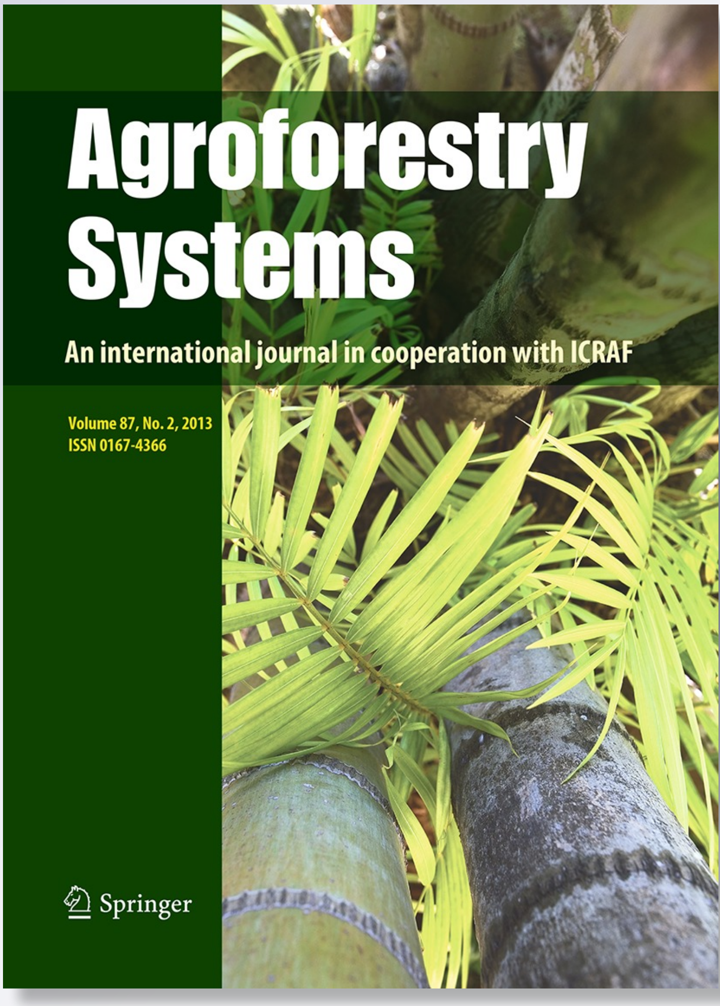
Silvopastoral use of Nothofagus antarctica *in Southern Patagonian forests, influence over net nitrogen soil mineralization*

Héctor A. Bahamonde, P. L. Peri, R. Alvarez, A. Barneix, A. Moretto & G. Martínez Pastur

Agroforestry Systems
An International Journal incorporating Agroforestry Forum

ISSN 0167-4366
Volume 87
Number 2

Agroforest Syst (2013) 87:259-271
DOI 10.1007/s10457-012-9541-5



Springer

Agroforest Syst (2013) 87:259–271
DOI 10.1007/s10457-012-9541-5

Silvopastoral use of *Nothofagus antarctica* in Southern Patagonian forests, influence over net nitrogen soil mineralization

Héctor A. Bahamonde · P. L. Peri · R. Alvarez · A. Barneix · A. Moretto · G. Martínez Pastur

Received: 11 January 2012 / Accepted: 13 June 2012 / Published online: 28 June 2012

Abstract In most temperate forest, nitrogen (N) is considered a limiting factor. This becomes important in extreme environments, as *Nothofagus antarctica* forests, where the antecedents are scarce. Thinning practices in *N. antarctica* forests for silvopastoral uses may modify the soil N dynamics. Therefore, the objective of this work was to evaluate the temporal variation of soil N in these ecosystems. The mineral extractable soil N, net nitrification and net N mineralization were evaluated under different crown cover and two site quality stands. The mineral N extractable (NH_4^+–N + NO_3^-–N) was measured periodically. Net nitrification and net N mineralization were estimated through the technique of incubation of intact samples with tubes. The total mineral extractable N concentration varied between crown cover and dates, with no differences among site classes. The lowest and highest values were found in the minimal and intermediate crown cover, respectively. In the higher site quality stand, the annual net N mineralization was lower in the minimal crown cover reaching 11 kg N ha^{-1} $year^{-1}$, and higher in the maximal crown cover (54 kg N ha^{-1} $year^{-1}$). In the lower site quality stand there was no differences among crown cover. The same pattern was found for net nitrification. Thinning practices for silvopastoral use of these forests, keeping intermediate crown cover values, did not affect both N mineralization and nitrification. However, the results suggest that total trees removal from the ecosystem may decrease N mineralization and nitrification.

Keywords Nitrogen dynamic · Ñire · Forest soil · Silvopastoral system · Patagonia

H. A. Bahamonde (✉) · P. L. Peri
Instituto Nacional de Tecnología Agropecuaria (INTA), cc 332, 9400 Río Gallegos, Santa Cruz, Argentina
e-mail: habahamonde@correo.inta.gov.ar

P. L. Peri
e-mail: pperi@correo.inta.gov.ar

H. A. Bahamonde · P. L. Peri
Universidad Nacional de la Patagonia Austral (UNPA), Lisandro de la Torre 1070, 9400 Río Gallegos, Argentina

P. L. Peri · A. Moretto · G. Martínez Pastur
Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina
e-mail: cadicmoretto@gmx.net

G. Martínez Pastur
e-mail: cadicforestal@cadic.gov.ar

R. Alvarez · A. Barneix
Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453, C1417DSE Buenos Aires, Argentina
e-mail: ralvarez@agro.uba.ar

A. Barneix
e-mail: barneix@agro.uba.ar

A. Moretto · G. Martínez Pastur
Centro Austral de Investigaciones Científicas (CADIC), Houssay 200, 9410 Ushuaia, Tierra del Fuego, Argentina

Introduction

Forest ecosystems generally base their long-term sustainability in the natural maintenance of nutrient cycles (Perry et al. 2008), forest structure and biodiversity conservation (Lencinas et al. 2011). Particularly nitrogen (N) is considered a limiting factor for growth in most temperate forests (Fisher and Binkley 2000), and particularly in extreme environments, as *Nothofagus antarctica* (ñire) forests (Peri et al. 2008a). Also, it is estimated that N mineralization is the major source of mineral N availability for plants in terrestrial ecosystems (Vitousek and Howarth 1991). Furthermore, the transformations of N in the soil, as mineralization and nitrification, are affected by environmental factors such as temperature, soil moisture, pH, light availability (MacDonald et al. 1995; Aciego Pietri and Brookes 2008), as well as litter-fall quality (Jarvis et al. 1996). Net nitrification rates can reflect the potential for N losses, either through leaching or by gaseous emission (Vitousek and Melillo 1979; Vitousek and Matson 1985).

There are evidences reporting that canopy opening modifies the biogeochemical cycle of elements (Caldentey et al. 2001; Jussy et al. 2004). Thinning practices in *N. antarctica* forests for silvopastoral uses may modify the soil N dynamics through changes in micro- environmental factors and lower potential N return from litterfall (Peri et al. 2008b) or due to increasing losses of nitrate by leaching (Feller et al. 2000). Furthermore, if the N absorption decreases in the thinned stands (less number of trees) it may be expected a higher availability of mineral N for the understory plants. However, major soil moisture due to an increase of water from rainfall in the forest gaps created after thinning could increase the losses by nitrate leaching. In Patagonian forests few studies in N mineralization have been conducted. Mazzarino et al. (1998a) and Satti et al. (2003) reported potential N mineralization of Patagonian soil forests, and Alauzis et al. (2004) evaluated the potential N mineralization of *N. pumilio* forests affected by fire in northern Patagonia. However all these studies were carried out under laboratory conditions and there are not antecedents of in situ N mineralization for *N. antarctica* forests.

