestation on C cycling have been the focus of a number of studies (Guo & Gifford 2002; Grünzweig *et al.* 2003; Laclau 2003; Berthrong *et al.* 2009), a mechanistic understanding of changes in N cycling that result from this land use change has only recently begun to be addressed (Grünzweig *et al.* 2007; Kirschbaum, Guo & Gifford 2008; Gelfand, Grünzweig & Yakir 2012). The productivity of most terrestrial, non-tropical ecosystems, managed and unmanaged, is limited by the supply of available N (Vitousek & Howarth 1991), and previous work suggests that Patagonian ecosystems are indeed N limited (Austin, Sala & Jackson 2006; Diehl, Mazzarino & Fontenla 2008; Gonzalez-Polo & Austin 2009). Replacement of native vegetation with pine plantations can significantly increase ecosystem productivity, which may be associated with increased plant N demand (Grünzweig *et al.* 2003, 2007; Rotenberg & Yakir 2010; Gelfand, Grünzweig & Yakir 2012). Diminished soil N stocks following afforestation with *Pinus* spp. support the idea of a transfer of N from soil to tree biomass and litter pools (Richter *et al.* 2000; Berthrong *et al.* 2012), although the impacts on soil C and N stocks may depend on annual rainfall and plantation age (Guo & Gifford 2002; Berthrong *et al.* 2012; Ecclesia *et al.* 2012).

The natural abundances of stable N isotopes ( $^{15}\text{N}$  and  $^{14}\text{N}$ ) can provide an integrated measure of N dynamics over time (Nadelhoffer & Fry 1994; Högberg 1997; Robinson 2001; Hobbie & Högberg 2012). Most reactions involving N favour the initial incorporation of the lighter isotope into the product, leaving the substrate enriched in  $^{15}\text{N}$ . As such, it is expected that most N lost from ecosystems through leaching and gaseous emissions are depleted in  $^{15}\text{N}$  relative to other ecosystem pools, and N loss should lead to  $^{15}\text{N}$  enrichment of remaining ecosystem pools. Ecosystem  $\delta^{15}\text{N}$  has been evaluated along environmental gradients, including rainfall gradients (Austin & Vitousek 1998; Handley *et al.* 1999; Schuur & Matson 2001; Amundson *et al.* 2003) and chronosequences (Brenner *et al.* 2001; Menge *et al.* 2011); these studies have demonstrated systematic variation in patterns of  $\delta^{15}\text{N}$  with respect to changes in annual precipitation and substrate age. Recent studies suggest that the presence and type of mycorrhizal association may additionally influence leaf  $\delta^{15}\text{N}$  values (Craine *et al.* 2009) and bulk soil  $\delta^{15}\text{N}$  (Hobbie & Högberg 2012).

Systematic variation of the natural abundance of  $^{15}\text{N}$  in ecosystems has been postulated to indicate the 'openness' of ecosystem N cycling, as greater N losses relative to total ecosystem N pools should lead to a greater degree of  $^{15}\text{N}$  enrichment (Evans & Ehleringer 1993; Austin & Vitousek 1998; Handley *et al.* 1999). Specifically, systematically

decreasing soil and plant  $\delta^{15}\text{N}$  values with increasing precipitation have been observed at local, regional and global scales, (Austin & Vitousek 1998; Austin & Sala 1999; Handley *et al.* 1999; Schuur & Matson 2001; Amundson *et al.* 2003; McCulley, Burke & Lauenroth 2009), which has been interpreted as an indication of increased N retention with increasing rainfall (Austin & Vitousek 1998; Handley *et al.* 1999; McCulley, Burke & Lauenroth 2009), and increased losses through denitrification in humid to wet ecosystems (Houlton, Sigman & Hedin 2006). However, changes in dominant vegetation occurring in tandem with changes in climate make it difficult to disentangle the effects of climate *per se* due to the fact that inter-specific variation in plant  $\delta^{15}\text{N}$  values can be substantial (Högberg 1997). Recent evidence suggests that inter-specific differences among plants may be due to differences in N assimilation and transport (Pardo *et al.* 2013), and plant mycorrhizal associations (Craine *et al.* 2009; Hobbie & Högberg 2012).

In this study, we examined effects of afforestation with *P. ponderosa* on C and N dynamics along a gradient from 250 to 2200 mm MAP in northwest Patagonia, Argentina. Our research took advantage of pine plantations established simultaneously between 1974 and 1976 in a wide range of ecosystems from steppe to native forest. The establishment of even-aged plantations of a single species across a broad precipitation gradient in Patagonia allowed us to evaluate the interaction between climate and vegetation controls on C and N dynamics, and the biogeochemical consequences of 35 years of afforestation with a single exotic species of *P. ponderosa*. In the pine plantations, invariant dominant vegetation allowed us to isolate the direct effects of climate on C and N dynamics, decoupling them from confounding variation in plant species composition along the precipitation gradient.

Given that net primary production is higher in afforested sites relative to sites with native vegetation along this gradient (Araujo 2012), we hypothesized that due to increased plant N demand, N cycling would be more conserved in afforested sites relative to those with native vegetation. As such, we predicted lower leaf and soil  $\delta^{15}\text{N}$  values in afforested sites relative to native vegetation across the precipitation gradient. We evaluated various aspects of C and N cycling, including soil and vegetation pools, and the natural abundance of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in foliage, roots, EcM and soil in natural and afforested

ecosystems along a contiguous precipitation gradient of 250–2200 mm MAP. This experimental design allowed us to evaluate the relative importance of and the interaction between direct climate and vegetation controls on N dynamics in the temperate ecosystems of Patagonia.

