

Tree–grass interactions for N in *Nothofagus antarctica* silvopastoral systems: evidence of facilitation from trees to underneath grasses

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Received: 20 September 2013 / Accepted: 14 June 2014 / Published online: 27 June 2014
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Abstract *Nothofagus antarctica* forests in south Patagonia are usually used as silvopastoral systems but how grasses and trees compete for specific resources, such as nitrogen in these systems is unknown. To understand interactions between grasses and *N. antarctica* trees for N, an experiment with ^{15}N labeled fertilizer was carried out comparing N absorption by grasses growing under trees (silvopastoral system) with an open site. Labeled $^{15}\text{NH}_4^{15}\text{NO}_3$ fertilizer at 10 % atom excess was added in spring at both sites and ^{15}N was measured in herbage, soil and trees every 30 days during the growing season. Soil was the component that containing the greatest amount of N and greatest ^{15}N recovery. Grasses growing in the silvopastoral system absorbed almost double of the fertilizer applied than grasses in the open site (32.4 kg N ha $^{-1}$ derived from fertilizer based on ^{15}N recovery). Roots were also an important fate for N

absorbed, representing 50 and 63 % of total ^{15}N recovered in grass roots of open and silvopastoral sites, respectively. Trees absorbed 69 % less applied N than grasses in the silvopastoral system; being mainly allocated in small branches, sapwood and fine roots. Overall, ^{15}N recovery was 65 % higher in the silvopastoral system (tree + grasses) than in the open site (grasses). Silvopastoral system made more efficient use of the ^{15}N added. These results indicated that *N. antarctica* trees in the silvopastoral system may “facilitate” fertilizer N absorption of grasses by improving environmental conditions like water availability or by reducing competition for inorganic N between soil microorganisms and plants.

Keywords Understory · Nutrients · Native forest

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Introduction

Nothofagus antarctica is a native species of Patagonian forest that extends from 46° to 56° South Latitude in South of Argentine and Chile. It grows on a variety of sites reaching heights up to 20 m on the most suitable sites (Donoso et al. 2006) and on poorly drained or drier sites near Patagonian steppe as a shrubby 2–3 m tall tree (Veblen et al. 1996). In south Patagonia (Santa Cruz Province) *N. antarctica* forest mainly develop on sites of low quality and mature trees are scarcely used for wood extraction, being their

main use as silvopastoral system, where natural grasses grown under the tree canopy are grazed by cattle and sheep (Peri et al. 2005).

In several ecosystems trees coexist with grasses (like agroforestry systems or savannas) and there is evidence that positive (facilitation) and negative (competition) interactions occur between woody and nonwoody (herbaceous, understory) components. For example, in savannas it has been reported that trees and grasses compete for light, nutrients and water, but trees can increase soil fertility, microbial activity or improve soil structure, and also soil water availability by reducing water loss from evapotranspiration in shade (i.e. Belsky et al. 1993; Scholes and Archer 1997; Archer et al. 2001; Ludwig et al. 2001; Mordelet and Le Roux 2006; Simmons et al. 2008). Facilitation takes place when one species enhances the survival or growth of another (Callaway 1997). These positive mechanisms may act simultaneously with competitive mechanisms, and the overall effect of one plant species on another depends on which mechanisms are the most important in a given environment (Callaway and Walker 1997, Holmgren et al. 1997). Likewise, some authors reported that the importance of the facilitation process in plant communities increases with increasing abiotic stress, whereas competition becomes more important when environment stress are absent (Bertness and Callaway 1994; Callaway et al. 2002).

Little is known about the mechanics of tree-grass interactions in *N. antarctica* forests in South Argentina, where natural low density of mature forests allows to grow grasses, or thinned young forests being used as silvopastoral systems. Peri et al. (2005) reported a first approach of these interactions where in *N. antarctica* sites with better water balance and soil condition, grass productivity was lower under trees compared with open areas whereas in drier sites, the opposite pattern was observed. This agree with Callaway et al. (2002) on the idea of facilitation increasing with increasing abiotic stress, being water possibly the main limiting factor in these *N. antarctica* forests. Furthermore it is recognized that nitrogen (N) is another important limiting resource in *N. antarctica* forests (Diehl et al. 2003; Bertiller and Mazzarino 2006), but the dynamics of this nutrient in these complex tree-grass systems is unknown. The utilization of labelled fertilizer enables the evaluation of N transformations in soils, N routes and N