Recently we have reported results related to the effect of silvicultural practices of *N. antarctica* forest under silvopastoral use on decomposition rates of grass and tree leaves in different site qualities (Bahamonde et al. 2012a). For a whole approach of nutrient cycling in these ecosystems, it is necessary to know the soil N dynamics related to the silvopastoral use of these forests.

The aims of this study were to (1) quantify the effects of different crown cover on the concentration of extractable mineral soil nitrogen (NH_4^+–N + NO_3^-–N) and their temporal variation in two *N. antarctica* stands growing in different sites qualities; and (2) asses the net nitrification and net N mineralization in these stands at different times of the year.

Materials and methods

Study sites

The study sites were located in pure *N. antarctica* forests under silvopastoral use growing in two site qualities (site classes) in the southwestern Patagonia (Argentina): (1) site class IV (SCIV) (51° 13′ 23″S–72° 15′ 39″W) where the mean dominant height (H) of mature trees reached to 8.0 m and (2) site class V (SCV) (51° 19′ 05″S–72° 10′ 47″W) where H reached to 4.7 m (Ivancich et al. 2011). In each stand, an adjacent area was selected without trees representing the ecotone zone between forest and steppe.

The climate of the area is described as cold temperate humid with a mean annual temperatures 5.5 and 8.0 °C, and precipitation (rain and snow) ranging between 400 and 800 mm year^{-1} (Soto 2004).

Composite samples of five soil cores were taken (0.3 m depth) at random under the tree crown projection (0.5 m from main trunk), between tree crowns (mean distances between trees of 8.2 m), and the adjacent area without trees at each studied sites for physical–chemical analysis.

Dasometric characteristics of the stands were estimated within three circular plots of 500 m^2. In each plot, total number of trees, frequency of crown classes (dominant, codominant, intermediate and suppressed), diameter at breast height (DBH) and total height were measured.

Environmental measurements

Through hemispherical photos, solar radiation intensities (direct and diffuse), light transmissivity and photosynthetically active radiation (PAR) reaching the soil were estimated in two contrasting crown cover situations (under and between crowns), and an adjacent area without trees in each study site. This provided 3 levels of crown cover.

Air and soil temperatures, and air relative humidity were measured continuously every 2 h with a datalogging system (HOBO H8 Family, Onset Computer Corporation, USA), using sensors located at each crown cover, in both studied site quality stands. Periodically (at the same time of incubation of soil) measurements of gravimetric soil moisture were obtained from the first 0.3 m ($n = 5$) in each study situation (crown cover and site class). Then, volumetric soil moisture was calculated using soil bulk density and the gravimetric measurements.

Details of soil analysis, dasometric characteristics of the stands and environmental data of the studied situations are provided in Bahamonde et al. (2012a).

Experimental design and biological measurements

In situ soil incubations

A factorial experiment design with crown cover, site class and time as main factors with 3, 2 and 6 levels, respectively was carried out. Five plots (replicates) of 2 × 2 m were setup at random in each crown cover. This design was repeated in the two site class (SC) stands. The concentration of mineral N extractable (NH_4^+–N + NO_3^-–N) was measured periodically at 30, 90, 150, 210, 360 and 450 days after 1st September 2005 through the technique of incubation of intact samples with tubes. Advantages and disadvantages of this method can be found in Raison et al. (1987). In situ N mineralization was determined in samples taken with PVC tubes (covered at the top), 20 cm high and 3.5 cm diameter, and incubated in the field over time. All soil samples were transported to the laboratory within 1-10 days after collection inside sealed plastic bags under cool (5.0 °C) conditions. Initial and incubated samples were analyzed for inorganic N (NH_4^+–N + NO_3^-–N) in fresh soil samples extracted with 2 M KCl. Net N mineralization was estimated as the difference between NH_4^+–N + NO_3^-–N in the incubated minus the initial samples. Similarly net nitrification was estimated as the difference in NO_3^-–N between successive samplings. The extractable mineral nitrogen concentration (NH_4–N + NO_3–N) of samples (before and after incubation) was obtained through the method of micro distillation by steam (Bremner and Keeney 1965) after extraction with KCl 2 M, using reagents Devarda and MgO (Mulvaney 1996). For each treatment, the amount of extractable nitrogen per unit area (kg ha^{-1}) was determined using soil bulk density data. Sub-soil samples from each date and location were dried in oven at 105 °C to determine gravimetric water content and then soil moisture volumetric content was calculated using the soil bulk density. Thus, the concentrations and availability of N were expressed on a dry basis. Annual nitrification and N mineralization rates (kg ha^{-1} $year^{-1}$) were estimated by adding partial values from each evaluation period and divided by the number of years of trial duration.

Soil incubations under laboratory conditions

Similarly to the in situ mineralization, a factorial design was implemented considering the crown cover, site class and sampling date as factors with 3, 2 and 3 levels each, respectively. In October 2005, February and April 2006, 5 samples (replications) were taken from the top 0.2 m of soil in each studied situation. All soil samples were kept cool (5.0 °C) in sealed plastic bags and transported to the laboratory within 6–24 h of collection. In the laboratory the samples were incubated to determine the potential net nitrogen mineralization under aerobic incubation method (Hart and Binkley 1985). Fifty grams of each soil sample were placed in containers of 250 ml, and incubated along 4 weeks in darkness and under optimal conditions of moisture (field capacity) and temperature (25 °C). The extractable inorganic N (NH_4^+–N + NO_3^-–N), the net N mineralization and nitrification, were measured and calculated in the same way as in situ mineralization.

Data analysis

Dasometric characteristics of each study stand were analysed by analysis of variance (ANOVA). Total transmitted radiation data were analysed by ANOVA with crown cover as a factor. Extractable N data of NH_4^+–N, NO_3^-–N and total (NH_4^+–N + NO_3^-–N) were analysed using ANOVA for repeated measures with crown cover and site class as between-subject factors and each sampling date as an within-subject factor. This analysis was done because the values are not independent of time. This type of analysis has been shown to be appropriate for these cases (Gurevitch and Chester 1986). Tukey tests were performed to define differences when *F* values were significant ($P < 0.05$). To avoid misinterpretations due to significant interaction between factors (Willems and Raffaele 2001) multiple comparisons were made concerning to between-subject effects, (crown cover and site class) for each sampling date. The same analyses were carried out for net N mineralization and nitrification in situ and under laboratory conditions.

Linear regression analysis between environmental variables and net N mineralization and nitrification in situ were carried out to determine the variable with more influence on the processes.

Results

Dasometric characteristics and micro-climate variation

The main dasometric characteristics of the studied stands and the micro-climate data are presented in Bahamonde et al. (2012a). Briefly, the total height of dominant trees, number of trees and basal area were higher in SCIV ($P < 0.05$) than in SCV. In both stands, light transmissivity and total photosynthetically active radiation reaching the forest floor increased ($P < 0.05$) in places where crown cover decreased.

Both air and soil temperatures showed maximum values during summer and minimum in winter in both stands. Air temperature did not differ between different crown cover in any of the two evaluated stands. In contrast, soil temperatures were higher in open sites in spring and summer during the first season in SCIV, but not in SCV. Volumetric soil moisture was higher in more open sites of the silvopastoral stands during spring and summer in SCIV, while in SCV no differences were found between crown cover. Relative air humidity was lower in the adjacent open areas at both stands.