## Materials and methods

### STUDY SITES

We identified a set of five paired sites in the northwest Patagonian region of Argentina, each with natural vegetation and an adjacent pine plantation, along a 60-km east–west precipitation gradient at  $40^\circ\text{S} \pm 0.5^\circ$  (for a map of study location, see Fig. S1 in Supporting Information). The sites cover an area ranging from Junin de los Andes, the watersheds of Quemquemtreu and Curruhue rivers, and Meliquina, Lolog, and Lacar lakes. Long-term MAP along the gradient ranges from 250 to 2200 mm, and native vegetation ranges from arid, shrub–grass steppe to closed-canopy forest (Araujo 2012; Table 1). The abrupt changes in precipitation in this region are due to the interaction between the cordillera and the westerly winds from the Pacific Ocean, which cause an exponential decline in precipitation over a distance of <100 km (Jobbágy, Paruelo & León 1995; Austin & Sala 2002). Precipitation is highly seasonal and concentrated between May and September, with dry summers. Average temperature varies between 8.9 and 12 °C independently of changes in MAP, with minimum temperatures below 0 °C in June and July. Parent material is volcanic in origin although the drier sites (<1400 mm MAP) are additionally influenced by sand and fluvial clay deposition (Etchevehere & Dimitri 1972).

At each of the five locations, we established a representative plot of 50 m × 50 m (10 plots in total), in an area of homogeneous vegetation on level ground in which all sampling was conducted. The slope (<5%) and orientation of the plots was similar in all cases, and with the exception of the most humid site, the pine plantation and natural vegetation were part of a continuous area, with the paired plots adjacent to one another. All pine plantations and their corresponding native vegetation were located within private property of large land holders, with the exception of the wettest site (2200 mm MAP), which is located within the boundary of Lanin National Park. Plant species with known nitrogen-fixing associations were not present in any site, with the exception of *Adesmia volckmannii*, which was a small proportion of the shrub cover in the driest site, and whose  $\delta^{15}\text{N}$  signature is not different from the non-fixing shrub species (Golluscio, Faigón & Tanke 2006). In the native woodlands and forests at the wetter end of the gradient, the dominant species are

**Table 1.** Site location and characteristics. Mean annual precipitation (MAP) is based on long-term averages (Araujo 2012)

Site	MAP (mm)	Location	MAT (°C) (min–max)	Altitude (m)	Soil type	Dominant vegetation type
1	250	40°15'S 70°48'W	12 (–0.5–29)	851	Natrixeralfs	Shrub–grass steppe
2	650	39°57'S 71°06'W	9.5 (–1.4–4.9)	1024	Argixerolls	Shrub–grass steppe
3	1100	40°26' S 71°13'W	8.9 (–0.9–2.6)	906	Dystrandeps	<i>Nothofagus antarctica</i> woodland
4	1350	40°04'S 71°20'W	11 (2–21.4)	1062	Vitrandraps	<i>N. antarctica</i> forest
5	2200	40°09'S 71°34'W	9.7 (–0.1–4.4)	804	Dystrandeps	<i>N. dombeyi</i> , <i>N. nervosa</i> , and <i>N. obliqua</i> forest <i>Chusquea culeou</i> understory

*Nothofagus* spp., which are dominant overstorey species of humid Andean–Patagonian forests (Veblen *et al.* 1996; Vivanco & Austin 2008). The initial planting of the pine plantations, all with a single species of *P. ponderosa*, occurred between 1974 and 1976, with the same initial planting density, such that all plantation sites had ages between 34–36 years. The establishment of the plantations did not involve land-clearing, application of synthetic chemicals including herbicides, or vegetation removal with the exception of the wettest site, where native forest vegetation had been selectively cut for two decades before the seeding of the pine plantations (E. Coliqueo, pers. comm.). Since that time, the plantations have not been irrigated or fertilized and were not pruned or thinned during the period of the study.

#### FIELD SAMPLING

Soils and leaves from all sites were collected in February 2010. Soil was sampled at five random points along four transects located at 10-m intervals in each 50 m × 50 m plot. Litter and the organic horizon were cleared prior to sampling. Soil cores were extracted with a push probe 2 cm in diameter and 30 cm in length, in sites 1 and 2, and 90 cm in sites 3–5 (reached with extension rods). In sites 1 and 2, sampling to 90 cm was impeded by rocky soil; small stones in cores to 30 cm in these sites were infrequent and removed by hand. Each core was separated by depth as follows: 0–5 cm, 5–15 cm, 15–30 cm, 30–60 cm (when applicable) and 60–90 cm (when applicable). Soil depth intervals in the first 30 cm were divided with a knife from a first core of 30 cm and removed carefully from the push probe to ensure as much accuracy as possible. Samples along each transect were composited, so there were four composite samples from each depth profile for each site. Roots were removed and soils were sieved before analyses.

Fully expanded, green sun leaves were also collected in February 2010. Leaves were collected from dominant native species in sites with native vegetation, and pines in pine plantations (for a list of species by site, see Table S2). In cases where sun leaves from overstorey trees were unreachable from the ground, trees were climbed, or poles with hooks were used to collect leaves. Five individuals of each dominant species were randomly chosen within the 50 × 50 m plot at each site: Site 1, two species: *Pappostipa* spp. ( $N = 4$ ) and *Mulinum spinosum* ( $N = 4$ ); Site 2, two species: *Pappostipa* spp. ( $N = 4$ ) and *Mulinum spinosum* ( $N = 4$ ); Site 3, three species: *Pappostipa* spp. ( $N = 4$ ) *Mulinum spinosum* ( $N = 4$ ) and *Nothofagus antarctica* ( $N = 4$ ); Site 4, one species: *Nothofagus antarctica* ( $N = 4$ ); Site 5, four species: *Chusquea culeou* ( $N = 4$ ), *Nothofagus dombeyi* ( $N = 4$ ), *Nothofagus obliqua* ( $N = 4$ ) and *Nothofagus nervosa* ( $N = 4$ ). A subsample of the leaves from each sample was used to calculate leaf mass per area (LMA). 5–20 leaves per sample were scanned with a Hewlett Packard ScanJet Scanner® to calculate leaf area, oven dried for 48 h and weighed. LMA was calculated as  $\text{g m}^{-2}$ .

