

Nitrogen addition stimulates forest litter decomposition and disrupts species interactions in Patagonia, Argentina

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Abstract

Nitrogen (N) deposition and biodiversity loss are important drivers of global change, with uncertain consequences for carbon (C) and nutrient turnover in terrestrial ecosystems. We evaluated the simultaneous effects of N deposition and plant diversity on litter decomposition within a temperate forest in Patagonia. We identified ‘tree triangles’ created by the intersection of three tree-canopies that directly controlled micro-environmental conditions on the forest floor, and combined it with an N addition treatment. Triangles were composed of one or three *Nothofagus* species (*N. dombeyi*, *N. obliqua* or *N. nervosa*). We placed litterbags containing litter of each of the *Nothofagus* species and litterbags containing a mixture of the three species within all triangles and assessed mass loss over 2 years. We used a standard litter type in all triangles to independently evaluate triangle effects on decomposition. N addition had strong and positive effects on decomposition with an average 46% increase in the decomposition constant. Litter species significantly differed in their response to N addition; litter with higher lignin content and lower labile C content had larger increase in decomposition due to fertilization. Also, N addition disrupted two types of species interactions that control decomposition. The affinity relation between litter and decomposers, that enhanced decomposition of home litter (‘home-field advantage’) that was demonstrated to be significant for all three *Nothofagus* species, disappeared with N addition. Second, N addition modified litter species interactions, transforming neutral effects of litter mixtures to positive, nonadditive effects on mass loss. Finally, N addition stimulated N release from decomposing litter which was modulated by plant species effects. Together, these results suggest that N addition to unpolluted forests increases C loss, contrary to what has been observed for temperate forests in industrialized areas of the world, and that alterations in nutrient pools have effects on species interactions, including the elimination of affinity effects for decomposition.

Keywords: affinity effects, atmospheric N deposition, carbon cycle, home-field advantage, litter mixtures, litter nitrogen dynamics, *Nothofagus*, plant diversity, plant–soil feedbacks, southern beech, species interactions, specificity effects, temperate forest

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Introduction

The amount of biological reactive nitrogen (N) has doubled globally in the last century (Martinelli *et al.*, 2006; Galloway *et al.*, 2008) and biodiversity has declined worldwide as a result of human activities (Chapin *et al.*, 2000). Given the co-occurring changes in N availability and plant diversity, it is crucial to understand their interactive effects to predict ecosystem response to global change. Biodiversity encompasses several components such as species identity, species assemblages, species richness and species interactions. Evaluating which components of biodiversity have an impact on ecosystem functioning and how they interact with N deposition would give us a better understand-

ing of the mechanisms by which human-induced environmental change affects natural ecosystems.

The ability to predict ecosystems’ ability to store carbon (C) relies on our understanding of how N deposition and biodiversity change independently and interactively affect decomposition (De Deyn *et al.*, 2008; Adair *et al.*, 2009). Decomposition is one of the main pathways of C release to the atmosphere from natural ecosystems. The effects of N deposition and plant diversity on litter decomposition have been assessed independently, but their effects are poorly understood. Traditionally, it has been proposed that N availability limits organic matter decomposition (Swift *et al.*, 1979). The C:N ratio of leaf litter (~40) is much higher than the C:N ratio of decomposer microorganisms (~5–17) (Paul & Clark, 1996), suggesting that N concentration in leaf litter is not sufficient to satisfy microbial requirements. Accordingly, many studies have shown that microorganisms import N from the soil solution to the

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decomposing litter (Hart & Firestone, 1991; Frey *et al.*, 2000), and that the degree of immobilization and the point of nutrient release depend on the initial N content of the litter (Parton *et al.*, 2007). Similarly, it has been frequently reported that decomposition rates are positively correlated with total N content or C:N ratio of the litter (Aerts, 1997). However, N fertilization experiments in temperate forests and grasslands have shown widely variable effects on decomposition, with evidence for increased mass loss (Hunt *et al.*, 1988; Hobbie, 2000), neutral effects (Prescott, 1995; Hobbie & Vitousek, 2000) or inhibition of decomposition (Magill & Aber, 1998). Recent meta-analyses concluded that responses to N addition depend on fertilization doses, levels of background N atmospheric deposition, and litter quality of different species (Knorr *et al.*, 2005), and that overall, there is little evidence to suggest a strong directional effect of N addition on litter decomposition in forest ecosystems (Janssens *et al.*, 2010).

Plant species diversity effects on litter decomposition have been evaluated independently of N deposition effects, largely through litter mixture experiments (Hättenschwiler *et al.*, 2005). These experiments allowed for testing the effects of plant species identity, assemblage and richness (Wardle *et al.*, 1997; Chapman & Koch, 2007). At the same time, other components of plant species diversity such as species interactions within litter mixtures or plant–decomposer interactions have received much less attention, but can be important in affecting decomposition (Hättenschwiler & Gasser, 2005). For example, affinity relationships between plant litter and the decomposer community result in a faster litter mass loss when litter decomposes in its place of origin than in other microsites within the same ecosystem (Vivanco & Austin, 2008; Ayres *et al.*, 2009). This ‘home-field advantage’ for decomposition highlights aspects of biodiversity that are unlike the usual species-richness approximation. Moreover, plant species diversity effects have been studied as a result of variation in leaf litter traits but indirect effects through abiotic and biotic changes in the soil environment have been evaluated on only a few occasions (Hector *et al.*, 2000). In particular, the study of tree species diversity effects in old-growth temperate forests has been constrained by challenges in experimental design and confounding effects (Vivanco & Austin, 2008).

Deciduous temperate forests are among the biomes that have been most heavily impacted by air pollution and land-use change (Reich & Frelich, 2002). Europe and North America host the largest expanse of deciduous temperate forests, which have received chronic and increasing inputs of N since the industrial revolution (Galloway *et al.*, 2004; Dentener *et al.*, 2006). N deposition effects on forest ecosystems have been

primarily evaluated in the Northern Hemisphere where N levels have increased with chronic deposition over more than a century, making it difficult to use these studies to predict future impacts on undisturbed ecosystems. There is still an important fraction of temperate forests that are under low, natural levels of N deposition including the northwestern United States, southeast Australia and temperate South America, and some of these regions are projected to receive increasing amounts of N deposition in the future (Dentener *et al.*, 2006). At the same time, the vast majority of N addition experiments have been conducted in regions which are, or have been, subject to atmospheric N deposition from human activity (Janssens *et al.*, 2010). As such, the study of pristine temperate forests can provide insights into the biogeochemical conditions that constrain ecosystem functioning before human domination of natural systems and provide an opportunity to evaluate biogeochemical theories that have been developed in areas subject to strong human influences (e.g. Hedin *et al.*, 1995; Perakis & Hedin, 2002). In addition, the responses of these unpolluted systems to experimental N additions would increase our understanding of the control of N on ecosystem functioning and its potential impact on the global C balance (Reay *et al.*, 2008).