Extractable N concentration

The concentration of N as NH_4^+–N significantly varied between crown cover and dates, with no differences among site class (Table 1). The lowest concentration was found between crowns ($P < 0.001$). Considering dates, the highest values of NH_4^+–N were measured in December 2006 ($P < 0.001$) reaching an average of 17 and 11 μg g^{-1} dry soil in SCIV and SCV, respectively (Fig. 1a, b). There was a significant interaction ($P < 0.001$) between crown cover and dates, and between site class and date ($P = 0.015$). In SCIV, during summer (February) there was a higher concentration of ammonium in the area without trees, while at the beginning of the growing season (September) there was a major concentration in the soil under crown (Fig. 1a). In SCV, the highest concentration of NH_4^+–N were found in the location without trees in December 2005, while in autumn (April) were measured under crown (Fig. 1b).

The NO_3^-–N concentration significantly varied between crown cover, site class and dates (Table 1). Averaging the two site class (data not shown), the highest concentration (2.8 μg g^{-1} dry soil) was measured under crown and the lowest values between crowns (1.9 μg g^{-1} dry soil). Comparing sites, the highest concentration occurred in SCV (data not shown). The lowest values of NO_3–N were measured in April and September (Autumn–Winter) (Fig. 1c, d).

When the proportions of each form of N were calculated, it was observed that the predominant form of N in all situations was the NH_4^+–N representing on average 75 % of the total extractable inorganic N.

The total mineral extractable N concentration (NH_4^+–N + NO_3^-–N) varied between crown cover and dates, with no differences among site class (Table 1). The lowest and highest ($P < 0.001$) values were found in the location without trees and between crowns, respectively (data not shown). The highest concentration of NH_4^+–N + NO_3–N was measured in December 2006. However, the interactions among crown cover and dates, and site class and dates were significant (Table 1). For example, in SCIV, in February 2006 the highest concentration was measured in the location without trees, while in SCV there were not differences among crown cover at the same date. In December 2006 the highest concentration was obtained under crown in both site class (Fig. 1e, f).

The net N mineralization varied significantly between crown cover and dates, with no differences among site class (Table 2). Averaging the two site class, the highest values of mineralized N was measured between crowns reaching 6.7 kg ha^{-1} 30 days^{-1} (data not shown). The period with highest mineralization rate was December 2006-February

Table 1 Repeated measures ANOVA for extractable nitrogen as ammonium (NH_4^+–N), nitrate (NO_3^-–N) and total (NH_4^+–N + NO_3^-–N) measured at three crown cover, two site class, and six dates in *Nothofagus antarctica* forests under silvopastoral use

Source	*df*	NH_4^+–N F (*P*)	NO_3^-–N F (*P*)	NH_4^+–N + NO_3^-–N F (*P*)
Between subject effects				
Crown cover (CC)	2	15.11 (<0.001)	3.92 (0.033)	11.93 (<0.001)
Site class (SC)	1	2.23 (0.148)	8.69 (0.007)	0.08 (0.777)
Within subject effects				
Date (D)	5	13.09 (<0.001)	10.71 (<0.001)	12.44 (<0.001)
Interactions				
CC × SC	2	2.08 (0.146)	2.01 (0.156)	0.99 (0.385)
CC × D	10	4.09 (<0.001)	2.10 (0.072)	3.55 (0.002)
SC × D	5	2.94 (0.015)	4.76 (0.006)	3.19 (0.021)
CC × SC × D	10	1.70 (0.088)	0.89 (0.496)	1.74 (0.109)

2007 with a mean mineralization value of 19 kg ha^{-1} 30 $days^{-1}$ (Fig. 2a, b). However, all the interactions between main effects were significant (Table 2). For example, the differences among crown cover were evident in SCIV during two periods (Fig. 2a), while in SCV there were not differences (Fig. 2b). When we compared the values of annual N net mineralization per unit area at each site we found different results depending of site class. In SCIV annual net mineralization was lower in the location without trees, while in SCV there were not differences among crown cover (Table 3).

The simple linear regression analysis showed that the volumetric soil moisture was the only parameter that explained variation in N mineralization at the studied sites ($P < 0.05$; $R^2 = 0.13$).

In situ net nitrification significantly varied only between periods (Table 2) with the highest values measured in the period December 2006–February 2007 (Fig. 3a, b). Multiple comparisons between radiation levels for the same period showed that in SCIV the highest values of nitrification occurred between crowns only during the December 2006–February 2007 period (Fig. 3a). In contrast, in SCV significant differences between radiation levels were found in the period April-September 2006 with higher nitrification under crown than "between crowns" cover condition (Fig. 3b).

Annual in situ nitrification at area basis was lower at SCIV in the area without trees, while in SCV there were no differences between radiation levels (Table 4).The simple linear regression between nitrification and environmental variables showed that the initial availability of NH_4 was the only measured variable that explain variation in nitrification between periods and crown cover in both site classes ($P < 0.01$; $R^2 = 0.26$).

Net nitrogen mineralization measured under laboratory conditions significantly varied among crown cover, site class and sampling date (Table 2). The highest values of mineralized nitrogen occurred between crowns averaging 17.4 kg ha^{-1} 30 $days^{-1}$, while there were no differences under crown and without trees conditions. Comparing sites and sampling dates, the highest values of mineralization occurred in SCIV during February. However, as the interactions were significant (Table 2), multiples comparisons gave us more detailed information. In SCIV the mineralized N was higher between crowns in October and February (Fig. 4a), while in SCV only there was differences in samples taken in April with values close to zero (Fig. 4b). Net nitrogen mineralization measured under laboratory conditions was higher than in situ for both site class stands in October 2005 and February 2006, while in April 2006 there were no differences (Figs. 2a, b, 4a, b). Thus, potential mineralization in SCIV was up to 15 and 4 times higher in the laboratory than those obtained in the field in October and February, respectively. In SCV N mineralization was up to 2 and 7 times higher in the laboratory compared with the measured in situ for the October and February period, respectively.

Nitrification under laboratory conditions varied between crown cover and sampling date, with no significant differences among sites class (Table 2). Highest value of nitrification occurred between crown