Ectomycorrhizae were collected in natural vegetation and pine plantations in May 2010 for all sites except sites 1 and 2 with native vegetation, where no native ectomycorrhizal trees were present. Ectomycorrhizae were collected for *Nothofagus* spp. in sites with native vegetation, and *P. ponderosa* in pine plantations. In each site, four trees were randomly selected for sampling. Soil blocks approximately 10 × 10 × 10 cm were removed from 2–3 points around each tree, approximately 1 m from the trunk. Soil was stored in zippered plastic bags at 4 °C until processed in Buenos Aires. Fine roots were removed from soil, washed in sieves and air dried. Ectomycorrhizal tips were collected and stored at room temperature until analysis. Roots of mixed grasses were also sampled from native vegetation in

sites 1, 2 and 3. Roots of *M. spinosum* were not collected, as each plant has a thick, woody tap root that extends to depth (Fernández & Paruelo 1988) and fine roots in accessible soil layers were not sufficiently abundant for analysis. As such, EcM tips and grass roots were used for the regression of root N concentrations and precipitation in sites with native vegetation.

#### LABORATORY ANALYSIS

All samples were dried at 60 °C, ground using a Thomas Wiley® Mini-Mill (Thomas Scientific, Swedesboro, New Jersey, USA), passed through a 40- $\mu\text{m}$  mesh and stored at room temperature until loaded into 9 mm × 5 mm tin capsules. Leaf, root, EcM and soil samples were sent to the Duke Environmental Stable Isotope Laboratory in April 2011 for analysis of  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , %C and %N.

Isotope ratios are expressed in delta notation ( $\delta$ ) as the per mil deviation from the isotopic ratio of a standard ( $R_{\text{std}}$ ), where  $R$  denotes the ratio of stable isotopes (for C,  $^{13}\text{C}/^{12}\text{C}$  and for N,  $^{15}\text{N}/^{14}\text{N}$ ). For example, for N,

$$\delta^{15}\text{N} = (R_{\text{sample}} - R_{\text{standard}}) / (R_{\text{standard}}) \times 1000$$

The V-PDB ( $\delta^{13}\text{C} = 0$ ) and atmospheric N ( $\delta^{15}\text{N} = 0$ ) serve as the international standards for stable C and N isotope ratios (Faure & Mensing 2005). Presence of carbonates in soils was tested by adding several mL 1 M HCl to 1 g soil, for each soil sample, and observing formation of bubbles. Using this method, no carbonates were detected.

#### TOTAL SOIL C AND N

Soil C and N for each depth increment were calculated as:

$$\text{g C (N) m}^{-2} = \% \text{C (N)} \times \text{BD} \times \text{D} \times 10000$$

where

$$\% \text{C (N)} = \text{Concentration of soil C (N)} (\text{g C (N)}/\text{g soil} \times 100)$$

BD, bulk density ( $\text{g cm}^{-3}$ ) of depth increment; D, depth increment (cm).

Total soil C and N to 30 cm depth were calculated as the sum of relevant depth increments.

Bulk density was calculated using the equation adapted from Post & Kwon (2000):

$$\text{BD} = \frac{100}{(\% \text{SOM}/0.244) + ((100 - \% \text{SOM})/1.64)}$$

where %SOM is the percentage of soil organic matter assuming that organic matter is equal to percent soil C divided by 0.58 (Mann 1986). Due to the nature of the sampling instrument and compression of the soils, we were not able to get accurate in-field estimates of bulk density, and for this reason, felt it was more consistent to apply this equation across all sites.

#### STATISTICAL ANALYSIS

All statistical analyses were performed using R 2.15.1 (R Development Core Team 2012). Regression analysis was used to detect relationships between response variables with MAP in sites with natural vegetation and pine plantations, respectively. For all regressions of vegetation and precipitation, data were averaged by species and by site. Data were tested for linear and quadratic relationships with MAP; in the case that both linear and polynomial relationships were



significant, an *F* test was conducted to determine whether the second-order terms contributed significantly to the model. Where regressions were significant in both natural vegetation and pine plantations, 95% confidence intervals for the slopes of the relationships were calculated to determine whether slopes were significantly different. If confidence intervals did not overlap, slopes were considered significantly different.

Three-factor ANOVA (MAP, vegetation and plant part) was used to identify differences between leaf and ectomycorrhizal  $\delta^{15}\text{N}$ . Significant differences between depth-integrated total soil C, total soil N and C:N ratios between pine plantations and sites with native vegetation were identified with two-factor (MAP and vegetation) ANOVA. Differences in soil  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  between pine plantations and sites with native vegetation, and variation with depth (to 30 cm) and MAP were identified with three-factor ANOVAs. Data were log- or Box-Cox-transformed when necessary to meet conditions of normality and homoscedasticity. For all analyses,  $\alpha = 0.05$ .

## Results

### LEAF CHARACTERISTICS IN NATURAL VEGETATION AND PINE PLANTATIONS

A number of leaf traits showed significant relationships with precipitation in native vegetation, but these patterns were not observed in *P. ponderosa*. Leaf N concentrations in native vegetation increased with MAP, while no significant relationship was observed in *P. ponderosa* (Fig. 1a,b). Fine root/EcM N concentrations showed a positive trend with MAP in both native vegetation and *P. ponderosa* (Fig. 1c,d), and the slopes of the relationship were not significantly different between the two vegetation types. LMA and leaf C:N both showed a negative relationship with MAP in native vegetation, while no relationship was observed in *P. ponderosa* (Fig. 2).

Leaf  $\delta^{15}\text{N}$  of native vegetation showed a negative, linear relationship with MAP, while that of pines showed no relationship with MAP (Fig. 3a,b). Leaf  $\delta^{13}\text{C}$  showed a negative relationship with precipitation in both native vegetation and *P. ponderosa* (Fig. 3c,d), and the slopes were not significantly

different between the two vegetation types. Ectomycorrhizal roots were significantly enriched in  $^{15}\text{N}$  relative to leaves in *Nothofagus* spp. and *P. ponderosa*, and EcM and leaf  $\delta^{15}\text{N}$  were more positive in *P. ponderosa* relative to *Nothofagus* spp. ( $P < 0.001$ , Fig. 4 and Table S3). There was a significant negative effect of MAP on ectomycorrhizal  $\delta^{15}\text{N}$  (as well as leaf  $\delta^{15}\text{N}$ ), but regressions between ectomycorrhizal  $\delta^{15}\text{N}$  and MAP were not significant (Fig. 4 and Table S3).