allocation patterns among different components (e.g. trees, grasses, microorganisms) (Cheng and Bledsoe 2004; Soethe et al. 2006; Rimski-Korsakov et al. 2012). There are few studies involving ^{15}N in silvopastoral systems (Goh et al. 1996; Buchmann et al. 1996; Rowe et al. 2001), and no antecedents in Patagonian. For this, we performed a field experiment using ^{15}N isotopes to compare grass performance and N absorption in an area where trees and grasses coexist (silvopastoral system) compared with grass in adjacent open grassland. This kind of information would improve the understanding of N dynamics in these complex systems and determine if trees facilitate grass grow or, in contrast, grass and trees compete for N. This knowledge also could help to design sustainable managing practices for Patagonian *N. antarctica* silvopastoral systems, since some authors postulate that silvopastoral systems can be designed to optimize the use of spatial, temporal and physical resources by maximizing positive interactions and minimizing negative interactions between components (Jose et al. 2004). In this context, the aims of this work were: (1) to evaluate N dynamics among *N. antarctica* silvopastoral components (trees, grasses and soil) and (2) to detect if *N. antarctica* trees and grasses compete for N, or if presence of trees “facilitates” grass N absorption. We hypothesized that in the investigated *N. antarctica* silvopastoral system facilitation from trees to grasses predominates, allowing underneath grasses to a higher absorption of the N added compared with grasses growing in an open grassland.

Methods

Study site

The study was carried out in a naturally regenerated *N. antarctica* young forest (66 ± 7 years old) with an original density of 4,750 trees ha^{-1} , 9 cm of diameter at breast height (DBH) and a total mean height of 5.8 m. Trees grew accompanied with a natural understory layer (silvopastoral system) of 80–100 % vegetation cover being grasses the predominant group, where *Agrostis capillaris*, *Festuca magellanica*, *Deschampsia flexuosa* and *Dactylis glomerata* were the most abundant species. To compare grasses growing without trees, an adjacent grassland was selected (open site) with a vegetation cover of 95 % mainly

constituted by grasses like *A. capillaris*, *Festuca gracillima*, *F. magellanica*, and *Carex sp.* This grassland was located just besides to the forest limit. Both sites were located in Cancha Carrera ranch (51°13'21" SL, 72°15'34" WL) in SW of Santa Cruz province, Argentina. The climate is cold temperate with 6 °C of mean annual temperature and 563 mm of mean annual rainfall.

Experimental design and Nitrogen applications

In late winter of 2007 two plots of 25 m² (5 × 5 m) were established in the silvopastoral system and four trees inside the plot were selected covering different crown classes (1 dominant, 1 codominant, 1 intermediate and 1 suppressed). The remainder trees in the plot and those located immediately next to the plots was harvested (6–7 trees in total). Likewise, to avoid N absorption of trees from outside and also avoid fertilizer lateral movement, plots were isolated with a polyethylene barrier up to a depth of 1 m. To evaluate grasses, 1.8 m² grazing exclosures (n = 3) within each plot were located under tree crowns and between tree crowns (n = 3) to obtain different light regimes. In open grassland three exclosures of the same characteristics were located and also protected with a polyethylene barrier to a depth of 0.8 m.

Before fertilizer application grasses in plots were clipped to 2 cm to homogenize the pasture and soil samples were taken to characterize initial conditions in silvopastoral system and open site. Bulked soil samples (n = 15) were taken at random every 20 cm layer to a final depth of 70 cm, where a clay layer impeded roots growth. Samples were sent to laboratory for texture, pH, total N, nitrate content, and organic carbon were determination.

¹⁵N labeled fertilizer was applied over the whole plots at rates of 103 and 130 kg N ha⁻¹ for open site and silvopastoral system, respectively. The silvopastoral received more fertilizer because of the expected additional tree N uptake. In spring (early November 2007), isotopically labeled N was applied as ¹⁵NH₄¹⁵NO₃ solution at 10 at.% diluted. Fertilizer was diluted in deionized water and it was applied by hand with a watering can evenly distributed all over the plots. After application, plots were watered with deionized water simulating a 1 mm rainfall to facilitate ¹⁵N incorporation into the soil.

Measurements and ¹⁵N recovery

Herbage in exclosures was harvested to 1 cm at 30, 60, 90, 120 and 150 days after fertilizer application using six 0.1 m² quadrants (three per plot) to estimate aerial biomass. Clipped herbage was dried at 55 °C to constant weight, weighed and then three 5 g subsamples per quadrant were ground in a mill containing 1 mm stainless steel screen for chemical analysis. At every harvest date, also three 50 g samples of grass roots at 0–30 cm depth were taken with a 250 ml cylinder, washed with deionized water, dried at 55 °C to constant weight and three 5 g subsamples were ground in a mill for chemical ¹⁵N analysis. Total grass root biomass was determined at the last harvest date where all roots from the 0.1 m² area were collected at 0–30 cm depth, washed with deionized water, dried at 55 °C to constant weight and weighed.