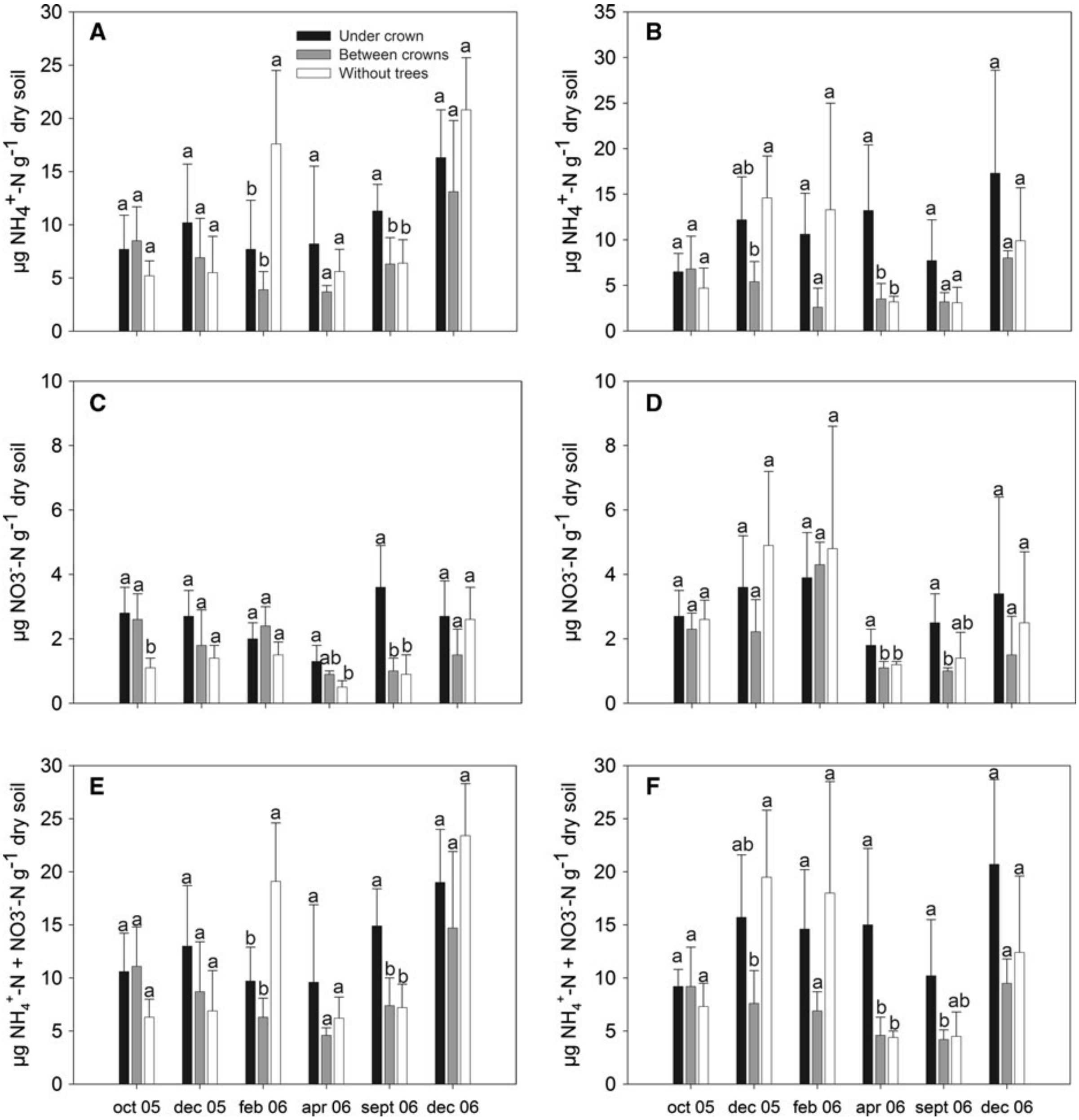


Fig. 1 Extractable mineral N concentration (NH_4^+–N, NO_3^-–N and NH_4^+–N + NO_3^-–N) measured in soil (0–0.2 m) located at 3 crown cover: under crown, between crowns and without trees, of ñire forests growing in site class IV (**a**, **c**, **e**) and site class V (**b**, **d**, **f**). The *bars* indicate the standard deviation. The mean of each radiation level, at each sampling date followed by the *same letter* are not significantly different ($P < 0.05$)

cover averaging 8.9 kg ha^{-1} 30 days^{-1}, while under crown and without trees averaged 5.0 and 4.3 kg ha^{-1} 30 days^{-1}, respectively with no significant differences between these conditions (data not shown). Comparing sampling dates, the lowest value of nitrification was obtained for samples taken in April with values near zero (Fig. 4c, d). Multiples comparisons showed that in SCIV the nitrification was lower ($P < 0.001$) in the location without trees in October with no differences among crown cover for the others dates (Fig. 4c). In contrast, in SCV the lowest values occurred under crown in April (Fig. 4d).

Table 2 Repeated measures ANOVA for in situ net nitrogen mineralization (Net N Min), in situ net nitrification (Net Nit); in laboratory net nitrogen mineralization (Net N Min) and in laboratory net nitrification (Net Nit) measured at 3 crown cover, two Site Class, and different dates (6 in situ, 3 in laboratory) in *Nothofagus antarctica* forests under silvopastoral use

Source	*df*	In situ		*df*	In laboratory	
		Net N Min F (*P*)	Net Nit F (*P*)		Net N Min F (*P*)	Net Nit F (*P*)
Between subject effects						
Crown cover (CC)	2	3.72 (0.039)	1.41 (0.262)	2	19.96 (<0.001)	4.15 (0.042)
Site class (SC)	1	1.07 (0.310)	4.11 (0.054)	1	13.38 (0.003)	0.07 (0.796)
Within subject effects						
Date (D)	5	36.69 (<0.001)	60.48 (<0.001)	2	56.06 (<0.001)	4.07 (0.030)
Interactions						
CC × SC	2	4.09 (0.029)	2.75 (0.084)	2	19.61 (<0.001)	4.68 (0.031)
CC × D	10	3.62 (0.003)	1.42 (0.251)	4	5.94 (0.002)	2.32 (0.129)
SC × D	5	4.01 (0.010)	2.03 (0.154)	2	1.23 (0.309)	0.062 (0.844)
CC × SC × D	10	2.19 (0.053)	1.46 (0.238)	4	3.19 (0.031)	0.43 (0.688)

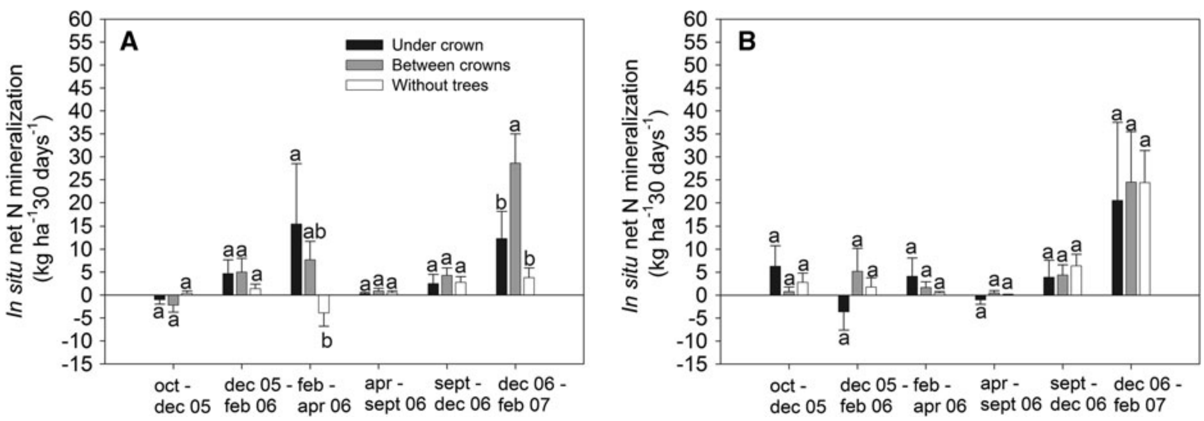


Fig. 2 In situ net N mineralization measured in soil (0–0.2 m) located at 3 crown cover: under crown, between crowns and without trees, of ñire forests growing in site class IV (**a**) and site class V (**b**). The *bars* indicate the SD. The mean of each radiation level, at each sampling date followed by the same letter are not significantly different ($P < 0.05$)

Nitrification under laboratory conditions from samples taken in October and February were 5 and 4 times higher than those measured in the field for SCIV and SCV, respectively (Figs. 3, 4).