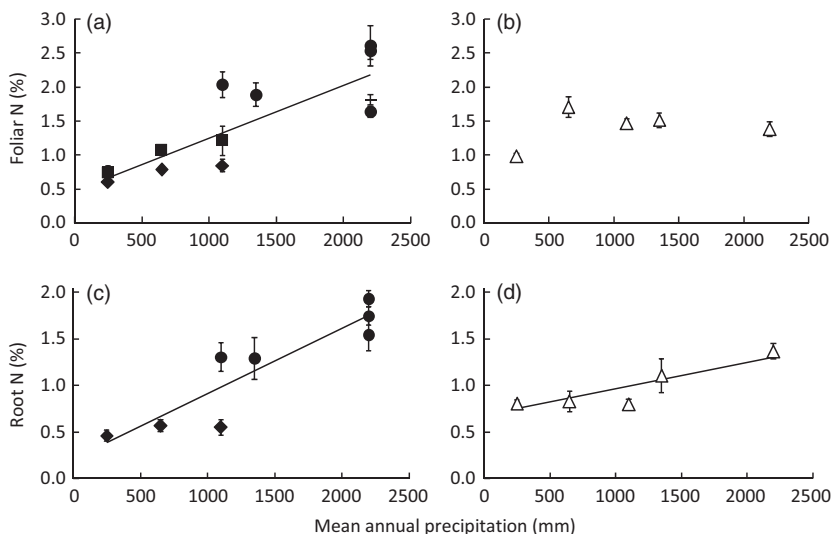
### SOIL CHARACTERISTICS IN NATURAL VEGETATION AND PINE PLANTATIONS

In pine plantations and sites with native vegetation, total soil C and N to 30 cm depth increased with MAP, although statistically, the regressions were only marginally significant in the native vegetation (Fig. 5). The slopes of the relationships between total soil C and N, respectively, and MAP were not significantly different between the native vegetation and pine plantations. Total soil C and N were significantly lower in pine plantations relative to sites with native vegetation (Table S3). Soil C:N ratios showed a significant second-order relationship with MAP in the natural vegetation and a linear relationship with MAP in pine plantations (Fig. 6), although significant differences were not detected between pine plantations and sites with native vegetation (Table S3).

Soil  $\delta^{15}\text{N}$  values did not show any detectable relationship with MAP. In contrast, there was a marginally significant positive effect of pine plantations on 0–30 cm soil  $\delta^{15}\text{N}$  signatures (Table S3,  $P < 0.06$ ), as well as  $^{15}\text{N}$  enrichment with depth (Table 2 and Table S3).

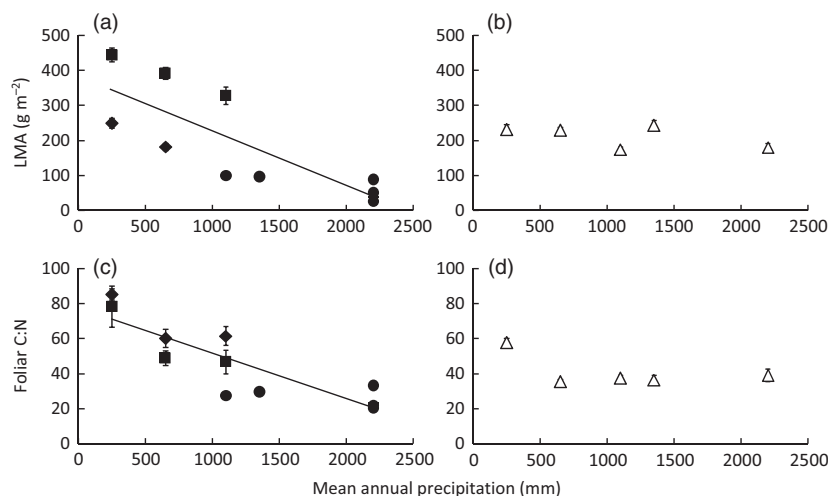
## Discussion

Natural precipitation gradients have been useful in identifying climatic controls on ecosystem processes, including net primary production (Sala *et al.* 1988; Austin & Sala 2002), litter decomposition (Murphy, Klopatek & Klopatek 1998; Austin & Vitousek 2000; Schuur 2001) and N dynamics (Austin &

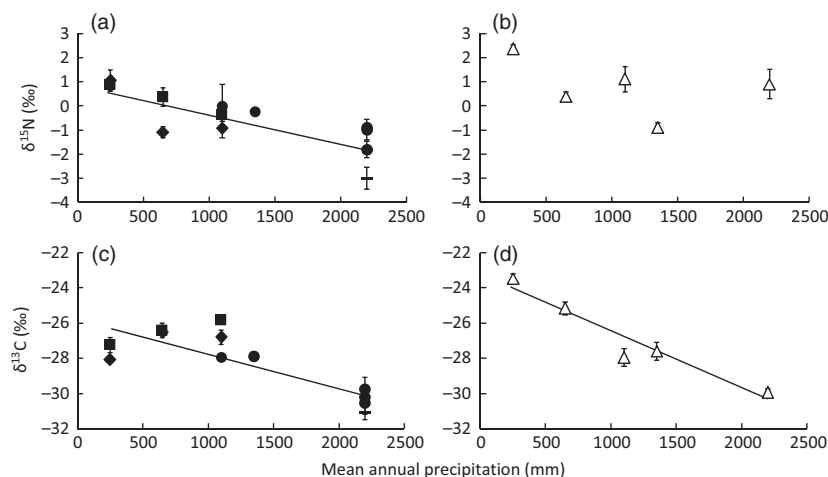


**Fig. 1.** Leaf and root N concentrations in native vegetation and *Pinus ponderosa* along the precipitation gradient. (a) Variation in leaf N with mean annual precipitation (MAP) for native vegetation, which includes *Pappostipa* spp. ( $\blacklozenge$ ), *Mulinum spinosum* ( $\blacksquare$ ), *Nothofagus* spp. ( $\bullet$ ) and *Chusquea culeou* ( $\blacktriangle$ ). Foliar N =  $7.7 \times 10^{-4} \times \text{MAP} + 0.51$ , adjusted  $r^2 = 0.68$ ,  $P < 0.001$ . (b) *P. ponderosa* ( $\triangle$ ) leaf N, which showed no relationship with precipitation. (c) Variation in root N with MAP for native vegetation, with symbols the same as in (a). Root N =  $7.0 \times 10^{-4} \times \text{MAP} + 0.21$ , adjusted  $r^2 = 0.80$ ,  $P < 0.005$ . (d) *Pinus ponderosa* root N vs. MAP. Root N =  $3.1 \times 10^{-4} \times \text{MAP} + 0.64$ , adjusted  $r^2 = 0.75$ ,  $P < 0.05$ . Error bars represent standard error.