Coinciding with each grass harvest date, 150 g of new full expanded tree leaves and small branches (<1 cm) were taken from the top and middle of the crown of every tree in the plots. These samples were dried at 55 °C to constant weight, weighed and ground for ¹⁵N chemical analysis. Samples from different dates were averaged to get the mean values. All components were weighed in fresh and five subsamples of 150 g were taken for biomass estimations. These sub-samples were dried at 55 °C to constant weight, weighed and separated for ¹⁵N chemical analysis. At the end of the growing period (150 days after fertilizer applications, in May) all trees (n = 8) were harvested and separated in components: leaves, small branches, trunk and roots. All components were weighed in fresh and five sub-samples of 150 g were taken for biomass estimations. These sub-samples were dried at 55 °C to constant weight, weighed and separated for ¹⁵N chemical analysis.

Three soil samples, composed of sub samples, were taken from silvopastoral system and open site at 30, 60, 90 120 and 150 days after fertilizer application at 0–20 cm depth in each treatment. At the last date, also three compound samples were taken at 20–40, 40–60 and 60–70 cm to describe ¹⁵N movement through the soil profile.

All grass and tree samples were analyzed for total N and ¹⁵N abundance at CATNAS Laboratory (Montevideo, Uruguay). Total N was determined by Kjeldhal (Axmann et al. 1990) and ¹⁵N abundance in plant components was determined by emission spectrometry

with NOI-6EPC (Fischer Analysen Instrumente, GMBH, Alemania, 1988). Soil N and ^{15}N were determined by mass spectrometry with a continuous-flow IRMS system coupled to an elemental analyzer FLAS EA 1112 (Milan, Italy) and ConFlo III (Finnigan MAT, Bremen, Germany, 2001).

^{15}N enrichment (\hat{a}) was calculated by subtracting ^{15}N natural abundance ($a = 0.369\%$) reported for soil and *N. antarctica* leaves for this site from the total ^{15}N of each organ. For grasses, the atmospheric constant value was used ($a = 0.3666\%$) since there are no data about its natural abundance available. Standard calculations were:

N derived from fertilizer (Ndf) (%)

$$= \left[\frac{^{15}\text{N} \% \text{ atom. exc. in the plant organ}}{^{15}\text{N} \% \text{ atom. exc. in the fertilizer}} \right] \times 100$$

N yield (g) = dry matter yield (g) \times % N/100

N fertilizer yield (NFY) (g) = N yield (g) \times Ndf.

Grass total N yield (kg N ha^{-1}) was determined by multiplication of N concentration and biomass production ($\text{kg dry matter ha}^{-1}$). For silvopastoral systems grass N yield was estimated by weighing the area of herbage growing under trees and the area between trees. Tree N yield ha^{-1} was estimated by multiplication of individual N values by tree density and respecting natural field crown class proportions. For N soil contents, N concentrations were obtained by multiplication of N concentrations by soil bulk densities at every depth.

Data analysis

Biomass, N concentrations, N amount and also N derived from fertilizer data were analysed with means and standard deviations to show general patterns in this ecosystem. Inferential statistics were not applied since pseudo-replicates were used, thus the “ecosystem level” were not replicated (silvopastoral system and open grassland). We believe that it was important to do this study with tall trees and natural grasses growing in field conditions, but this kind of study, as many others in ecology, are difficult and costly to replicate (Carpenter 1990). Our study approach agrees with Cottenie and De Meester (2003) who postulated that large scale experiments may for certain questions be essential in order to maximize the applicability to

the natural situation. In this sense, when practical considerations make replicated experiments difficult to obtain, the resulting experiment can still be used as a check of existing theory on the particular system (Cottenie and De Meester 2003).

Results

Soil

The analysis of soils before N application showed that silvopastoral system had lower organic carbon and total nitrogen values in shallow layers (0–20 and 20–40 cm depth), but higher nitrate contents and carbon/nitrogen ratios compared with open site (Table 1). Soil N concentration at 0–20 cm depth was higher in the open site (Fig. 1a) but nitrogen derived from fertilizer (Ndf) always was higher in silvopastoral system (Fig. 1b). N concentration decreased with depth being higher in the open site higher (Fig. 2a) and ^{15}N atom excess also decreased with depth, however, at 70 cm no ^{15}N excess was detected (Fig. 2b). Ndf found in soil varied between 0.11 and 1.09 %, depending on date and site (data not shown). In general, at 20 cm depth the gradient under trees > between trees > open grasses was found for % Ndf.

Grasses

Grasses in the open site always had higher biomass amounts than grasses growing in the silvopastoral system. The peak of aerial biomass accumulation occurred 90 days after fertilizer application (February) and cumulative yield varied from 1,953 kg DM ha^{-1} for grasses growing under tree canopy to 3,165 kg DM ha^{-1} for grasses growing at the open site (Table 2). Roots accounted for an important fraction of total grass biomass and also increased with light incidence. Grasses in the open site accounted with 15,136 kg DM roots ha^{-1} with a root/shoot ratio of 4.8, whereas grasses growing in silvopastoral system yielded 5,269 and 6,433 kg DM roots ha^{-1} , under and between tree canopy, respectively, with a root/shoot ratio of 2.7 and 2.9, respectively (Table 2).