Discussion

Extractable mineral N concentration

The values of extractable mineral N (NH_4^+–N + NO_3^-–N) measured in this work were lower than those reported in soils of *N. pumilio* forests in Tierra del Fuego (Argentina) (Moretto et al. 2004) and *N. betuloides* forests in southern Chile (Huygens et al. 2008). The difference between our values and the cited bibliography is likely due to contrasting forests productivity in terms of aerial biomass. For example, the dominant height of trees cited for *N. pumilio* forest in Tierra del Fuego overcomes 14 m (Martínez Pastur et al. 2000) and for *N. betuloides* trees reach 24 m of height (Godoy et al. 2001), while our better evaluated site for *N. antarctica* trees do not overcome 8 m (Bahamonde et al. 2012a).

Table 3 Total annual net nitrogen mineralization (NH_4 + NO_3) (kg N ha^{-1} $year^{-1}$) measured in *N. antarctica* forest soils (0–0.2 m) at three crown cover and two site classes (SC)

	Crown cover		
	Under crown	Between crowns	Without trees
SCIV	54.4a	72.4a	11.0b
SCV	51.6a	59.5a	59.1a

Different letters in the same site class indicate significant differences ($P < 0.05$) between radiation levels

The greater concentrations of NH_4^+–N in the location without trees during summer in both site classes would be due to a major uptake of NH_4^+–N inside the forest. There are not antecedents about the main nitrogen source for *N. antarctica*, however is well known that ammonium is the most important N compound uptake by tree species (Rennenberg et al. 2009). Recent studies in these forests (Gargaglione 2011) have shown a facilitation effect of trees on grasses by increasing the N uptake rate of the herbaceous component inside the forest compared with an adjacent area without trees. This pattern was inverted, with greater concentrations of NH_4^+-N under trees in September (beginning of growing season) and April (autumn) for SCIV and SCV, respectively. We speculate that this occurred because of the contribution of decomposing litterfall in autumn and a decrease of nitrogen uptake after winter.

Despite of differences among site classes on NO_3^-–N concentrations (Table 1) the values were lower than NH_4^+–N. The major proportion of NH_4^+–N found in this work, also documented by Moretto et al. (2004) in *N. pumilio* forests soils, may be given mainly by the pH values prevailing in these soils (4.9–5.6) that could inhibit nitrification (Stark and Hart 1997; Aciego Pietri and Brookes 2008), or because nitrates were absorbed in higher proportions by plants or microbes (Kaye and Hart 1997), or due to N leaching (Ritter et al. 2005). Our results suggest that a combination of these factors may occur in the studied ecosystems. Averaging the two site class, it is likely that the highest values found under crown were due to relative low leaching and absorption, whilst the lowest concentrations between crowns could be caused by higher leaching in gaps (Ritter et al. 2005) and greater accumulation of N in the understorey stratum (Gargaglione 2011).

Considering total mineral extractable N (NH_4^+–N + NO_3^-–N) the differences among crown covers depending over time could be caused by a combination of processes. For example, higher amounts of extractable mineral N in the location without trees during summer (e.g., February 2006 in SCIV) would be due to nitrogen release from decaying grasses (Bahamonde et al. 2012a) and that soil N mineralization could exceed N demand from grassland, mainly due to higher temperatures (Clarkson et al. 1986). In contrast the highest values of extractable N under crown at the end of winter (e.g., September 2006 in SCIV) could be due the contribution of litterfall decomposition in the previous period (Bahamonde et al. 2012a) and because

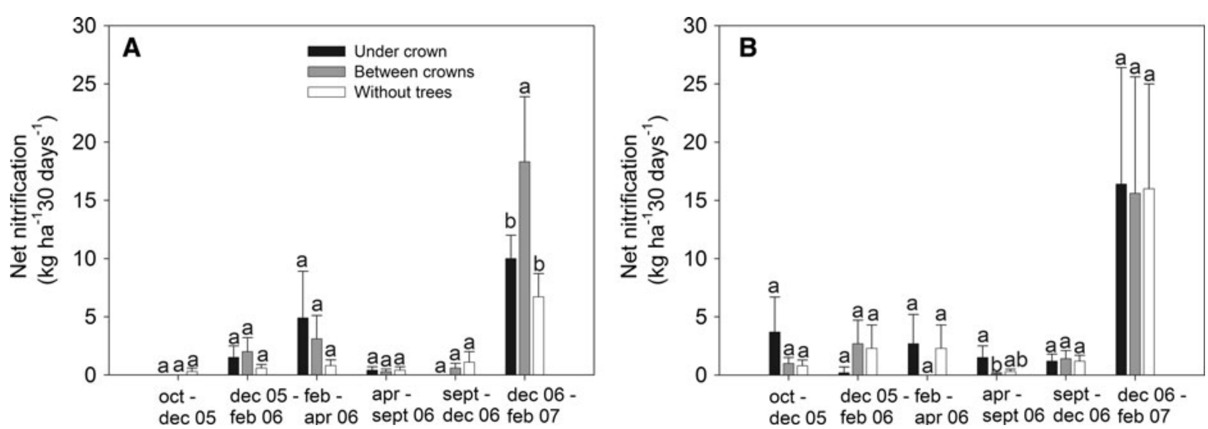


Fig. 3 In situ net nitrification measured in soil (0–0.2 m) located at 3 crown cover: under crown, between crowns and without trees, of ñire forests growing in site class IV (**a**) and site class V (**b**).The *bars* indicate the SD. The mean of each radiation level, at each sampling date followed by the *same letter* are not significantly different ($P < 0.05$)