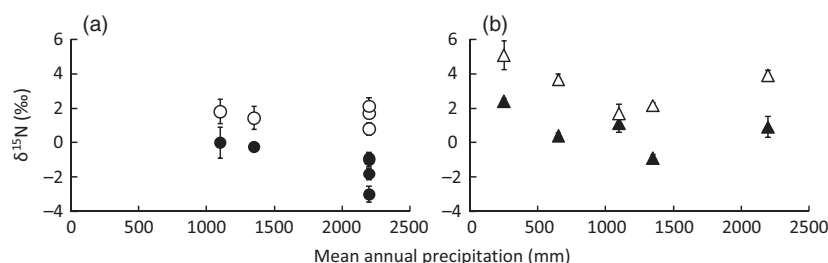
**Fig. 2.** Leaf characteristics of native species and *Pinus ponderosa* in sites along the precipitation gradient. (a) Variation in leaf mass per area (LMA) in native vegetation with MAP.  $LMA = 380 - 0.15 * MAP$ , adjusted  $r^2 = 0.62$ ,  $P < 0.005$ . (b) LMA of *P. ponderosa*, which showed no relationship with precipitation. (c) Variation in leaf C:N in native vegetation with MAP.  $C:N = 78 - 2.6 * 10^{-2} * MAP$ , adjusted  $r^2 = 0.70$ ,  $P < 0.001$ . (d) *P. ponderosa* leaf C:N, which showed no relationship with precipitation. Symbols are the same as in Fig. 1. Error bars represent standard error.



**Fig. 3.** Leaf  $\delta^{15}N$  and  $\delta^{13}C$  for native vegetation and *Pinus ponderosa* in sites along the precipitation gradient. (a) Variation in leaf  $\delta^{15}N$  with MAP in native vegetation. Foliar  $\delta^{15}N = 0.87 * MAP - 0.0012$ , adjusted  $r^2 = 0.58$ ,  $P < 0.005$ . (b) *Pinus ponderosa* leaf  $\delta^{15}N$ , which showed no relationship with precipitation. (c) Variation in leaf  $\delta^{13}C$  with MAP in native vegetation. Foliar  $\delta^{13}C = -1.9 * 10^{-3} * MAP - 26$ , adjusted  $r^2 = 0.62$ ,  $P < 0.005$ . (d) Variation in *Pinus ponderosa* foliar  $\delta^{13}C$  with MAP. Foliar  $\delta^{13}C = -3.3 * 10^{-3} * MAP - 23$ , adjusted  $r^2 = 0.90$ ,  $P < 0.01$ . Symbols are the same as in Fig. 1. Error bars represent standard error.



**Fig. 4.** Leaf and ectomycorrhizal (EcM)  $\delta^{15}N$  in native and pine trees in sites along the precipitation gradient. (a) *Nothofagus* spp. leaf (●) and EcM (○)  $\delta^{15}N$ . (b) *P. ponderosa* leaf (▲) and EcM (Δ)  $\delta^{15}N$ . Error bars represent standard error.

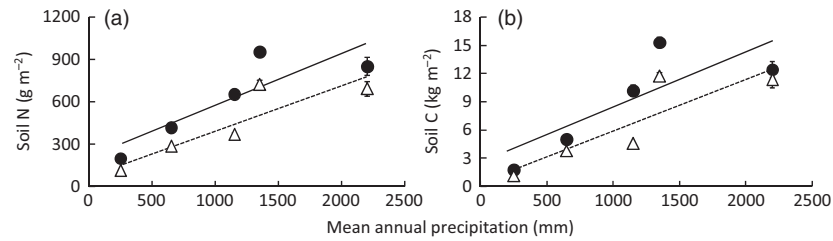


Vitousek 1998; Aranibar *et al.* 2004; McCulley, Burke & Lauenroth 2009). The gradient in this study is unique in that the establishment of even-aged pine plantations across a broad range of annual rainfall allowed for the evaluation of the interaction between the impacts of climate and vegetation on C and N pools, as well as the cumulative impact of 35 years of afforestation with a single exotic species of *P. ponderosa*.

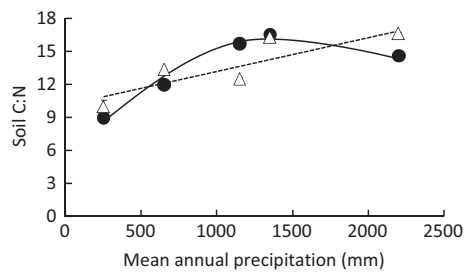
#### HOW IMPORTANT IS SPECIES COMPOSITION IN AFFECTING N DYNAMICS?