Total N concentration in grasses varied according to site, date and plant component (Table 3). Aerial components always had higher N concentrations than

Table 1 Initial soil characteristics from *Nothofagus antarctica* silvopastoral system (SP) and an open site dominated by grasses without trees in South Patagonia, Argentine

Depth (cm)	0–20		20–40		40–60		60–70	
	SP system	Open site	SP system	Open site	SP system	Open site	SP system	Open site
Clay (%)	8.0	16.5	8.3	18.5	14.5	22.0	12.5	16.0
Silt (%)	36.2	53.6	35.9	41.3	24.8	34.1	21.2	31.1
Sand (%)	55.8	29.9	55.8	40.2	60.7	43.9	66.3	52.9
Bulk density (g cm ⁻³)	0.51	0.4	0.75	0.93	0.93	1.4	1.4	1.5
pH	4.9	5.3	4.9	5.2	4.7	5.3	4.9	5.3
Organic carbon (%)	5.6	6.6	3.5	5.5	2.6	3.0	0.7	0.4
Total nitrogen (%)	0.6	1.2	0.6	0.6	0.2	0.3	0.05	0.04
NO ₃ -N (mg kg ⁻¹)	4.6	2.9	4.7	2.1	3.9	1.7	6.1	1.2
C/N ratio	9.3	5.5	11.7	9.2	13.0	10	14	10

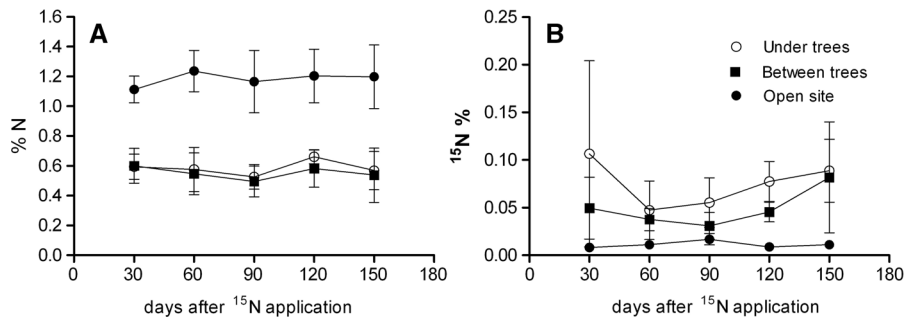


Fig. 1 N total concentration (a) and ¹⁵N atom excess (b) in soil at 20 cm depth along the growing season for open grass (filled circle) and grasses growing in a *Nothofagus antarctica*

silvopastoral system under canopy trees (filled square) and between canopy (empty circle) in SW of Santa Cruz province, Argentine. Vertical bars indicate standard deviation values

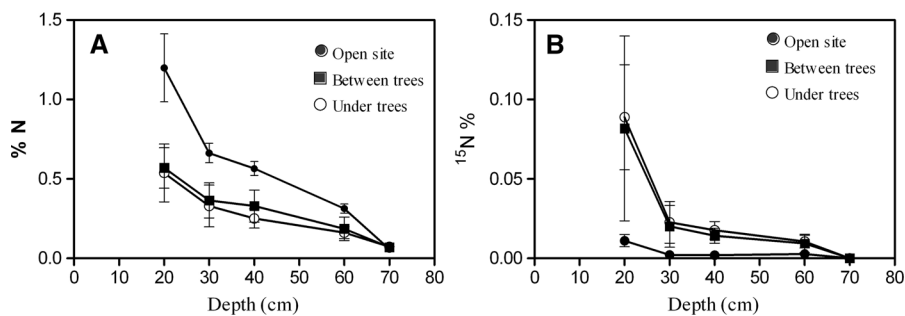


Fig. 2 N concentration (a) and ¹⁵N atom excess (b) in soil profile at 150 days after ¹⁵N fertilizer application for open grass (filled circle) and grasses growing in a *Nothofagus antarctica*

silvopastoral system under canopy trees (filled square) and between canopy (empty circle) in SW of Santa Cruz province, Argentine

roots and grasses in the open site had lower values compared with grasses in the silvopastoral system (Table 3). Aerial N concentrations varied from 2.26 %

(grasses under trees at 30 days after fertilizer application) to 0.72 % (grasses in open site at 120 days after application). Concentrations in roots varied from

0.63 to 1.06 % N with no big differences between sites (Table 3).

Recovery of the applied N varied according to sites. Grasses in the open site had lower values of N derived from fertilizer than grasses growing in the silvopastoral system. Thus, after 30 days of fertilizer application, N found in grasses (^{15}N concentrations) showed the gradient: under trees > between trees > open

grasses (Table 3). Likewise, Ndff showed values around 60–69, 47–61 and 20–31 % for grasses growing under trees, between trees and in open site, respectively (Table 4). During the growing season, grasses in silvopastoral system had always significant higher values of Ndff than grasses in open site. Roots also were a sink for N applied obtaining 33–57, 28–57 and 6–10 % of Ndff for grasses growing under trees, between trees and in open site, respectively (Table 4).