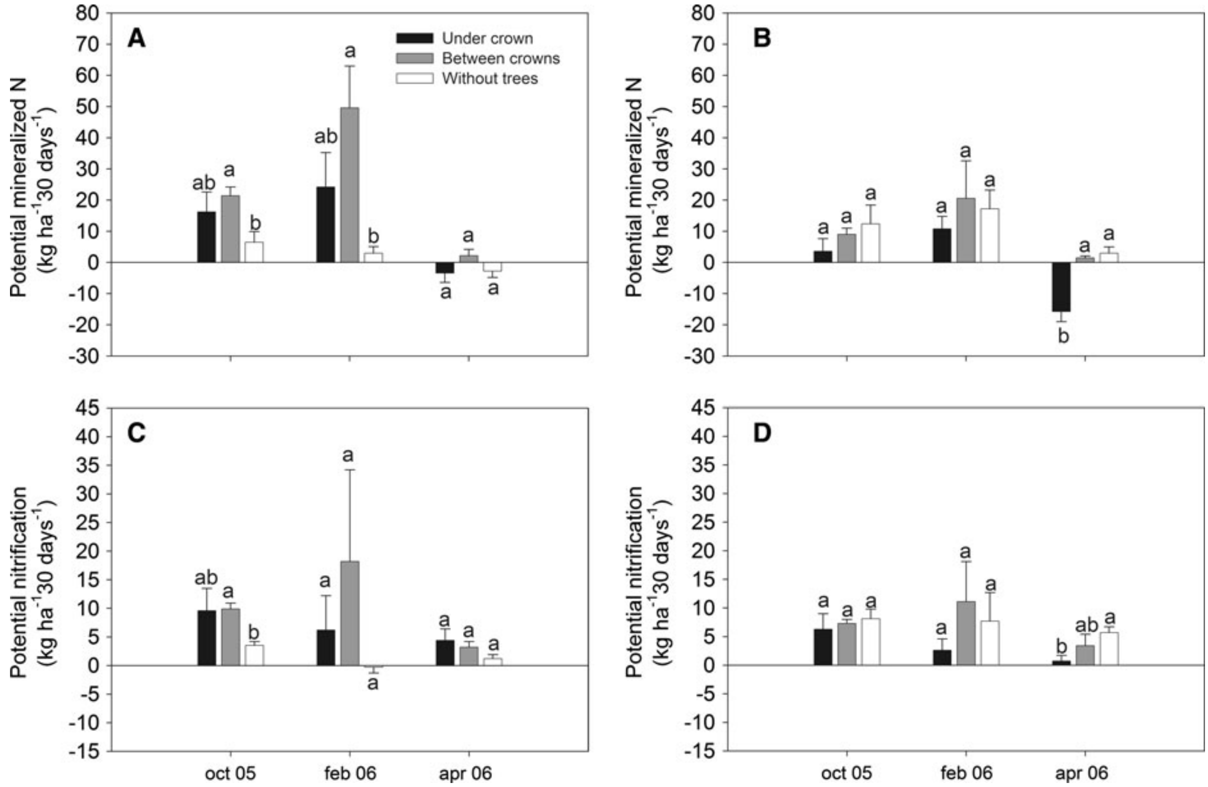


Fig. 4 Potential net N mineralization and nitrification measured under laboratory conditions within soil (0–0.2 m) from 3 crown cover: under crown, between crowns and without trees, of ñire forests growing in site class IV (**a**, **c**) and site class V (**b**, **d**)

Table 4 Net nitrification (kg ha^{-1} year^{-1}) measured in ñire forest soils soils (0–0.2 m) at 3 radiation levels and two site class (SC)

	Crown cover		
	Under crown	Between crowns	Without trees
SCIV	26.0ab	37.7a	17.0b
SCV	43.2a	32.2a	36.3a

Different letters in the same site class indicate significant differences ($P < 0.05$) between radiation levels. The data were analysed separately for each site

N uptake in winter months would be minimal due to low soil temperatures (Dong et al. 2001).

In situ net N mineralization and nitrification

The net mineralization values estimated in this study are within the range of those found in temperate forests of *Nothofagus* and *Fitzroya* of southern Chile (Pérez et al. 1998, 2003) and were higher than those reported for soils of steppe in Patagonia (Yahdjian et al. 2006). Nevertheless, there are antecedents suggesting that the method used in the present study may underestimate N mineralization rate in soils due to oxygen depletion inside the container that depresses microbial activity (Abril et al. 2001). Therefore, the interpretation of results should emphases the relative differences among treatments rather than absolute values.

The lower net N mineralization within the adjacent area without trees in SCIV (Table 3) is difficult to explain considering that the environmental conditions (temperature and soil moisture) were more favorable. However, other factors that impact the processes of nitrification and mineralization of N such as the C:N ratio of soil and decomposing litterfall (Mazzarino et al. 1998a; Bengtsson et al. 2003; Booth et al. 2005; Bertiller et al. 2006) may explain the process in SC IV. High C:N ratios may limit microbial activity due to lack of nitrogen. Gallardo and Schlesinger (1992) reported a positive correlation between microbial biomass and soil C:N ratio to values less than 20. In this study, the low soil C:N ratio (8.1) in the adjacent area with no trees in SCIV could be the cause of a lower nitrification and mineralization rate compared to values obtained inside the forest. Also, it has been found positive correlations between microbial biomass and potential nitrogen mineralization (Mazzarino et al. 1998a; Satti et al. 2003; Bertiller et al. 2006). Therefore, we speculate that low C:N ratio (as mentioned above) may partly explain the lower values of nitrification and N mineralization found in the situation with no trees.

Considering the results from the correlation between soil moisture and N mineralization found in this study different results have been reported in the literature. While Burke et al. (1997); Mazzarino et al. (1998b) and Xueling et al. (2008) have reported positive correlations, other studies found no correlations (Hook and Burke 2000; Yahdjian et al. 2006). These differences related to the effect of soil moisture on N mineralization could be due to strong interactions between temperature and soil moisture affecting the process (Zak et al. 1999). Also there is evidence indicating that excess of soil moisture reduce the activity of micro organisms in a lack of oxygen condition (Linn and Doran 1984) which promote a denitrification process. In our study, the volumetric soil moisture values (Bahamonde et al. 2012a) mostly were in the range between water-holding capacity and permanent wilting point for the studied soils (Bahamonde et al. 2012b).

In general the nitrification values were low and similar to those reported by Mazzarino et al. (1998b) in soils of the steppe in Patagonia. In our study the low values of nitrification may be given by the values of pH (4.9–5.6) that would limit the process (Stienstra et al. 1994) as mentioned above. The correlation found between nitrification and ammonium availability (as mentioned in the "Results") has been reported previously (Lamb 1980; Robertson 1984), where low levels of NH_4 may limit nitrification process. Also this correlation could explain the apparent not significant effect of soil temperature on nitrification as it has been reported by others authors (Breuer et al. 2002; Dalias et al. 2002). Also, it is known that soil moisture can act as an inhibitor of nitrification by either excess or defect, since the nitrifiers need water and oxygen, being the water-filled pore space a better predictor of nitrification (Grundmann et al. 1995), which could explain the no significant correlation between volumetric soil moisture and nitrification in this study.

Potential net N mineralization and nitrification

The highest values of N mineralization and nitrification measured in this study were lower than those reported for forests of *N. pumilio* (Moretto et al. 2004) and *N. antarctica* (Mazzarino et al. 1998a) in Tierra del Fuego, and in the range of *N. antarctica* in northern Patagonia (Satti et al. 2003). These differences could be due to different soil characteristics between sites. For example, the values reported by Satti et al. (2003) had soils with higher pH (6.1) than our study soils, which would favor nitrification.

The fact that both mineralization and nitrification values measured under laboratory conditions were higher than those observed in situ suggest that there are environmental factors in the field that limit these processes. For example, low soil temperature would be the limiting factor (<13 °C) during October and February. Similar results were reported by Pérez et al. (1998) in forests of southern Chile attributing the lower values obtained in field measurements to the effect of low temperature. Different studies have reported the direct effect of temperature on N mineralization with the highest rates at temperatures near 25 °C (Dalias et al. 2002; Knoepp and Swank 2002; Bagherzadeh et al. 2008).

On the other hand, the no differences between N mineralization and nitrification obtained in the laboratory and In situ samples collected in April would indicate that other factors rather than low temperature are affecting the processes. As mentioned previously there is evidence that N mineralization is directly related to microbial biomass of soil. Thus, the carbon source may limit the microbial biomass (Gallardo and Schlesinger 1992; Allen and Schlesinger 2004). In the studied forests the main contribution of organic matter to soil litterfall occurs mainly in April (Peri et al. 2008b). Most decomposition of ñire and grasses leaves probably start to decompose after winter (September) when temperatures increase. Similarly, in the adjacent area with no trees, senescent material accumulated since April begins to decompose in the next season. Probably, these aspects related to decomposition could affect microbial biomass in April and therefore N mineralization and nitrification.

From the practical point of view, Peri et al. (2008b) reported that the removal of trees through thinning practices for silvopastoral use of *N. antarctica* forest in Patagonia caused a decrease of 35–50 % in the contribution of litter to soil forest. Our results suggest that this reduction of litter in stands with less crown cover would not affect the N mineralization rates.

Conclusions

According to the results, we speculate that concentration of total extractable mineral N ($NH_4 + NO_3$) was not related to crown cover, depending mainly on environmental variation between seasons.

The results suggest that thinning practices for silvopastoral use of *N. antarctica* forests (keeping intermediate crown cover values) growing under the Patagonian environment did not affect both N mineralization and nitrification. However, lower values of N mineralization and nitrification were found in the location without trees in the more productive site class, which suggest that total trees removal from the ecosystem may decrease N mineralization and nitrification.