Patagonian forests, located at the southern end of the South American continent, are far from urban areas and protected from anthropogenic sources of N (Hedin *et al.*, 1995). These forests offer the opportunity to test the effects of N deposition on litter decomposition in unpolluted conditions. Additionally, long-term coexistence of tree species is the ideal situation to evaluate how long-term interactions between plant species and their environment are able to modulate the effects of N deposition on C and nutrient cycling. In a previous study, we reported results from an experiment that simultaneously evaluated direct effects of leaf litter diversity (litter effects) and indirect effects of living plant species on the decomposition microenvironment (microsite effects) in a natural temperate forest ecosystem (Vivanco & Austin, 2008). Here we show how N addition to the soil interactively modifies plant species controls on decomposition through litter and microsite effects in a 2-year experiment in the same study site. Specifically, we tested the following hypotheses: (1) N addition increases C and N turnover rates because it alleviates biogeochemical restrictions on microbial activity in unpolluted temperate forests; (2) Plant species composition modulates N addition effects on decomposition directly through litter quality, and indirectly through plant species-specific changes created in the soil environment; (3) N addition alters leaf litter mixture effects on mass loss due to alterations of interactions among litter types.

Materials and methods

Study site and experimental design

We designed our experiment within a natural mosaic of tree species diversity in a *Nothofagus* mixed forest, in northern Patagonia, 30 km west from San Martín de los Andes (40°08'S, 71°30'W), Neuquén, Argentina. Average annual precipitation in this area is 2300 mm and mean monthly temperatures range from 3 °C in winter (July) to almost 15 °C in summer (January). Soils are Inceptisols, suborder Distrandepts, derived from post-glacial volcanic ashes (Ferrer & Irrisarri, 1990). The overstorey vegetation of the study site was entirely dominated by three tree species from the genus *Nothofagus*, which were evenly represented in terms of basal area (Vivanco & Austin, 2008). Understorey vegetation was dense and monospecific, almost completely dominated by *Chusquea culeou* (caña colihue).

Within the forest mosaic, we identified microsites with different tree species composition. These microsites or 'tree triangles' were defined by the intersection of three tree-canopies that directly controlled micro-environmental conditions on the forest floor (Vivanco & Austin, 2008). The effective area on the forest floor beneath the tree triangles was 4 m². Monospecific triangles were composed of single *Nothofagus* species: *N. dombeyi*, *N. obliqua* or *N. nervosa*. Plurispecific triangles were delineated by three trees from different species; single tree of *N. dombeyi*, a single tree of *N. nervosa* and a single tree of *N. obliqua*. Ten replicates of each microsite type were identified; the microsites were distributed throughout the 6 ha study site.

We combined the microsite diversity treatment with a N addition treatment (82.5 kg N ha⁻¹ yr⁻¹ the first year and 60 kg N ha⁻¹ yr⁻¹ the second year). We used urea (coated sulfur urea, 39% N) to fertilize five replicates of each of the four microsite types. We chose slow-release urea as the fertilizer in an attempt to mimic N atmospheric deposition effects that would occur chronically over time. Urea was applied on the soil surface distributed at three times during the year (January, May and October) over a 2-year period at a dose of 20 kg N ha⁻¹ at each time point. The first fertilization was approximately two times higher (42.4 kg N ha⁻¹) to create a situation of higher N availability at the onset of the experiment. The fertilization treatment represented an average of 30% increase in the total amount of N that entered to the system through annual net mineralization (annual net mineralization averaged between the 2 years of the experiment was 240 kg N ha⁻¹ yr⁻¹ (Vivanco, 2008)).

Litter decomposition experiment

Leaf litter from three *Nothofagus* species was collected in the fall using nets suspended 2 m from the soil surface in the study site. We prepared litterbags (2 mm fiberglass mesh) containing 2 g of air-dried leaf litter of *N. dombeyi*, *N. nervosa*, *N. obliqua* or an equivalent mixture of the three species (mixed litter). We also prepared litterbags with litter of black poplar (*Populus nigra*) (2 g), a nonnative tree species which did not grow in the study area and whose litter was collected in an adjacent black poplar plantation. Litterbags were 15 cm × 15 cm, except for *N. dombeyi* alone, where we used 10 cm × 10 cm bags because of

the smaller leaves of this species. The idea was to create the same litter density conditions among all litter treatments. Litterbags were placed in the center of the plot on the forest floor in all microsites (5 litter types × 4 microsites × 2 N treatments × 5 sampling dates × 5 replicates = 1000 litterbags), where all detritus and understorey vegetation had been removed. Litter accumulation on the litterbags was prevented by hand cleaning during most of the year to avoid confounding effects of overstorey litter on decomposition.

The experiment started in January (mid-summer) and litterbags were harvested at 43, 122, 268, 366 and 730 days. Litterbags were collected from the field and brought to the laboratory for processing. Samples were dried for 48 h at 65 °C; soil and debris were carefully removed from litter and were oven-dried again for the determination of dry mass. For the last sampling date (730 days), litter from the mixed litter treatment was sorted by species on the basis of color, texture and patterns of leaf margins. Ash-free dry mass was determined for all samples to correct for soil contamination from the field (Harmon *et al.*, 1999). Mass loss over time was approximated using a single exponential decay model using $\ln(M_t/M_0) = -kt$, where M_0 is the initial ash-free dry mass, M_t is the ash-free dry mass at time t and k is the decomposition constant (Swift *et al.*, 1979). Linear regressions were performed setting the intercept to zero. Data for the first year only of decomposition from the nonfertilized microsites are reported elsewhere (Vivanco & Austin, 2008).

Litter N dynamics was assessed by determining total N content of the litter at the beginning of the experiment and after 1 year in the field by dry combustion with a Carlo-Erba[®] NA 2500 elemental analyzer (Haake Buchler Instruments Inc., Saddle Brook, NJ, USA) at the Pontificia Universidad Católica de Chile. Percent N remaining was calculated as: $\%N_t M_t / \%N_0 M_0 \times 100$, where $\%N_0$ is the initial N content, $\%N_t$ is the N content after 1 year (Harmon *et al.*, 1999). Values higher than 100% indicated net accumulation of N in the litter, whereas values lower than 100% indicated net N release.