Table 2 Biomass accumulated (kg ha^{-1}) during the growing season 2007 for grasses growing under and between canopy trees in a *Nothofagus antarctica* silvopastoral system and in a near grassland (open site) in SW of Santa Cruz province, Patagonia, Argentine

	Silvopastoral system		Open site
	Under canopy	Between canopy	
Shoots (kg ha^{-1})	1,953 (± 539)	2,200 (± 208)	3,165 (± 580)
Roots (kg ha^{-1})	5,269 (± 425)	6,433 (± 971)	15,136 (± 626)
Total (kg ha^{-1})	7,222 (± 964)	8,633 (± 951)	18,301 (± 934)

In parenthesis standard deviations are presented

Table 3 Total N and ^{15}N atom excess expressed in % of aerial and roots components of grasses growing in a *Nothofagus antarctica* silvopastoral system (under canopy tree and

DAA*	Total N (%)			% ^{15}N atom excess		
	Under trees	Between trees	Open grasses	Under trees	Between trees	Open grasses
30						
Aerial	2.26 (± 0.22)	1.80 (± 0.34)	1.13 (± 0.13)	6.55 (± 0.46)	5.47 (± 0.18)	2.65 (± 0.68)
Roots	1.06 (± 0.19)	0.94 (± 0.09)	0.80 (± 0.12)	3.53 (± 0.42)	3.02 (± 0.67)	0.96 (± 0.03)
60						
Aerial	1.67 (± 0.04)	1.58 (± 0.24)	1.03 (± 0.09)	6.55 (± 0.28)	6.16 (± 0.40)	3.05 (± 0.89)
Roots	0.84 (± 0.07)	0.83 (± 0.26)	0.87 (± 0.43)	3.30 (± 0.54)	2.85 (± 0.65)	0.96 (± 0.37)
90						
Aerial	1.55 (± 0.27)	1.53 (± 0.21)	0.91 (± 0.17)	6.91 (± 0.35)	5.63 (± 0.57)	3.07 (± 0.20)
Roots	0.75 (± 0.12)	0.92 (± 0.30)	0.67 (± 0.08)	3.76 (± 0.43)	3.78 (± 0.80)	1.02 (± 0.33)
120						
Aerial	1.23 (± 0.01)	1.35 (± 0.09)	0.72 (± 0.03)	6.03 (± 0.94)	5.68 (± 0.34)	2.35 (± 0.97)
Roots	0.88 (± 0.09)	0.86 (± 0.20)	0.63 (± 0.09)	5.25 (± 0.42)	3.68 (± 0.86)	0.98 (± 0.17)
150						
Aerial	1.32 (± 0.10)	1.51 (± 0.12)	0.95 (± 0.05)	6.22 (± 0.44)	4.66 (± 0.85)	2.02 (± 0.18)
Roots	1.03 (± 0.09)	1.06 (± 0.13)	1.04 (± 0.08)	3.04 (± 0.42)	2.34 (± 0.58)	0.60 (± 0.29)

Standard deviation of the means are shown in parenthesis

DAA days after fertilizer application

Trees

Total N concentrations in *N. antarctica* leaves were 1.5 %, meanwhile those of trunk and roots were around 0.24 %. Likewise, N derived from fertilizer was higher in small branches with a value of 5 % meanwhile other components had Ndff around 3.5 % (Table 5). Total N varied from 57 to 226 g N tree $^{-1}$ for suppressed and dominant trees, respectively (Table 6). Boles were the component that accounted with the highest N amounts, with values around 41 and 169 g N tree $^{-1}$ for suppressed and dominant trees, respectively. Roots also were an important sink for total N, varying from 15.8 to 56.4 g N tree $^{-1}$, according to the crown class (Table 6).

between canopy trees) and in a near open grassland in SW of Santa Cruz province, Argentine

Table 4 Nitrogen derived from fertilizer (Ndff) expressed in % of aerial and roots components of grasses growing in a *Nothofagus antarctica* silvopastoral system (under canopy tree and between canopy trees) and in a near open grassland in SW of Santa Cruz province, Argentine

DAA*	% Ndff		
	Under trees	Between trees	Open grasses
30			
Aerial	65.5 (±4.6)	54.7 (±1.76)	26.6 (±6.80)
Roots	35.3 (±4.2)	30.2 (±6.7)	9.6 (±0.28)
60			
Aerial	65.5 (±2.8)	61.6 (±4.0)	30.5 (±8.9)
Roots	33.0 (±5.4)	28.5 (±6.5)	9.6 (±3.7)
90			
Aerial	69.1 (±3.5)	56.3 (±5.7)	30.6 (±2.0)
Roots	37.6 (±4.3)	27.8 (±8.0)	10.2 (±3.3)
120			
Aerial	60.3 (±9.4)	56.8 (±3.4)	23.5 (±6.7)
Roots	52.5 (±4.2)	36.8 (±8.6)	9.8 (±1.7)
150			
Aerial	62.1 (±4.4)	46.6 (±8.5)	20.2 (±1.76)
Roots	30.4 (±8.9)	23.4 (±5.8)	6.0 (±2.9)