Data of N mineralization and nitrification obtained under laboratory conditions suggest that both processes would be limited by low soil temperature in the field.

References

Abril A, Caucas V, Bucher EH (2001) Reliability of the in situ incubation methods used to asses nitrogen mineralization: a microbiological perspective. Appl Soil Ecol 117:125–130. doi:10.1016/S0929-1393(01)00128-7

Aciego Pietri JC, Brookes PC (2008) Nitrogen mineralisation along a pH gradient of a silty loam UK soil. Soil Biol Biochem 40:797–802. doi:10.1016/j.soilbio.2007.10.014

Alauzis MV, Mazzarino MJ, Raffaele E, Roselli L (2004) Wildfires in NW Patagonia: long-term effects on a *Nothofagus* forest soil. Forest Ecol Manag 192:131–142. doi:10.1016/j.foreco.2003.11.014

Allen AS, Schlesinger WH (2004) Nutrient limitations to soil microbial biomass and activity in loblolly pine forests. Soil Biol Biochem 36:581–589. doi:10.1016/j.soilbio.2003.12.002

Bagherzadeh A, Brumme R, Beese F (2008) Temperature dependence of nitrogen mineralization and microbial status in O_H horizon of a temperate forest ecosystem. J For Res 19:37–43. doi:10.1007/s11676-008-0006-7

Bahamonde HA, Peri PL, Alvarez R, Barneix A, Moretto A, Martínez Pastur G (2012a) Litter decomposition and nutrients dynamics in *Nothofagus antarctica* forests under silvopastoral use in Southern Patagonia. Agrofor Syst 84:345–360. doi:10.1007/s10457-012-9479-7

Bahamonde HA, Peri PL, Alvarez R, Barneix A (2012b) Producción y calidad de gramíneas en un gradiente de calidades de sitio y coberturas en bosques de *Nothofagus antarctica* (G. Forster) Oerst. en Patagonia. Ecol Austral 22:62–73

Bengtsson G, Bengtsson P, Mansson KF (2003) Gross nitrogen mineralization-, immobilization-, and nitrification rates as a function of soil C/N ratio and microbial activity. Soil Biol Biochem 35:143–154. doi:10.1016/S0038-0717(02)00248-1

Bertiller MB, Mazzarino MJ, Carrera AL, Diehl P, Satti P, Gobbi M, Sain CL (2006) Leaf strategies and soil N across a regional humidity gradient in Patagonia. Oecologia 148:612–624. doi:10.1007/s00442-006-0401-8

Booth MS, Stark JM, Rastetter E (2005) Controls on nitrogen cycling in terrestrial ecosystems: an synthetic analysis of literature data. Ecol Monogr 75:139–157. doi:10.1890/04-0988

Bremner JM, Keeney ER (1965) Steam distillation methods for determination of ammonium, nitrate and nitrite. Anal Chem Acta 32:485–495

Breuer L, Kiese R, Butterbach-Bahl K (2002) Temperature and moisture effects on nitrification rates in tropical rain-forest soils. Soil Sci Soc Am J 66:834–844. https://www.soils.org/publications/sssaj/articles/66/3/834. Accessed 19 May 2011

Burke I, Lauenroth K, Parton W (1997) Regional and temporal variation in net primary production and nitrogen mineralization in grasslands. Ecology 78:1330–1340

Caldentey J, Ibarra M, Hernández J (2001) Litter fluxes and decomposition in *Nothofagus pumilio* stands in the región of Magallanes, Chile. For Ecol Manag 148:145–157. doi:10.1016/S0378-1127(00)00532-6

Clarkson DT, Hopper MJ, Jones LHP (1986) The effect of root temperature on the uptake of nitrogen and the relative size of the root system in *Lolium perenne*. I. Solutions containing both NH_4^+ and NO_3^-. Plant Cell Environ 9:535–545. doi:10.1111/1365-3040.ep11616282

Dalias P, Anderson JM, Bottner P, Couteaux MM (2002) Temperature responses of net nitrogen mineralization and nitrification in conifer forest soils incubated under standard laboratory conditions. Soil Biol Biochem 34:691–701. doi:10.1016/S0038-0717(01)00234-6

Dong S, Scagel CF, Cheng L, Fuchigami LH, Rygiewicz PT (2001) Soil temperature and plant growth stage influence nitrogen uptake and amino acid concentration of apple during early spring growth. Tree Physiol 21:541–547. doi:10.1093/treephys/21.8.541

Feller MC, Lehmann R, Olanski P (2000) Influence of forest harvesting intensity on nutrient leaching through soil in southwestern British Columbia. J Sustain For 10:241–247

Fisher RF, Binkley D (2000) Ecology and management of forest soils, 3rd edn. Wiley, New York

Gallardo A, Schlesinger WH (1992) Carbon and nitrogen limitations of soil microbial biomass in desert ecosystems. Biogeochemistry 18:1–17. doi:10.1007/BF00000423

Gargaglione V (2011) Dinámica de macronutrientes en bosques de *Nothofagus antarctica* de Patagonia Sur. Doctoral Thesis in Agricultural Sciences University of Buenos Aires (UBA)

Godoy R, Oyarzún C, Gerding V (2001) Precipitation chemistry in deciduous and evergreen *Nothofagus* forests of southern Chile under a low-deposition climate. Basic Appl Ecol 2:65–72

Grundmann GL, Renault P, Rosso L, Bardin R (1995) Differential effects of soil water content and temperature on nitrification and aeration. Soil Sci Soc Am J 59:1342–1349

Gurevitch J, Chester ST Jr (1986) Analysis of repeated measures experiments. Ecology 67:251–255. doi:10.2307/1938525

Hart SC, Binkley D (1985) Correlations among indices of forest soil nutrient availability in fertilized and unfertilized loblolly pine plantations. Plant Soil 85:11–21

Hook PB, Burke IC (2000) Biochemistry in a shortgrass landscape: control by topography, soil texture, and microclimate. Ecology 81:2686–2703. doi:10.1890/0012-9658(2000)081[2686:BIASLC]2.0.CO;2]

Huygens D, Boeckx P, Templer P, Paulino L, Van Cleemput O, Oyarzún C, Müller C, Godoy R (2008) Mechanisms for retention of bioavailable nitrogen in volcanic rainforests soils. Nat Geosci 1:543–548. doi:10.1038/ngeo252

Ivancich H, Martínez Pastur G, Peri PL (2011) Modelos forzados y no forzados para el cálculo de índice de sitio en bosques de *Nothofagus antarctica* en Patagonia Sur. Bosque 32: 135–145. http://www.scielo.cl/pdf/bosque/v32n2/art04.pdf. Accessed 12 Dec 2011

Jarvis SC, Stockdale EA, Shepherd MA, Powlson DS (1996) Nitrogen mineralization in temperate agricultural soils: processes and measurement. Adv Agron 57:187–235. doi:10.1016/S0065-2113(08)60925-6

Jussy JH, Ranger J, Bienaimé S, Dambrine E (2004) Effects of a clear-cut on the in situ nitrogen mineralization and the nitrogen cycle in a 67-year-old Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) plantation. Ann For Sci 61:397–408. doi:10.1051/forest:2004033

Kaye JP, Hart SC (1997) Competition for nitrogen between plant and soil organisms. Tree 12:139–143

Knoepp JD, Swank WT (2002) Using temperature soil and moisture to predict forest soil nitrogen mineralization. Biol Fertil Soils 36:177–182. doi:10.1007/s00374-002-0536-7