Initial physico-chemical parameters associated with the C quality of the litter and tensile strength are reported elsewhere (Vivanco & Austin, 2008). Initial water content of the litter was assessed by drying samples at 65 °C for 48 h. The amount of ash in initial litter was determined by combusting samples in a muffle furnace at 500 °C for 4 h. Initial litter Ca, K, Mg, Mn, Zn, Fe and Cu content were determined by inductively couple argon plasma emissions spectrometry (JY2000 ICP-AES, Horiba Jobin Yvon, Longjumeau, France) following digestion in nitric acid at Brown University.

Statistical analysis

We assessed the dependence of N addition on litter quality calculating the 'N addition effect' on decomposition as the percent change of the decomposition constant due to N fertilization for each litter type in each microsite. We calculated the 'N addition effect' (%) = $(k_N - k_C) / k_C \times 100$, where k_C is the average decomposition constant in control plots ($n = 5$) and k_N is the average decomposition constant in N addition plots ($n = 5$). Then, we assessed the correlation of the N addition effect for *Nothofagus* species with initial litter quality parameters of each litter type ($n = 12$).

N addition and biodiversity effects on decomposition for the native *Nothofagus* species were analyzed with a three-way analysis of variance (ANOVA), with fertilization, litter and microsite as main factors. N addition and microsite effects for the common litter substrate, *Populus nigra*, were analyzed by two-way ANOVA. Affinity effects were tested by a two-way ANOVA, with individual litter species (*N. dombeyi*, *N. nervosa* and *N. obliqua*) and location (*in situ* vs. *ex situ*) as the main factors for each fertilization treatment. The *in situ* location corresponded to individual litter species decomposing in their own monospecific microsite (3 species × 5 replicates). *Ex situ* decomposition corresponded to individual litter species decomposing in monospecific microsites of different species (3 species × 2 microsites × 5 replicates). Plurispecific triangles were not considered in these analyses.

Nonadditive effects of tree species mixture on decomposition were analyzed at the litter level (mixed litter) and at the microsite level (mixed tree species present in a microsite),

under N-fertilized and nonfertilized conditions. For the litter level, we assessed the effects of litter mixtures for (1) the whole litter mixture and for (2) individual litter species within the mixture. For the first analysis, we compared the observed decomposition constants (*k*) of mixed litter against the predicted *k* values for each microsite and N treatment. Predicted values of *k* for each plot were calculated by regressing the log of the fraction of the average organic matter remaining of the three litter species decomposing alone in the same plot against time. For the second analysis, we compared mass loss of each *Nothofagus* species after 2 years in the field when the litter was decomposing alone against litter decomposing within the litter mixture. For these analyses, we used a three-way ANOVA, with a nested block design. Main effects were fertilization (control vs. N-fertilization), condition (predicted vs. observed), microsite (*N. dombeyi*, *N. nervosa*, *N. obliqua* and three species triangles), and plot. Plots were nested within fertilization × microsite interaction (each plot could be only under a

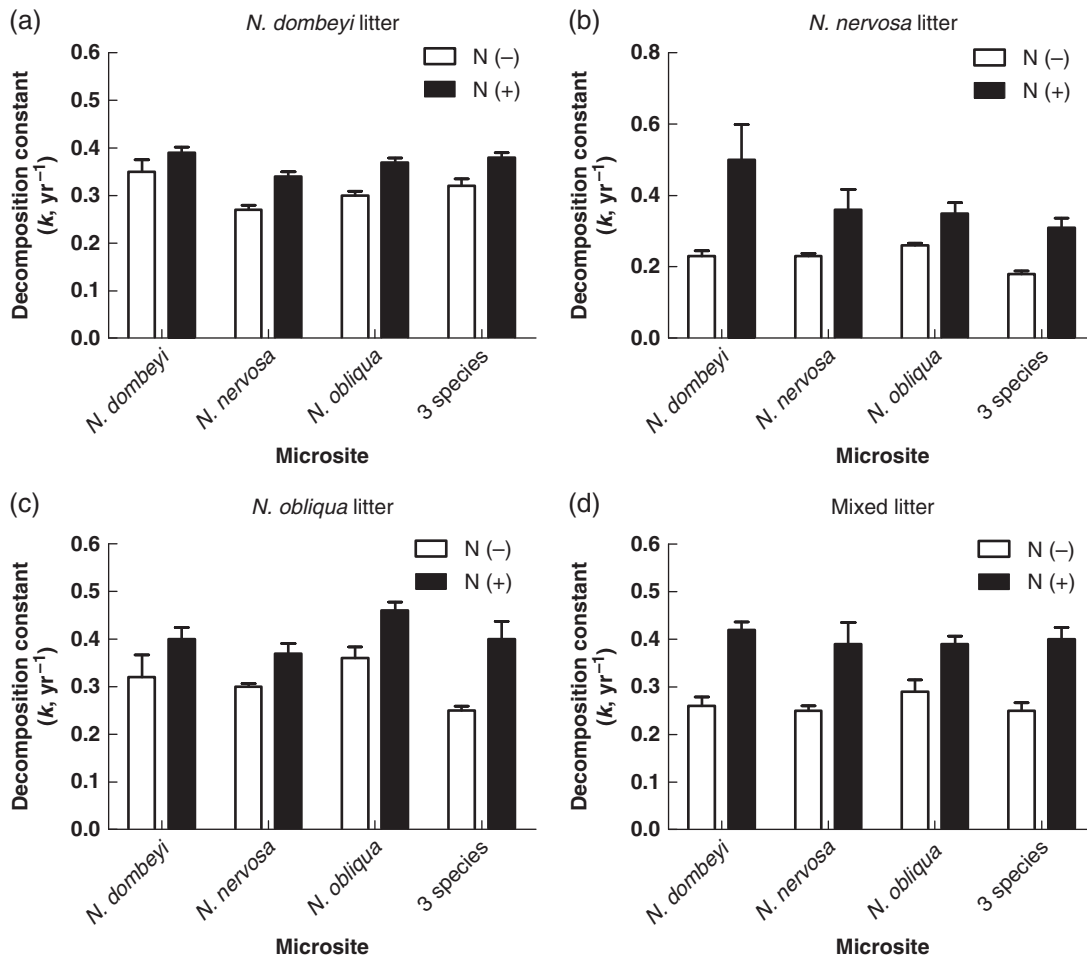


Fig. 1 Nitrogen (N) addition and plant species' effects on leaf litter decomposition. Leaf litter decomposition constants of three different *Nothofagus* species and their mixture across microsities dominated by different *Nothofagus* species under control and N-fertilized conditions. (a) *N. dombeyi*, (b) *N. nervosa*, (c) *N. obliqua*, (d) Mixed litter of the three *Nothofagus* species. Bars are means for each leaf litter type (*n* = 5); 1 SE is presented. Decomposition constants differed significantly between N addition treatments, litter species and microsities ($P < 0.001$, $P < 0.01$, $P < 0.01$, respectively). The interactions litter species × N addition treatment and litter species × microsite were also significant ($P < 0.05$, $P < 0.01$, respectively).