Standard deviation of the means are shown in parenthesis
DAA days after fertilizer application

Table 5 Mean N, ^{15}N atom excess concentrations (%) and Nitrogen derived from fertilizer (% Ndff) for *N. antarctica* components collected 150 days after ^{15}N fertilizer application in a silvopastoral system in SW Santa Cruz province, Argentine

Component	% N	% ^{15}N átom. excess	% Ndff
Leaves	1.52 (±0.52)	0.34 (±0.17)	3.4 (±1.7)
Small branches	0.75 (±0.20)	0.51 (±0.26)	5.1 (±2.6)
Trunk	0.24 (±0.06)	0.35 (±0.20)	3.6 (±2.0)
Roots	0.23 (±0.09)	0.33 (±0.24)	3.3 (±2.4)

In parenthesis standards errors are presented

N and ^{15}N recovery at system level

Soils accounted for the highest N amounts in the system with values of 13,656 and 30,098 kg N ha⁻¹ for silvopastoral system and open site, respectively

(Table 7). N contents also were different in depth between both systems, where open site had the highest amount at 20–40 cm (39 %) meanwhile silvopastoral system had the highest amount at 0–20 cm (43 %).

The tree-grass system had higher total N amounts than grasses in the open site (290 vs. 186 kg N ha⁻¹), partially due to the tree contribution (199 kg N ha⁻¹) (Table 7). Comparing only herbaceous components, grasses in the open site had twice as much N than grasses in the silvopastoral system and a high proportion (85.5 %) was derived from roots (Table 7).

^{15}N added was recovered in higher proportions in the silvopastoral system (Table 7). For example, while grasses in silvopastoral system recovered 32.4 kg ha⁻¹ from the applied N, grasses in the open site assimilated only 15 kg ha⁻¹ from the applied N. In the silvopastoral system an additional portion was taken up by trees (10 kg N ha⁻¹ from the applied N) although the component with highest ^{15}N retention was the soil (68 kgN ha⁻¹ from the applied N) at 0–20 cm depth. Roots were also an important fate for ^{15}N absorbed (50 and 63 % of total ^{15}N recovery was in grass roots of open site and silvopastoral sites, respectively). Tree roots also accumulated ^{15}N , but in smaller proportion than grasses (19 %). In general, adding all components, the silvopastoral system recovered almost three folds of the N added than the open site (110 vs. 38 kg N ha⁻¹ from the fertilizer applied).

Discussion

Biomass, N and ^{15}N recovery in grasses

Grasses growing in the silvopastoral system had lower biomass accumulation probably due to a lower light exposure than open grasses. This is concordant with Peri et al. (2005) who reported that in wet high fertility sites, herbaceous production under *N. antarctica* trees decreased linearly as light decreased. Furthermore, grasses growing in the open site of this study had higher allocation to roots (root/shoot ratio = 4.8) compared with grasses in the silvopastoral system. This change in allocation pattern could be associated with differences in the water regime, since grasses growing in open sites are more exposed to strong winds and consequently to drier soils than grasses growing under tree protection. This is concordant with

Table 6 Mean total N values (g tree^{-1}) for *Nothofagus antarctica* trees of different crown classes growing in a silvopastoral system in SW of Santa Cruz province, Argentine

Component (gr tree^{-1})	Dominants	Codominants	Intermediate	Suppressed
Leaves	44.6 (± 6)	22.0 (± 7)	6.7 (± 4)	11.4 (± 6)
Bole	124.6 (± 49)	79.2 (± 19)	44.3 (± 23)	29.6 (± 20)
Roots	56.4 (± 21)	33.9 (± 11)	29.2 (± 9)	15.8 (± 5)
Total	225.6 (± 75)	135.1 (± 23)	80.2 (± 7)	56.8 (± 25)

Standards errors are given in parenthesis

Table 7 Total N and ^{15}N based fertilizer N (kg ha^{-1}) in a *Nothofagus antarctica* silvopastoral system vs. grasses growing in open site in SW of Santa Cruz province, Argentine 90 days after application of labeled fertilizer