In sites of native vegetation, leaf variables demonstrated strong significant relationships with precipitation, including leaf N concentrations, LMA and leaf C:N. These relationships

suggest increasing leaf decomposability (Cornelissen *et al.* 1999) and corresponding rates of C and N cycling (Vitousek 2004) with increasing precipitation. These patterns are consistent with other temperate climatic gradients in Patagonia (Austin & Sala 2002), North American grasslands (McCulley, Burke & Lauenroth 2009) and the Kalahari region of southern Africa (Aranibar *et al.* 2004), all of which demonstrate increased C and N pools and soil C:N ratios with increasing MAP. In addition, a significant negative relationship between leaf  $\delta^{15}N$  values and MAP in native plant species was consistent with other studies of precipitation gradients (Austin & Vitousek 1998; Austin & Sala 1999; Schuur & Matson 2001; Aranibar *et al.* 2004; McCulley, Burke & Lauenroth 2009), including in the same region as this study, in southern beech



**Fig. 5.** Average total soil N and C to 30 cm depth in sites with native vegetation (●) and pine plantations (Δ) across the precipitation gradient. (a) Variation in total soil N with MAP. In sites with native vegetation, soil N =  $0.36 \cdot \text{MAP} + 220$ , adjusted  $r^2 = 0.65$ ,  $P = 0.06$  (solid line). For pine plantations, soil N =  $0.32 \cdot \text{MAP} + 85$ , adjusted  $r^2 = 0.72$ ,  $P < 0.05$  (dashed line). Total soil N was significantly lower in pine plantations relative to sites with native vegetation ( $P < 0.001$ ). (b) Variation in total soil C with MAP. In sites with native vegetation, soil C =  $6.0 \cdot \text{MAP} + 2200$ , adjusted  $r^2 = 0.55$ ,  $P = 0.09$  (solid line). For pine plantations, soil C =  $5.6 \cdot \text{MAP} + 240$ ,  $r^2 = 0.70$ ,  $P < 0.05$  (dashed line). Total soil C was significantly lower in pine plantations relative to sites with native vegetation ( $P < 0.01$ ). Error bars represent standard error.



**Fig. 6.** Variation in soil C:N ratios to 30 cm depth with MAP in sites with native vegetation and pine plantations. C:N ratios showed a significant second-order relationship with MAP in sites with native vegetation ( $\text{C:N} = -4.5 \cdot 10^{-6} \cdot \text{MAP}^2 + 1.4 \cdot 10^{-2} \cdot \text{MAP} + 5.4$ , adjusted  $r^2 = 0.96$ ,  $P < 0.05$ ), and a significant linear relationship with MAP in pine plantations ( $\text{C:N} = 3.2 \cdot 10^{-3} \cdot \text{MAP} + 10$ , adjusted  $r^2 = 0.68$ ,  $P = 0.05$ ). Symbols are the same as in Fig. 5. Error bars represent standard error.

**Table 2.** Soil  $\delta^{15}\text{N}$  by depth  $\pm$  standard error, in sites with native vegetation and pine plantations, along the precipitation gradient. Soil  $\delta^{15}\text{N}$  values were not significantly different between pine plantations and sites with natural vegetation

Depth (cm)	Mean annual precipitation (mm)				
	250	650	1100	1350	2200
<b>Native vegetation</b>					
0–5	3.1 $\pm$ 0.2	2.3 $\pm$ 0.3	1.4 $\pm$ 0.3	1.9 $\pm$ 0.2	3.0 $\pm$ 0.2
5–15	4.5 $\pm$ 0.3	4.1 $\pm$ 0.3	2.9 $\pm$ 0.2	3.6 $\pm$ 0.2	4.3 $\pm$ 0.2
15–30	5.4 $\pm$ 0.3	5.4 $\pm$ 0.2	4.3 $\pm$ 0.4	5.1 $\pm$ 0.1	5.2 $\pm$ 0.2
30–60	–	–	4.3 $\pm$ 0.2	5.6 $\pm$ 0.3	5.5 $\pm$ 0.1
60–90	–	–	3.9 $\pm$ 0.4	6.2 $\pm$ 0.3	6.0 $\pm$ 0.1
<b>Pine plantations</b>					
0–5	3.0 $\pm$ 0.5	2.3 $\pm$ 0.2	3.2 $\pm$ 0.7	2.8 $\pm$ 0.1	3.1 $\pm$ 0.2
5–15	4.2 $\pm$ 0.2	4.1 $\pm$ 0.2	4.5 $\pm$ 0.2	4.1 $\pm$ 0.0	3.5 $\pm$ 0.1
15–30	5.0 $\pm$ 0.6	5.3 $\pm$ 0.3	5.2 $\pm$ 0.2	5.0 $\pm$ 0.1	5.3 $\pm$ 0.1
30–60	–	–	5.1 $\pm$ 0.1	5.5 $\pm$ 0.1	5.3 $\pm$ 0.2
60–90	–	–	4.6 $\pm$ 0.8	6.7 $\pm$ 0.1	4.8 $\pm$ 0.2

forests in Patagonia (Peri *et al.* 2012). These patterns of leaf traits, and in particular leaf  $\delta^{15}\text{N}$  signatures, appear to be robust across many terrestrial ecosystems, and the native plant

species in this study demonstrated a similar range of variation with rainfall as has been observed in regional and global analyses of leaf  $\delta^{15}\text{N}$  and rainfall (Handley *et al.* 1999; Amundson *et al.* 2003; Craine *et al.* 2009).

In contrast, variation in leaf traits and  $\delta^{15}\text{N}$  signatures was nearly absent in the afforested sites along the precipitation gradient (Figs 1–3). The introduction of pine plantations essentially eliminated all variation in life form and species composition among sites of widely varying MAP. This homogenization of the aboveground vegetation translated into the elimination of climate signals on variables associated with N dynamics in vegetation, and demonstrates how plant species composition can directly control C and N dynamics in terrestrial ecosystems.

In natural ecosystems, observed changes in leaf traits and  $\delta^{15}\text{N}$  signatures with MAP have been interpreted as indicators of climate controls on N dynamics. In particular, climate controls on soil and ecosystem processes have been inferred from variation in  $\delta^{15}\text{N}$  signatures, including rates of N mineralization at soil depth (Vervaeke *et al.* 2002; Boeckx *et al.* 2005), denitrification losses (Houlton, Sigman & Hedin 2006) or higher N retention relative to turnover as MAP increases (Austin & Vitousek 1998; Austin & Sala 1999; McCulley, Burke & Lauenroth 2009). However, comparisons of relationships in natural vegetation and afforested sites in this study challenge the direct importance of climate and suggest that changes in response variables with MAP, particularly in leaf traits, may be largely driven by plant species composition. There was a positive relationship between leaf N concentrations and MAP observed in the native vegetation (Fig. 1a), which occurred both across dominant vegetation types and within species which were present in more than one site. Nevertheless, none of the species present in the natural vegetation were found in all sites along the gradient. Grasses and *M. spinosum*, found at the drier end of the gradient, had generally low leaf N concentrations, while *Nothofagus* spp., found at the wetter end of the gradient, had relatively higher leaf N concentrations. This pattern was similar for LMA and leaf  $\delta^{15}\text{N}$  signatures; while there appears to be a response of each species to changes in precipitation, changes in species composition were most important in determining the significant

relationships across all sites. These patterns are consistent with global analyses of variation in leaf traits with climate, which demonstrated that variation in co-existing species across climate gradients dictated large changes in LMA and leaf N per area, while single species varied less across this same range of environmental variation (Wright *et al.* 2005).