single fertilization and microsite combination). For the microsite level of analysis, we compared average k -values obtained in monospecific microsites with the average k -values obtained in the plurispecific microsite for all litter types under both N

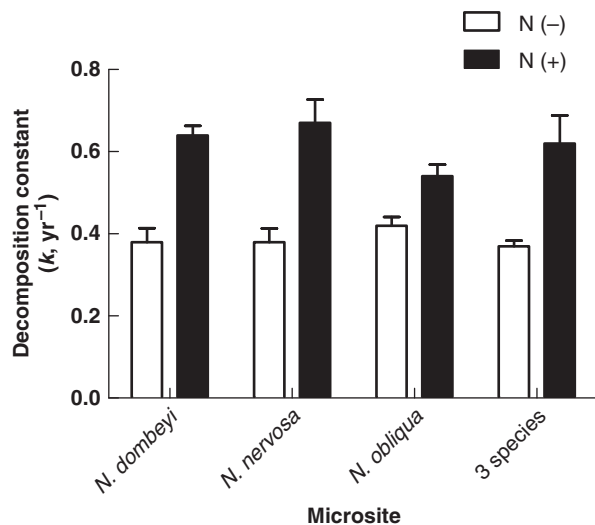


Fig. 2 Nitrogen (N) addition and microsite effects on decomposition of a common substrate. Leaf litter decomposition constants of *Populus nigra* across microsites dominated by different *Nothofagus* species under control and N-fertilized conditions. Bars are means for each microsite ($n = 5$); 1 SE is presented. Decomposition constants differed significantly between nitrogen addition treatments ($P < 0.001$).

treatments using a two-way ANOVA. All *post hoc* comparisons were performed with a Tukey's HSD test, and an α of 0.05 was used for all the statistical analysis.

Correlations between decomposition constants and litter quality, and N remaining in the litter and litter quality were performed using data for the *Nothofagus* species for each microsite (3 litter types \times 4 microsites, $n = 12$). Correlations between N remaining in the litter and mass remaining were performed using data for the *Nothofagus* species for each N treatment (3 litter types \times 4 microsites \times 5 replicates, $n = 60$). An α of 0.05 was used in all the statistical analyses.

Results

N effects on litter decomposition

N addition significantly and consistently stimulated leaf litter decomposition in this Patagonian temperate forest. Decomposition constants (k , yr^{-1}) increased 46% on average across all litter types (Figs 1 and 2). Litter mass loss increased significantly after 120 or 270 days depending on the litter type (Supporting Information, Fig. S1).

Litter identity

Plant species identity modulated N stimulation effects on decomposition directly through litter identity effects.

Table 1 Initial litter quality of three native *Nothofagus* species of the Patagonian forest and *Populus nigra*, species absent in the area that was used as common substrate in this study

	<i>N. dombeyi</i>		<i>N. nervosa</i>		<i>N. obliqua</i>		<i>P. nigra</i>	
Litter chemistry	Evergreen broad-leaf		Deciduous broad-leaf		Deciduous broad-leaf		Deciduous broad-leaf	
<i>Carbon fraction</i>								
Total carbon (%)	52 ^a	0.3	52 ^a	0.3	49 ^b	0.2	43 ^c	0.4
Soluble carbohydrates (%)	64 ^a	0.6	51 ^b	0.6	57 ^c	0.2	53 ^b	1.1
Soluble polyphenols (%)	14.4 ^a	0.4	5.9 ^b	0.1	11.6 ^c	0.6	4.9 ^b	0.6
Lignin (%)	19.3 ^a	0.8	29.2 ^b	0.9	27.6 ^{bc}	2.3	21.5 ^{ac}	0.7
<i>Nutrient content</i>								
N (%)	0.57 ^a	0.004	0.99 ^b	0.05	0.94 ^b	0.02	0.66 ^a	0.02
Ca (%)	1.66 ^a	0.05	1.76 ^a	0.08	2.01 ^a	0.07	4.13 ^b	0.21
K (%)	0.20 ^a	0.01	0.30 ^{ab}	0.02	0.45 ^b	0.02	0.69 ^c	0.08
Mg (%)	0.29 ^a	0.01	0.28 ^a	0.01	0.27 ^a	0.01	0.56 ^b	0.00
Mn (ppm)	555 ^a	9	298 ^b	6	331 ^b	15	137 ^c	6
Zn (ppm)	32 ^a	1	80 ^b	6	85 ^b	2	61 ^c	5
Fe (ppm)	60 ^a	4	83 ^{ab}	11	172 ^b	36	140 ^{ab}	11
Cu (ppm)	3.0 ^a	0.2	6.1 ^b	0.2	5.4 ^b	0.3	3.7 ^a	0.1
C:N ratio	91 ^a	1	53 ^b	2	52 ^b	1	65 ^c	2
Lignin:N ratio	34	1	29	1	29	3	32	0
Tensile strength (N m^{-2})	1575 ^a	132	544 ^b	72	626 ^b	55	369 ^b	35

Values are means with 1 SE. Different letters indicate significant differences among species ($P < 0.05$). Carbon fractions, N content and tensile strength values are published data (Vivanco & Austin, 2008).

The decomposition constants of the three *Nothofagus* species significantly differed in their response to N addition (N addition \times litter type, $P < 0.05$, Fig. 1). *N. nervosa* showed a 70% increase under N addition across all microsites, whereas *N. dombeyi* only showed a 20% increase. *P. nigra*, the common litter substrate, showed a 60% increase in its decomposition constant with N addition (Fig. 2).

Variation in decomposition responses to N addition among *Nothofagus* species was most strongly associated with the C quality of the litter. Although *Nothofagus* species differed significantly in many aspects of their litter chemistry (Table 1), including initial N content, only C fractions showed significant associations with N effects on mass loss (Table 2). Notably, N concentration did not have a significant effect on the mass loss response to N addition. In contrast, litter with high lignin content and low labile C content (soluble carbohydrates and polyphenols) experienced a larger effect on mass loss due to N addition than litter with low recalcitrant C (Table 2). Together, the magnitude of N stimulation of mass loss was most related to the C quality of the litter.