Component	Silvopastoral system		Open site	
	Total N (kg ha^{-1})	Total fertilizer N (kg ha^{-1})	Total N (kg ha^{-1})	Total fertilizer N (kg ha^{-1})
<i>Tree</i>				
Aerial	144.8 (± 23.5)	8.0 (± 0.9)	–	–
Roots	54.2 (± 6.7)	1.9 (± 0.2)	–	–
Total	199.0 (± 30.0)	9.9 (± 1.5)	–	–
<i>Grasses</i>				
Aerial	30.4 (± 9.2)	16.0 (± 4.7)	28.9 (± 2.3)	5.5 (± 1.0)
Roots	60.7 (± 7.5)	16.4 (± 1.8)	157.2 (± 15.6)	9.4 (± 3.4)
Total	91.1 (± 6.1)	32.4 (± 5.5)	186.1 (± 17.1)	14.9 (± 2.8)
<i>Soil</i>				
0–20 cm	5,873 ($\pm 1,408$)	54 (± 39)	9,502 ($\pm 1,694$)	13 (± 3)
20–40 cm	4,626 ($\pm 1,287$)	10 (± 7)	11,624 (± 287)	6 (± 1)
40–60 cm	3,157 (± 938)	4 (± 3)	8,972 (± 655)	5 (± 1)
Total	13,656 ($\pm 3,621$)	68 (± 49)	30,098 (± 883)	24 (± 3)
Total system	13,946.1	110.3	30,284.1	38.9

In parenthesis the standard deviations of the means are shown

Bahamonde et al. (2012), who reported that in sites where *N. antarctica* trees reached heights less than 8 m, water availability is the main limiting factor for grass production, and trees play a beneficial role decreasing wind speed and increasing relative humidity, creating a more favorable environment for the understory compared with open grasses. Likewise, differences in root/shoot allocation in grasses between both systems may be related with soil texture, since open grasses were growing in a soil with higher proportion of silt meanwhile soil in silvopastoral system had higher proportion of sand at 0–20 cm depth (Table 1).

Root/shoot ratios found in this work were similar to the mean value reported by Mokani et al. (2006) for cold temperate grasslands (4.5) and with those reported by Fernández et al. (2004) for *Festuca*

pallescens in North Patagonia growing under *Pinus* shade or in open sites.

Grasses growing in the silvopastoral system had higher N concentrations in the aboveground components than grasses in the open site. Several studies reported an increase in N concentration under deficient light conditions (i.e. Lin et al. 2001; Perry et al. 2009) including grasses growing under *N. antarctica* (Peri et al. 2005). Despite of differences in N concentrations, grasses in the open site accumulated larger amounts of total N (186 kg ha^{-1}) than grasses in silvopastoral systems, mainly due to differences in biomass. Because grasses in the silvopastoral system allocated more N to aerial components, no differences in total aerial N amounts were found between both sites ($30 \text{ vs. } 28.9 \text{ kg N ha}^{-1}$). This compensation may be partially explained by the tree shade that had stimulated a higher allocation to

photosynthetic organs to acquire the limiting factor light, and because these grasses had less water stress than grasses in open site, exposed to the strong winds (Bloom et al. 1985; McCarthy and Enquist 2007; Bahamonde et al. 2012). In concordance with this, Treydte et al. (2008) found that grasses growing under trees had lower water stress and that increased their water use efficiency in shade. In grasses, labeled N was preferentially allocated to the aerial components, where grasses growing in silvopastoral system accounted with 60–70 % of Ndff. These values are concordant with Aanderud and Bledsoe (2009) who reported values of 73.5 and 51.4 % Ndff in *Bromus diandrus* according to fertilizer type (ammonium or nitrate) and with Logan and Thomas (1999) with recovery values around 42–74 % in aerial ryegrass depending on soil type. In contrast, grasses growing in the open site absorbed much less of the ^{15}N added (21–35 %). Several aspects may explain this. First, it is possible that differences in mineralization-immobilization process could exist between both sites. Kaye and Hart (1997) concluded that microorganism and plant compete actively for inorganic N and the occurrence of mineralization or immobilization will depend on N availability in the soil and in the decomposing litterfall. Decomposing litterfall with high N concentration may promote net N mineralization from microorganisms, whereas with low N concentration, N immobilization may predominate since microorganisms acquire N for their own structures (Kaye and Hart 1997). In our study, litterfall from silvopastoral system would have higher quality, since trees contribute annually with leaves and small branches which have N concentrations higher than grasses (Peri et al. 2006, 2008) and also grasses growing in this system had higher N concentrations, in consequence, net N mineralization process could predominate. Bahamonde et al. (2013) comparing two class sites in *N. antarctica* reported that in the best site net N mineralization was higher in the silvopastoral system than in the open site without trees. In agreement with this, we suspect that in the open site of our study the N immobilization process may predominate, reducing N available for grasses. Furthermore, it is important to note that differences in soil texture between systems may have influenced grass N absorption capacity. The finer soil texture of the open site could retain water in micro pores making it not available for plant, and in consequence, reduce N absorption, since N acquisition is closely related with water absorption. (Hsiao 1973; Lawlor and Cornic 2002).

With regards to the Ndff of roots found in this study for open site grasses (6–10 %) they were similar to those reported for others grasses (Logan and Thomas 1999; Partala et al. 2001; Cheng et al. 2004; Aanderud and Bledsoe 2009). However, Ndff values in grasses growing in silvopastoral systems were quite higher (23–50 %).