A notable exception to the contrasting relationships with MAP in natural and afforested ecosystems is the pattern of leaf  $\delta^{13}\text{C}$  signatures, which demonstrated a highly significant, negative linear relationship with rainfall in both native plant species and *P. ponderosa* (Fig. 3b,d). Increasingly negative leaf  $\delta^{13}\text{C}$  with increased precipitation has been observed in a number of other studies (Vitousek, Field & Matson 1990; Austin & Vitousek 1998; Luo *et al.* 2009). This pattern has been associated with decreasing water-use efficiency and canopy-scale changes in vapour pressure deficit with increasing rainfall (Bowling *et al.* 2002; Diefendorf *et al.* 2010), although photosynthetic  $^{13}\text{C}$  discrimination can also contribute to variation in  $\delta^{13}\text{C}$  values independently of water-use efficiency (Seibt *et al.* 2008). It is possible, due to the close association of water availability and its demonstrated effects on leaf  $\delta^{13}\text{C}$ , that changes in this response variable were more closely linked to changes in MAP (Fig. 3b,d) and may be primarily determined by climatic variables, independent of inter-specific differences.

Taken together, we suggest that ecosystem-scale variation in dominant species and life form in the native vegetation along this precipitation gradient is the proximate driver of observed differences in LMA, C:N ratios, N content and stable isotope signatures of  $\delta^{15}\text{N}$ . Once the indirect effects of rainfall on species composition are eliminated, the climate 'footprint' is much reduced or entirely undetectable. It should be noted that the particular species characteristics of *P. ponderosa* may result in low phenotypic plasticity and thus exaggerate the impacts of converting the natural vegetation to a single species. However, it does seem clear that the control of species composition is comparable with direct effects of climate for determining several aspects of N dynamics in these sites. As such, this study highlights the importance of the indirect effects of precipitation in determining floristic composition, which had quantitative impacts on C and N inputs and turnover.

#### DOES AFFORESTATION ERASE THE SOIL CLIMATE FOOTPRINT?

Soil C and N pools demonstrated significant positive relationships with MAP, with increasing C, N and C:N ratios with increasing rainfall (Figs 5–6). In addition, there were some consistent effects of afforestation across all sites, with more positive soil  $\delta^{15}\text{N}$  values, and reduced total C and N pools (0–30 cm depth) in pine plantations (Fig. 5 and Table S3). It appears that the clearest effects of changes in floristic composition are observable in the most dynamic N pools, while slower turnover pools may reflect long-term climate effects (Austin & Sala 2002; McCulley *et al.* 2005; McCulley, Burke & Lauenroth 2009). These results further underscore that

natural gradients represent the sum of long-term, plant–soil interactions, and interpreting these differences in a current climate change context should be done with great care.

In addition to supporting other studies showing detectable climate effects on soil C and N pools, these data corroborate studies demonstrating that afforestation with *Pinus* spp. leads to overall decreases in soil C and N pools. For example, in a meta-analysis of the effects of afforestation on soil properties, Berthrong *et al.* (2009) found that soil C stocks declined by 15% and soil N stocks declined by 20% on average following afforestation with pine species across all climatic regimes. Evaluation of *Eucalyptus* afforestation along climatic gradients in South America, however, demonstrated that observed declines occurred only in systems with MAP >1150 mm (Berthrong *et al.* 2012). Guo and Gifford (2002) demonstrated a similar reduction in soil C stocks in pasture to forest conversion, but again only at MAP higher than 1200 mm. These results are partially consistent with the results of this study, where we observed a decline in total soil C and N as a result of afforestation across all levels of precipitation, although the losses were not restricted to higher precipitation regimes. (Fig. 5). In contrast, semi-arid shrublands in Argentina showed no changes in soil organic matter accumulation with 15 years of afforestation (Nosetto, Jobbágy & Paruelo 2006), while in Israel, there was a twofold increase in carbon content in the soil after 35 years of afforestation (Grünzweig *et al.* 2007).

Various mechanisms for the changes in soil C and N with afforestation have been postulated, including increases in recalcitrant litter, and slowed soil organic matter accumulation and nitrogen turnover (Gelfand *et al.* 2009; Gelfand, Grünzweig & Yakir 2012). At the same time, increased nitrogen-use efficiency has been postulated as a mechanism to sustain increased primary production and counteract effects of progressive nitrogen limitation over time (Norby *et al.* 2010; Gelfand, Grünzweig & Yakir 2012). The slow decomposition of pine litter (Gholz *et al.* 2000; Araujo 2012) compared to native species litter decomposition may also result in reduced inputs of C and N to the soil pool in a consistent manner across the entire precipitation gradient, which would be consistent with the results of the observed declines in soil pools in this study.

#### ROLE OF ECTOMYCORRHIZAE IN N DYNAMICS ALONG THE PRECIPITATION GRADIENT

We originally hypothesized that decreased leaf and soil  $\delta^{15}\text{N}$  signatures in pine plantations would indicate more conserved N cycling in afforested ecosystems, due to higher NPP and N demand at all sites along the precipitation gradient. There was little evidence to support increased N retention in pine plantations based on leaf  $\delta^{15}\text{N}$  signatures, however, which were generally more positive than site-average values of native vegetation (Fig. 3a,b).