Microsite identity

Plant species identity weakly modulated the N addition effects on decomposition through their influence in the surrounding soil environment (microsite effects). Microsite identity significantly affected decomposition for *Nothofagus* litter (Fig. 1) but not for the common substrate (Fig. 2). Although decomposition in the different microsites consistently increased in response to N addition, there was no significant interaction with microsite on patterns of mass loss, either for *P. nigra* (microsite \times N addition $P = 0.15$) or for *Nothofagus* species (Fig. 1, microsite \times N addition $P = 0.38$).

Affinity and diversity effects with N addition

N addition altered species interactions that affect litter decomposition in this temperate forest ecosystem. Under nonfertilized conditions, interactions between litter and microsite resulted in faster decomposition when litter was placed in its microsite of origin (*in situ*) than in other microsites (*ex situ*, Fig. 3). It seems that individual tree species produced effects on below-ground properties that enhanced decomposition of their own litter, defining affinity effects between litter and microsite, also observed after a single year of decomposition (Vivanco & Austin, 2008). Under N-fertilized conditions; however, there were no differences between *in situ* or *ex situ* location for decomposition (Fig. 3), demonstrating that N addition disrupted affinity rela-

tionships between plant species and their environment that affected mass loss.

N addition also significantly altered leaf litter mixture effects on mass loss (Fig. 4). Under nonfertilized conditions, decomposition of mixed litter was the same as expected from the average decomposition of the component species in the mixture in all microsites (additive effects). However, under N-fertilized conditions, decomposition of mixed litter was significantly faster than was expected from the single species decomposing under N addition (nonadditive effects, Fig. 4a). The assessment of mass loss of each species within the litter mixture after 2 years revealed that *N. obliqua* decomposed faster than expected based on single-species predictions under the fertilization treatment ($P < 0.05$, Fig. 4d), whereas the other species did not show significant mass loss changes due to litter mixing (Fig. 4b and c). Decomposition constants in the plurispecific microsite did not differ from the average decomposition in monospecific microsites in either of the fertilization treatments.

N effects on litter N dynamics

N addition significantly reduced N immobilization from the decomposing litter after 1 year in the field. Under nonfertilized conditions, N remaining in litter was 118% on average across all litter types, indicating slight net N accumulation. Under N-fertilized conditions, N remaining was unchanged, at 97% on average across all litter types. In general, N dynamics was significantly correlated with litter mass loss, with a higher degree of N release for more-decomposed litter either under both fertilized ($r = 0.56$, $P < 0.0001$) and nonfertilized conditions ($r = 0.46$, $P < 0.001$).

Litter identity

N addition effects on litter N dynamics varied significantly among *Nothofagus* species (N addition \times litter type, $P < 0.05$, Fig. 5). *N. nervosa* and *N. obliqua* litter showed a clear switch from N accumulation to N release under N fertilization in all microsites, whereas *N. dombeyi* litter did not reveal significant changes in its litter N dynamics with N addition (Fig. 5). N addition also significantly stimulated N release from *P. nigra* litter (Fig. 5). In general, litter N dynamics for the same substrate showed lower variability in N-fertilized plots than control plots.

Litter N dynamics of the *Nothofagus* species was associated with their litter quality, and the direction and strength of that association depended upon the N addition treatment. Under nonfertilized conditions, *Nothofagus* litter tended to accumulate N (Fig. 5) and litter N dynamics did not show a significant relation-

Table 2 Correlation coefficients between litter chemistry and litter decomposition and with litter nitrogen (N) dynamics

Litter chemistry	Litter decomposition (k , yr^{-1})			Litter N dynamics (N remaining %)	
	N (-)	N (+)	N effect [†]	N (-)	N (+)
<i>Carbon fraction</i>					
Total carbon	-0.37	-0.31	0.28	0.16	0.46
Soluble carbohydrates	0.64*	-0.11	-0.68*	-0.43	0.85***
Soluble polyphenols	0.73*	-0.02	-0.72*	-0.47	0.71*
Lignin	-0.50	0.19	0.58*	0.36	-0.93***
<i>Nutrient content</i>					
N	-0.47	0.20	0.56	0.34	-0.93***
Ca	0.14	0.32	0.03	-0.02	-0.69***
K	0.05	0.32	0.12	0.04	-0.76***
Mg	0.02	-0.31	-0.18	-0.08	0.81***
Mn	0.48	-0.20	-0.56	-0.34	0.93***
Zn	-0.34	0.25	0.46	0.27	-0.93***
Fe	0.23	0.32	-0.05	-0.07	-0.61*
Cu	-0.53	0.17	0.60*	0.38	-0.92***
C:N ratio	0.39	-0.23	-0.50	-0.30	0.94***
Lignin:N ratio	0.38	-0.23	-0.49	0.94***	0.38

Correlation analysis was performed with different datasets: N (-), nonfertilized conditions ($n = 12$); N (+), N-fertilized conditions ($n = 12$). Significant correlation coefficients are presented in bold.

* $P < 0.05$, *** $P < 0.001$.

[†]N effect, see 'Materials and methods' for details.

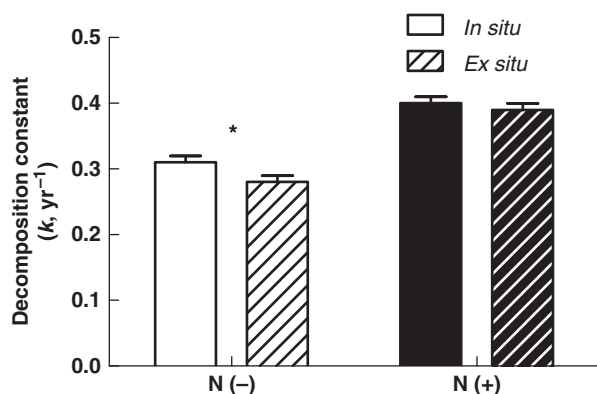


Fig. 3 Nitrogen (N) addition and plant–soil interactions effects on decomposition. Leaf litter decomposition constants *in situ* (litter and microsite from the same species) and *ex situ* (litter and microsite from different species) under control and N-fertilized conditions. Bars are means for the monospecific treatments (*in situ*, $n = 5$; *ex situ*, $n = 15$); 1 SE is presented (* $P < 0.05$).

ship with any of the litter quality parameters measured (Table 2). In contrast, under N-fertilized conditions, *Nothofagus* litter tended to release N (Fig. 5) and both C quality and nutrient content of the litter were significantly associated with litter N dynamics (Table 2). For example, litter with higher content of recalcitrant C (lignin) and less soluble C content (soluble carbohydrates and polyphenols) showed more N release from

the litter (less N remaining). Higher concentration of nutrients such as N, Ca, K, Zn, Fe and Cu also favored N release from the litter under N-fertilized conditions (Table 2). Collectively, litter with high C:N and lignin:N ratios tended to release less N (more N remaining) under N-fertilized conditions. Together, these results suggest that N release occurred when both litter N and soil N availability (through N-addition) were high.