N in trees

Ndff values found in trees were inferior to grasses, with a maximum value around 5 %. This is concordant with Buchmann et al. (1996) who found that *Picea abies* recovered only 3–7 % of labeled ammonium nitrate whereas understory absorbed around 9–15 %. Likewise, Cheng et al. (2004) in a pot study observed that grasses absorbed near 36 % of labeled nitrate whereas *Quercus* seedlings only accounted with 3–4 %. These authors also reported that trees increased N absorption by 94 % when they were growing without grasses. They indicated that grasses had higher root density and higher growth rates, being better competitors to acquire N than trees. This also could be the case for our study, since grasses had higher growth rates, higher root density to 0–20 cm depth and also, started to grow early in the season.

^{15}N in soil

Ndff values found in soil were, as expected, inferior to those found in vegetal components, due to a dilution effect that occurs in soil when ^{15}N is added. ^{15}N values in both sites decreased with depth and were lower in open site. Ndff values found in this study were consistent with those presented by Neto et al. (2008) for *Pyrus communis*. At the end of the growing season no major leaching of ^{15}N was detected, since at 70 cm depth ^{15}N atom excess was zero for all samples. This may indicate that precipitation was not enough to leach mobile elements as nitrates over the growing season, although leach may occur during the winter when soil profile is water refilled (Paruelo and Sala 1995).

Comparing total amounts: silvopastoral vs. open site

In both systems, soil was the component that retained highest ^{15}N amounts, with values around 62 % of total ^{15}N detected. This agrees with Buchmann et al. (1996) and Nadelhoffer et al. (1999) who reported that soil

was the main fate for ^{15}N added in forests of *Picea abies*, *Quercus* and *Pinus*.

In the silvopastoral system, trees absorbed 69 % less ^{15}N than grasses. This could be partly owing to the fact that trees start N absorption later in the growing season than grasses, and the first N used for leaves expansion comes from tree reserves (Muñoz et al. 1993; Neto et al. 2008). Furthermore, root systems of grasses and trees are different. Whereas grasses have a complex and dense root system capable to explore and acquire N from a larger soil volume (although shallow), trees have an important proportion of coarse and medium roots that usually are used for support and as reserve organ.

In this study we observed that grasses growing in the open accumulated more biomass and N amounts, but an important proportion of these resources were derived belowground. With respect to the aerial components, grasses in the silvopastoral system had 30–37 % less biomass but there were not differences in total N accumulation, mainly due to higher N concentrations in silvopastoral grasses that compensated the reduction in biomass. These results are concordant with Garrett et al. (2004) who reported that in general in silvopastoral systems herbaceous productivity decreased with tree cover, but N contents increased.

^{15}N recovery was 65 % higher in the silvopastoral system than in the open site. This was mainly due to a higher retention in soil and grasses and additional uptake by trees. An important aspect is that grasses in the silvopastoral system were able to absorb higher ^{15}N amounts. These results indicate a better and efficient use of applied N in silvopastoral system. Rowe et al. (2001) also reported that intercropping system of *Gliciridia sepium* and/or *Peltophorum dasyrrachis* with maize was more efficient in N use than maize alone. Our results agree with the hypothesis proposed since *N. antarctica* trees in this site would not strongly compete with grasses for N absorption and, also, indirectly could favor it by facilitating a better environment (wind protection and less water stress) or by high quality litterfall contribution that reduces competition with microorganism for inorganic N. However, future research involving microorganism biomass and its function in *N. antarctica* silvopastoral systems are needed to confirm this aspect. Despite that, both analyzed systems were near each other and had a similar land use history (sheep

grazing), there were not true replications, and so is possible that other pre-existing differences could be interfering in our results. However, we consider that the present study provided a starting point in the understanding of N dynamics of these austral Patagonian ecosystems, where silvopastoral systems may be more efficient for N recovery than pure grassland systems. This aspect should be taken into account when management practices such as high intensity thinning may reduce the benefits that trees concede to the overall N system. Research about optimal *N. antarctica* densities to maximize these benefits and increase grass productivity is needed, since several authors have shown that tree-grass-cattle interactions can be manipulated to enhance grass productivity without losing benefits from trees (Lehmkuhler et al. 1999; Garrett et al. 2004).

Conclusion

The present study provided a starting point in the understanding of N dynamics in Patagonian *N. antarctica* silvopastoral systems. We observed that the silvopastoral system in our study site was more efficient in N use since it retained more ^{15}N added than grasses growing alone. Grasses in silvopastoral system absorbed twice as much ^{15}N amounts than grasses in the open site, indicating that trees may not strongly compete for N with grasses. Conversely, we suspect that trees may “facilitate” N absorption by grasses improving environmental conditions like lower water stress (by protection of strong winds) or by reducing competition between soil microorganisms and grasses for inorganic N, since litterfall improves decomposing litter quality.

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