Lamb D (1980) Soil nitrogen mineralization in a secondary rain forest succession. Oecologia 47:257–263. doi:10.1007/BF00346829

Lencinas MV, Martínez Pastur G, Gallo E, Cellini JM (2011) Alternative silvicultural practices with variable retention to improve understory plant diversity conservation in southern Patagonian forests. Forest Ecol Manag 262:1236–1250. doi:10.1016/j.foreco.2011.06.021

Linn DM, Doran JW (1984) Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. Soil Sci Soc Am J 48:1667–1672. http://phoenix.nal.usda.gov/bitstream/10113/16745/1/GUA85010259.pdf. Accessed 17 Nov 2010

MacDonald NW, Zak DR, Pregitzer KS (1995) Temperature effects on kinetics of microbial respiration and net nitrogen and Sulphur mineralization. Soil Sci Soc Amer J 59:233–240

Martínez Pastur G, Cellini JM, Peri PL, Vukasovic RF, Fernández MC (2000) Timber production of Nothofagus pumilio forests by a shelterwood system in Tierra del Fuego (Argentina). Forest Ecol Manag 134:153–162

Mazzarino MJ, Bertiller M, Schlichter T, Gobbi M (1998a) Nutrient Cycling in Patagonian ecosystems. Ecol Austral 8:167–181. http://www.ecologiaaustral.com.ar/files/8-2-7.pdf. Accessed 11 Feb 2010

Mazzarino MJ, Bertiller MB, Sain C, Satti P, Coronato F (1998b) Soil nitrogen dynamics in northeastern Patagonia steppe under different precipitation regimes. Plant Soil 202:125–131. doi:10.1023/A:1004389011473

Moretto A, Lázzari A, Fernández O (2004) Calidad y cantidad de nutrientes de la hojarasca y su posterior mineralización en bosques primarios y bajo manejo con distintos sistemas de regeneración. Dirección de Bosques de la Secretaría de Ambiente y Desarrollo Sustentable de Nación (SAyDS). Proyecto BIRF 4085-AR. http://www.ambiente.gov.ar/archivos/web/PBVyAP/File/A3/PIARFON%20BAP/Descomposicion%20Lenga.pdf. Accessed 14 Nov 2011

Mulvaney RL (1996) Nitrogen inorganic forms. In: Sparks DL (ed) Methods of soil analysis. Part 3. Chemical methods, pp 1123–1184. SSSA-ASA, Madison, pp 1123–1184

Pérez CA, Hedin LO, Armesto JJ (1998) Nitrogen mineralization in two unpolluted old-growth forests of contrasting biodiversity and dynamics. Ecosystems 1:361–373. doi:10.1007/s100219900030

Pérez CA, Carmona MR, Armesto JJ (2003) Non-symbiotic nitrogen fixation, net nitrogen mineralization and denitrification in evergreen forests of Chiloé Island, Chile: a comparison with other temperate forest. Gayana Bot. 60: 25–33. doi:10.4067/S0717-66432003000100005

Peri PL, Gargaglione V, Martínez Pastur G (2008a) Above—and belowground nutrients storage and biomass accumulation in marginal *Nothofagus antarctica* forests in Southern Patagonia. Forest Ecol Manag 255:2502–2511. doi:10.1016/j.foreco.2008.01.014

Peri PL, Bahamonde HA, Monelos LH, Martínez Pastur G (2008b) Producción de Hojarasca en Bosques Primarios y bajo Manejo Silvopastoril de *Nothofagus antarctica* en la Provincia de Santa Cruz, Argentina. In: Bava J, Picco OA, Pildain MB, Bernal PL, Orellana I (eds) Segunda reunión sobre *Nothofagus* en la Patagonia, EcoNothofagus 2008.Esquel, Argentina, Abril de 2008, pp 149–155

Perry DA, Oren R, Hart SC (2008) Biogeochemical Cycling. In: Perry DA, Oren R, Hart SC (eds) Forest ecosystems, 2nd edn. The Johns Hopkins University Press, Baltimore, pp 347–370

Raison RJ, Connell MJ, Khanna PK (1987) Methodology for studying fluxes of soil mineral N in situ. Soil Biol Biochem 19:521–530. http://www.planta.cn/forum/files_planta/ok_185.pdf. Accessed 15 Sept 2011

Rennenberg H, Dannenmann M, Gessler A, Kreuzwieser J, Simon J, Papen H (2009) Nitrogen balance in forest soils: nutritional limitation of plant under climate change stresses. Plant Biol 11:4–23. doi:10.1111/j.1438-8677.2009.00241.x

Ritter E, Starr M, Vesterdal L (2005) Losses of nitrate from gaps of different sizes in a managed beech (*Fagus sylvatica*) forest. Can J For Res 35:308–319

Robertson GP (1984) Nitrification and nitrogen mineralization in a lowland rainforest succession in Costa Rica, Central America. Oecologia 61:99–104. doi:10.1007/BF00379093

Satti P, Mazzarino MJ, Gobbi M, Funes F, Roselli L, Fernández H (2003) Soil N dynamics in relation to leaf litter quality and soil fertility in north-western Patagonian forests. J Ecol 91:173–181. doi:10.1046/j.1365-2745.2003.00756.x

Soto J (2004) Clima. In: González L, Rial P (eds) Guía Geográfica Interactiva de Santa Cruz. Editorial Instituto Nacional de Tecnología Agropecuaria, Buenos Aires, Argentina, pp 24–28

Stark JM, Hart SC (1997) High rates of nitrification and nitrate turnover in undisturbed coniferous forests. Nature 385: 61–64. http://www.biology.usu.edu/files/uploads/Faculty/Stark-J/Stark1997-High%20rates%20of%20NO3%20turnover.pdf. Accessed 17 Oct 2010

Stienstra AW, Gunnewiek PK, Laanbroek HJ (1994) Repression of nitrification in soils under a climax grassland vegetation. Microbiol Ecol 14:45–52

Vitousek PM, Howarth RW (1991) Nitrogen limitation on land and in the sea: how can it occur? Biogeochemistry 13:87–115. doi:10.1007/BF00002772

Vitousek PM, Matson P (1985) Disturbance, nitrogen availability, and nitrogen losses in an intensively managed loblolly pine plantation. Ecology 60:1360–1376. http://www.jstor.org/pss/1939189. Accessed 29 Mar 2011

Vitousek PM, Melillo JM (1979) Nitrate losses from disturbed forests: patterns and mechanisms. Forest Sci 25:605–619

Willems P, Raffaele E (2001) Implicancias del efecto de las interacciones en la interpretación de experimentos factoriales en biología. Ecol Austral 11:131–138. http://www.ecologiaaustral.com.ar/files/70fdc341ce.pdf. Accessed 31 Oct 2009

Xueling Z, Qibing W, Linghao L, Xingguo H (2008) Seasonal variations in nitrogen mineralization under three land use types in a grassland landscape. Acta Oecol 34:322–330. doi:10.1016/j.actao.2008.06.004

Yahdjian L, Sala OE, Austin AT (2006) Differential controls of water input on litter decomposition and nitrogen dynamics in the patagonian steppe. Ecosystems 9:128–141. doi:10.1007/s10021-004-0118-7

Zak DR, Holmes WW, MacDonald NW, Pregitzer KS (1999) Soil temperature, matric potential, and the kinetics of microbial respiration and nitrogen mineralization. Soil Sci Soc Am J 63:575–584. https://www.agronomy.org/publications/sssaj/abstracts/63/3/SS0630030575?access=0&view=pdf. Accessed 14 Nov 2011