Microsite effects

The effects of N addition on litter N dynamics significantly varied among microsites. For example, N addition affected N dynamics differently in *P. nigra* litter across microsites (microsite \times N addition $P = 0.02$). There was a 48% difference in N accumulation in *P. nigra* between N addition treatments in *N. nervosa* microsites, whereas there was only 27% difference in *N. obliqua* microsites (Fig. 5).

Discussion

Here we show that N addition had strong and positive effects on litter decomposition in an unpolluted temperate forest in Patagonia. We demonstrate that plant species composition can modulate N addition effects on decomposition directly through litter and indirectly through plants effects on their immediate environment. Also, we show for the first time that increased avail-

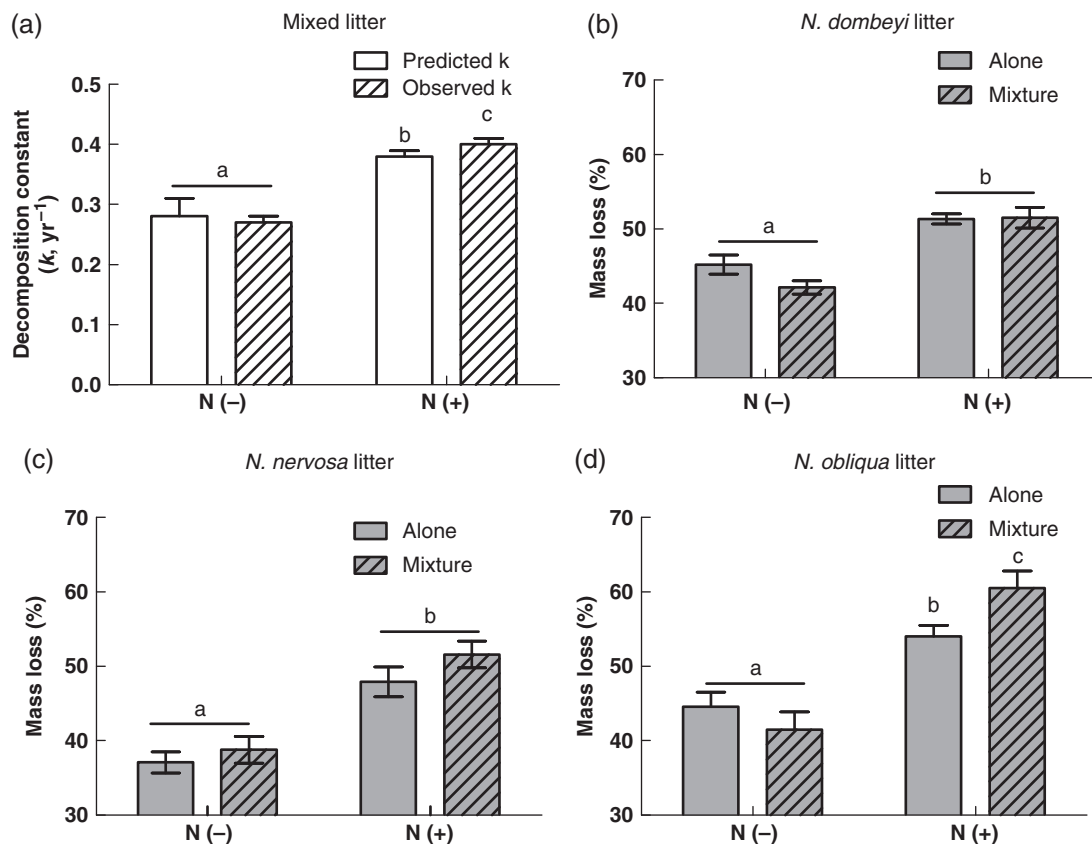


Fig. 4 Nitrogen (N) addition and litter mixture effects on decomposition. (a) Predicted and observed decomposition constants of the litter mixture of three *Nothofagus* species (mixed litter) under control N (-) and N-fertilized conditions N (+) over 2 years average across all microsites (triangles). Predicted values are the average decomposition constant of each single *Nothofagus* species decomposing alone. Litter mass loss of (b) *N. dombeyi*, (c) *N. nervosa* and (d) *N. obliqua* decomposing alone or within the mixture after 2 years of incubation under nonfertilized conditions and N-fertilized conditions. Bars are means across all control microsites ($n = 20$) and N-fertilized microsites ($n = 20$); 1 SE is presented. Different letters indicate significant differences among treatments.

ability of N disrupts the effects of two types of species interactions on decomposition. One type of interaction, the affinity relation between litter and decomposers, that enhanced decomposition of home litter ('home-field advantage'), was eliminated with N fertilization. Second, N addition modified litter species interactions transforming additive effects to nonadditive effects of litter mixtures on mass loss. Finally, N addition stimulated N release from decomposing litter which was also modulated by plant species effects through their litter and the microenvironment they create.

N effects on litter decomposition

The significant and decisive stimulation of decomposition with N addition supports the idea that N is a limiting resource for decomposers in this temperate Patagonian forest. Similar stimulation of rates of litter mass loss (17–18% change in litter mass remaining with N addition) were reported in studies with intermediate

N additions ($75\text{--}125 \text{ N kg ha}^{-1} \text{ yr}^{-1}$), areas under low N deposition ($<5 \text{ kg ha}^{-1} \text{ yr}^{-1}$), and incubation periods equal or shorter than 2 years (Knorr *et al.*, 2005). At the same time, N stimulation of litter decomposition observed in this Patagonian forest contrasted with neutral or inhibitory effects reported in another meta-analysis for temperate forests in the Northern Hemisphere (Janssens *et al.*, 2010), which include data from 20 N-fertilization experiments that differed in their duration time, fertilizer types use, trees species composition, stand age, background levels of N deposition. This range of responses to N deposition represents an important challenge to our understanding of the effects of increased atmospheric N deposition in terrestrial ecosystems.