In addition to changes in species composition in the natural vegetation highlighted in the previous section, there is a categorical shift in the type of mycorrhizal associations, with arbuscular mycorrhizal associations prominent in the grass



and shrub species of arid sites (Fontenla, Puntieri & Ocampo 2001), while the humid sites are dominated by EcM-forming broad-leaved trees (Diehl, Mazzarino & Fontenla 2008). It has been documented that ectomycorrhizal associations in particular can alter N acquisition, increasing the uptake of organic nitrogen (Orwin *et al.* 2011; Hobbie & Högborg 2012), and these differences in N acquisition have been postulated to be reflected in differences in leaf  $\delta^{15}\text{N}$  signatures (Craine *et al.* 2009; Hobbie & Högborg 2012). Leaf  $\delta^{15}\text{N}$  signatures are less enriched (positive) in ectomycorrhizal plants relative to arbuscular- and non-mycorrhizal plants at the global scale (Craine *et al.* 2009). This difference among plant species suggests at least two ways in which mycorrhizal associations may influence leaf  $\delta^{15}\text{N}$  signatures along a precipitation gradient: (i) through the influence of mycorrhizal association, particularly EcM associations, on the source and  $\delta^{15}\text{N}$  signatures of plant-absorbed nitrogen; or (ii) through the presence of species with ectomycorrhizal associations in particular climatic regimes (Hobbie & Högborg 2012). These possibilities are not mutually exclusive, and evidence from this study supports both possible mechanisms.

First, across all vegetation types and species, EcM were enriched in  $^{15}\text{N}$  relative to leaves (Fig. 4), in *Nothofagus* spp. in woodland and forest sites in the natural vegetation, as well as in *P. ponderosa* in all sites. This  $^{15}\text{N}$  enrichment of EcM supports the contention that ectomycorrhizal fungi are transferring  $^{15}\text{N}$ -depleted N compounds to plants and retaining  $^{15}\text{N}$ -enriched N compounds in their tissue (Hobbie & Colpaert 2003; Hobbie & Hobbie 2006; Craine *et al.* 2009; Hobbie & Högborg 2012). Second, while EcM have not been definitively linked to high rainfall ecosystems, EcM associations were only present in the natural vegetation at the three most humid sites, all associated with *Nothofagus* spp. Some indirect evidence suggests that the presence of ectomycorrhizae may be strongly associated with humid temperate ecosystems. For example, global diversity of EcM fungi is maximum in temperate and boreal forest ecosystems (Tedersoo *et al.* 2012), ectomycorrhizal colonization is also strongly adversely affected by drought (Swaty *et al.* 2004) and the incidence of species with ectomycorrhizal associations is higher in ecosystems characterized by high concentrations of soil organic matter (Read & Perez-Moreno 2003). Taken together, this indirect evidence suggests a strong spatial association of EcM colonization with sites of higher precipitation. In this study, it seems plausible that spatial variation in mycorrhizal associations in natural ecosystems contributes to the pattern of declining leaf  $\delta^{15}\text{N}$  with increasing MAP. This could arise through the transfer of  $^{15}\text{N}$ -depleted compounds to host plants from EcM fungi in humid sites, resulting in depleted leaf  $\delta^{15}\text{N}$  values with increasing precipitation. In contrast, *P. ponderosa* leaf  $\delta^{15}\text{N}$  did not show any patterns with precipitation (Fig. 3b), which supports the idea that the presence of EcM is an important control on leaf  $\delta^{15}\text{N}$  signatures in sites of variable precipitation.

A final reflection on the importance of EcM associations stems from the large differences in EcM and leaf  $\delta^{15}\text{N}$  in *Nothofagus* spp. and pines (Fig. 4). These differences among the EcM-associated species suggest that there is variation in

the quantity and source of N transfer from the EcM fungi to their host plant species. While the amount of N transferred to the plant by the EcM fungi can be as much as 85% of the plant's total N uptake (Hobbie & Hobbie 2006), there may be differences in the forms of N transferred by the EcM, ranging from ammonia to amino acids (Hobbie & Högborg 2012). The consistently positive values of leaf and EcM  $\delta^{15}\text{N}$  signatures of *P. ponderosa* suggest a source of N that is enriched in  $^{15}\text{N}$  and similar to the bulk soil  $\delta^{15}\text{N}$  values. This tight coupling in N sources between the EcM fungi and the pines themselves may help to explain the high nitrogen-use efficiency observed in pine species (Billings & Richter 2006; Gelfand, Grünzweig & Yakir 2012), and may contribute to a more conserved nitrogen cycle in sites of higher precipitation (Chapman *et al.* 2006; Orwin *et al.* 2011).

## Conclusions

Intentional planting of a single species along a steep climatic gradient in northwestern Patagonia allowed for the assessment of the importance of life form and species composition in controlling C and N dynamics along a natural gradient of precipitation. Replacement of natural vegetation with pine plantations had a strong homogenizing effect on leaf traits, N pools and leaf  $\delta^{15}\text{N}$  signatures across sites spanning an order of magnitude of MAP. In addition to the introduction of *P. ponderosa*, the simultaneous introduction of ectomycorrhizal symbionts may have contributed to the homogenization of N acquisition strategies in the modified ecosystems. As such, it seems that the impacts of afforestation on ecosystem carbon sequestration potential, mediated by changes in N cycling, must be evaluated in the context of the vegetation it replaces: the relative difference in litter quality, N-use efficiency and ectomycorrhizal association between the native and afforested species will likely determine the potential magnitude for change in C and N pools in soil organic matter. These results highlight the important control of plant–soil interactions in affecting C and N dynamics in natural ecosystems, and serve to demonstrate that altering native vegetation alone is sufficient to cause large, detectable impacts on N turnover independently of its interaction with climate.

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## Data accessibility

All original data for calculations of the figures and tables including leaf, root and soil data are available in the Dryad Digital Repository (Hess & Austin 2014).

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Map of study location.

**Table S1.** Geologic information for study sites.

**Table S2.** Plant species from which leaves and ectomycorrhizae were collected along the precipitation gradient in Patagonia, Argentina.

**Table S3.** ANOVA results for evaluation of mean annual precipitation (MAP), vegetation (native or pine plantations) and plant part [leaf or ectomycorrhizae (EcM)] and leaf and soil values of C and N and stable isotope signatures.

**Table S4.** Soil  $\delta^{13}\text{C}$  by depth  $\pm$  standard error, in sites with native vegetation and pine plantations, along the precipitation gradient.