Difference in responses to N addition on litter decomposition may be due to N legacy effects, site differences or biotic differences in soil and plant communities. In forests of the Northern Hemisphere with a long history of relatively high N atmospheric deposition

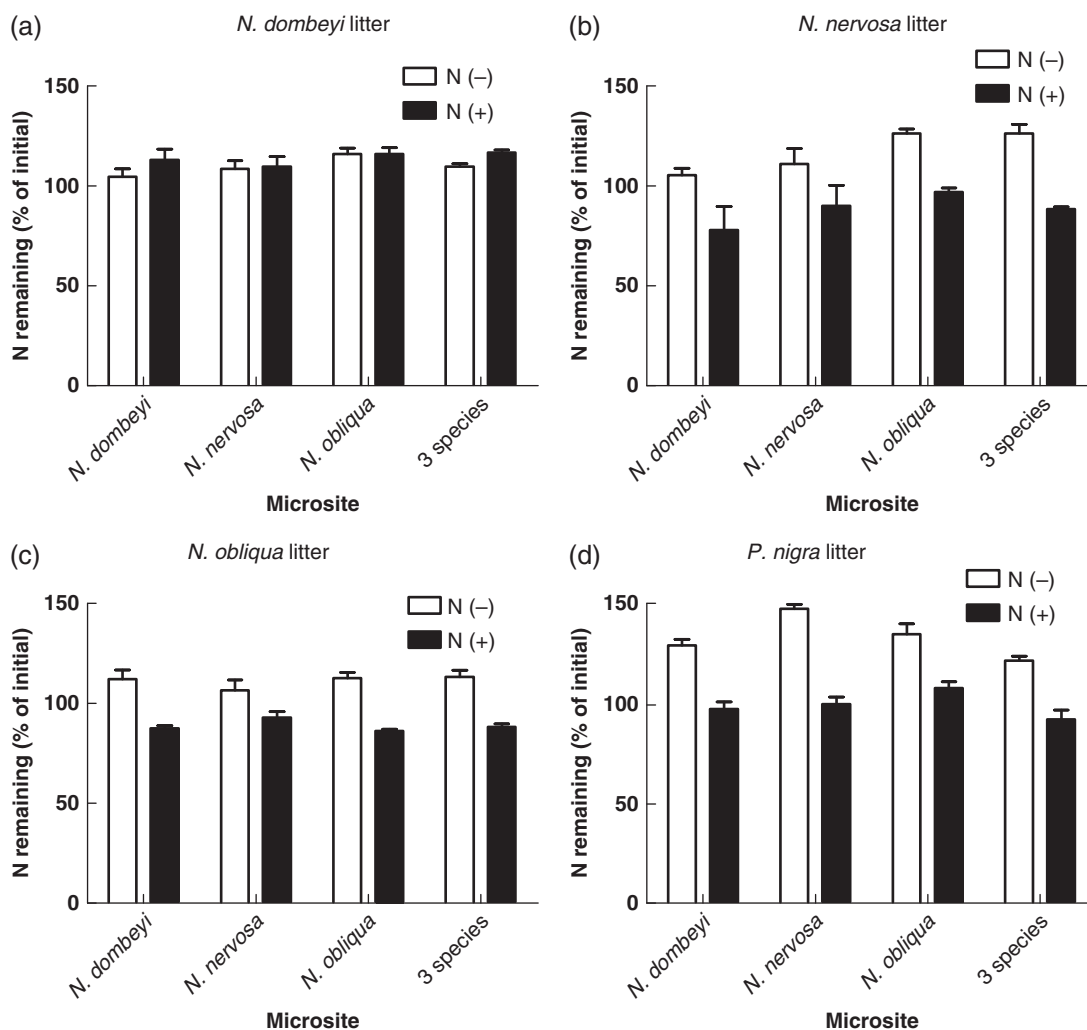


Fig. 5 Nitrogen (N) addition and plant species effects on litter N dynamics. Percent nitrogen remaining in leaf litter under control N (-) and N- fertilized N (+) conditions, across microsites dominated by different *Nothofagus* species after 1 year in the field. (a) *N. dombeyi* litter, (b) *N. nervosa* litter, (c) *N. obliqua* litter, (d) *Populus nigra* litter. Bars are means for each leaf litter type ($n = 5$); 1 SE is presented. Percent N remaining differed significantly between N addition treatments, litter species and microsites ($P < 0.001$, $P < 0.001$, $P < 0.01$, respectively). The interaction litter species \times N addition treatment was significant ($P < 0.001$) and litter species \times microsite was marginally significant ($P = 0.053$).

(10–20 kg ha⁻¹ yr⁻¹), it is possible that biogeochemical conditions at the onset of the experiment (in the non-fertilized plots) were fundamentally different than in this unpolluted Patagonian forest. Forests exposed to chronic elevated atmospheric N deposition might differ in total soil N availability, microbial community structure and microbial N requirements or different litter chemistry (Aber *et al.*, 1998). Additionally, N addition has been shown to change microbial community composition and decrease microbial diversity (Allison *et al.*, 2007) that could lead to a decreased decomposition rate due to the loss of certain decomposer guilds. Finally, C-use efficiency can vary with N availability (Agren *et al.*, 2001; Manzoni *et al.*,

2008), which could explain why litter decomposition in forests with higher soil N background availability might respond less to N fertilization than in forests with lower N availability (Hobbie, 2005). Taken together, our results demonstrate a consistent and clear response to N addition which may be more representative of undisturbed forests which at present are not experiencing high levels of N deposition from human activities.

Plant species effects: litter identity and N addition on decomposition

Plant species identity was an important regulator of N stimulation effects on mass loss through litter quality

effects. In this forest, as in most temperate forests, litter with more labile C compounds decomposed much faster than litter with more recalcitrant characteristics (Vivanco & Austin, 2008). At the same time and in contrast with other ecosystems (Fog, 1988; Knorr *et al.*, 2005), litter with a higher proportion of recalcitrant C (more lignin, less soluble polyphenols and carbohydrates) demonstrated a larger stimulation of mass loss (N addition effect) when compared with litter with low lignin content. Lignin content was positively correlated with the N addition effect, while soluble carbohydrates and soluble polyphenols were strongly negatively correlated (Table 2). N addition could have increased lignin-degrading enzyme production stimulating mass loss of recalcitrant C compounds but this possibility contradicts the few studies evaluating N effects on lignin degradation that have demonstrated no effect (Hobbie, 2008; Sinsabaugh *et al.*, 2008; Allison *et al.*, 2009) or inhibition (Carreiro *et al.*, 2000; Sinsabaugh *et al.*, 2002) of N fertilization on lignin-degrading enzyme in temperate ecosystems in North America. While it is not clear what are the mechanisms behind this litter quality relationship with N addition, this study highlights the range of responses in litter decomposition to N addition that are largely driven by species identity.

Diversity effects: species interactions and N addition on decomposition

N addition altered species interactions that affect litter decomposition. Long-term plant species and soil interactions in this temperate forest promoted affinity relationships that resulted in patches where litter decomposed faster in microsites from its own species than in other areas within the same forest (Vivanco & Austin, 2008). This home-field advantage for litter decomposition has been recently identified in a number of forests around the world (Ayres *et al.*, 2009), and has been suggested, but not demonstrated, that the specialization of the decomposer community to break down litter that is encountered more frequently could be the mechanism to explain the home-field advantage (Gholz *et al.*, 2000; Vivanco & Austin, 2008; Ayres *et al.*, 2009). Interestingly, N addition disrupted these affinity relationships between the litter and the decomposer community, eliminating the home field advantage for litter decay (Fig. 3). The advantage in specializing in a particular litter type or the capacity to degrade particular compounds in the litter might have been diluted by the higher N availability, thus eliminating the advantage. It is possible that unprecedented biogeochemical conditions created by N addition favored a different group of decomposers that were not specialized for a particular litter type, and responded

directly to differences in litter chemistry and environmental conditions.

N addition also altered litter interactions that occurred within litter mixtures. Interestingly, additive effects (where the rate is equal to the average of each species alone) of litter mixtures on decomposition transformed into synergistic effects (where the rate is enhanced in mixture when compared with each species alone) under N-fertilized conditions. Synergistic effects of litter mixtures were mainly driven by the increased mass loss of *N. obliqua* when it was exposed both to N fertilization and the presence of two other litter species. Although the mechanisms involved in litter species interactions have not been elucidated (Gartner & Cardon, 2004), our results demonstrate two important insights: (1) N availability alters litter species interactions; (2) individual litter species responses can lead to non-additive responses in litter mixture experiments as opposed to unidirectional change of all species within the mixture. To our knowledge, there are no other reports of litter mixture experiments with N addition, but studies aimed at the effects of plant species diversity and N addition have also shown synergistic effects on soil respiration (Madritch & Hunter, 2003), microbial composition and enzyme production (Chung *et al.*, 2007), but with weak effects on litter decomposition (Knops *et al.*, 2007).

Individual species responses within litter mixtures have been rarely assessed given the difficulty in identifying species within a decomposing litter mixture after a period of time. However, these few studies have shown that, within a litter mixture, individual species can respond differently to the presence of others. For example, while one litter showed an increased mass loss due to litter mixing, other species within the same mixture can be indifferent or decrease mass loss due to litter mixing (Hättenschwiler & Gasser, 2005; Chapman & Koch, 2007). It is interesting to note that this change in decomposition pattern in our study occurred only when there was an increase in N addition, while nonfertilized conditions demonstrated simple additive effects (Fig. 5, Vivanco & Austin, 2008). It is possible that once proximate N limitation was overcome, other limitations constrained mass loss, and synergistic effects were observed. For example, it is possible that soluble carbohydrates and polyphenols from *N. dombeyi*, that were positive correlated with decomposition (Table 2), stimulated *N. obliqua* and *N. nervosa* breakdown in litter mixtures once N availability was increased.

N effects on litter N dynamics

The stimulation of N release in decaying litter with N addition gives additional support to the idea that N

availability limits C and nutrient dynamics in this temperate Patagonian forest. Stoichiometric theory predicts that net mineralization of any element relates directly to the extent of availability of that element to the decomposer organisms (Swift *et al.*, 1979). In the circumstances of N limitation, net N accumulation tends to prevail, as observed under nonfertilized conditions (Fig. 5). Similarly, net N release occurs where N is at nonlimiting availability, which could have been represented by N-fertilized conditions (Fig. 5). At a global scale, though, patterns of litter N dynamics suggest that decomposers can degrade litter with low initial N by reducing their C-use efficiency [amount of new biomass C produced per unit organic resource C consumed (Manzoni *et al.*, 2008)]. This strategy is expressed in trends of decreasing critical litter C:N ratio (C:N ratio below which net N release occurs) with increases in initial litter C:N ratios (Manzoni *et al.*, 2010). In our study, C:N ratio of N-releasing and N-accumulating litter did not differ significantly (44 and 47, respectively), suggesting that at local scale, litter initial N is not the only controlling factor of N dynamics, in contrast to observed global patterns (Parton *et al.*, 2007). Rather, both litter initial N and inorganic N appear to determine litter N dynamics (Table 2). Given the tight association between mass loss and litter N dynamics, it is possible that the postulated changes in the microbial community to explain differences in decomposition due to N addition could also be involved in litter N dynamics, although this remains to be demonstrated.

Conclusions

One of the current scientific challenges is to understand the factors that control the C balance in order to predict the potential for C storage in terrestrial ecosystems. Given current scenarios of co-occurring changes in N deposition and biodiversity, it is imperative to understand their interactive effects on decomposition. Increases in N availability due to N deposition have stimulated forest growth (Magnani *et al.*, 2007; Thomas *et al.*, 2010) and decreased organic matter decomposition in temperate forests of industrialized areas of the Northern Hemisphere (Janssens *et al.*, 2010). However, unpolluted temperate forests seem to respond differently to N addition. Our results demonstrate that increases in N availability promote C loss from the forest floor in a Patagonian forest. In addition, N addition leads to a functionally homogeneous system due to the disruption of long-term plant–soil interactions that modulate litter decomposition and contribute to the functional diversity of ecosystem processes. Together, our results suggest that predictions of N deposition

effects on C storage are not wholly generalizable and that regional variations must be taken into account in order to accurately assess the C balance in terrestrial ecosystems.

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Supporting Information

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

Figure S1. Mass loss of four leaf litter types under control and N-fertilized conditions in an old-growth forest in Patagonia. Substrates are leaves from three native *Nothofagus* species (a) *N. dombeyi*, (b) *N. nervosa*, (c) *N. obliqua*; (d) a mixture of the three species in equal proportion; and (e) leaf litter from *Populus nigra*, a non-native species. Values are means for each leaf litter type across all microsites ($n = 20$); one standard error is presented.

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