Selective logging of lowland evergreen rainforests in Chiloé Island, Chile: Effects of changing tree species composition on soil nitrogen transformations

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ABSTRACT

Lowland evergreen rainforests in southern Chile growing on highly productive soils and accessible sites have been subjected to traditional and industrial logging of valuable timber trees. Old-growth rain forests in this area are characterized by highly conservative N cycles, which results in an efficient N use of ecosystems. We hypothesize that different logging practices, by changing forest structure and species composition, can alter the quantity and quality (i.e. C/N ratio) of litterfall and soil organic matter and soil microbial processes that determine N storage and availability. To test this hypothesis we investigated chemical properties, microbial N transformations, N fluxes and N storage in soils of lowland evergreen rainforests of Chiloé Island after 10 years since industrial selective logging (ISL) and in stands subjected to traditional selective logging (TSL) by landowners in small properties. We compared them to reference unlogged old-growth stands (OG) in the same area. Tree basal area was more reduced in the stands subjected to ISL than to TSL. Litterfall inputs were similar in both logging treatments as in OG stands. This was due to greater biomass of understory species after logging. In TSL understory tree species determined a higher litterfall C/N ratio than ISL. We found higher soil N availability and content of base cations in surface soils of logged forests than in OG. The litter horizon of OG forest had significantly higher rates of non-symbiotic N fixation than logged forests. In the ISL treatment there was a trend toward increasing soil denitrification and significantly higher $NO_3–N/N_t$ ratio in spring waters, which led to a stronger $\delta^{15}N$ signal in surface and deep soils. We conclude that massive understory occupation by the shade-intolerant native bamboo *Chusquea quila* in ISL led to enhanced litter quality (lower C/N ratios) relaxing the tightness of the N cycle, which increased soil N availability leading to a higher proportion of nitrate in spring waters and higher gaseous N losses. In contrast, under TSL a higher litterfall C/N ratio slowed decomposition and net N mineralization rates thus reducing the chances for N losses, and enhancing C and N storage in soil. We suggest that sustainable logging practices in these rain forests should be based on lower rates of canopy removal to enhance colonization of the understory by shade-tolerant trees, which are associated with a more efficient N cycle.

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1. Introduction

Old-growth temperate rainforests of southern South America are strongly nitrogen-limited. The nutrients are efficiently retained in soils and above ground biomass reflecting that theses ecosystems are very conservative in nutrient use (Hedin et al., 1995; Pérez et al., 1998; Vann et al., 2002; Perakis and Hedin, 2001; Satti et al., 2003; Diehl et al., 2008). Limited nitrogen (N) inputs to these southern forest ecosystems derive primarily from non-symbiotic nitrogen fixation in forest soils, at the same time low rates of internal nitrogen cycling and denitrification have also been reported (Pérez et al., 2003a).

Logging practices that alter forest structure and tree species composition can be detrimental to ecosystem functions, especially those that depend on the chemical quality (e.g. C/N ratio) and quantity of organic matter entering the soil. Processes that are highly sensitive to the chemical quality and quantity of litter are those controlled by heterotrophic soil bacteria, responsible for N inputs and transformations in soils; e.g. non-symbiotic N fixation, denitrification, and net N mineralization and nitrification. In fact, more than 99.9% of total nitrification came from soil organic matter in a Chilean Andisol (Rütting et al., 2008). In northern temperate and boreal forests, logging often increases net N mineralization rates, soil N availability (Reynolds et al., 2000; Thibodeau et al., 2000; Hope et al., 2003; Lindo and Visser, 2003; Inagaki et al., 2008) and litter decomposition rates (Prescott, 1997; Brais et al., 2002). Such effects are generally associated with increases in soil temperature and enhanced soil moisture as a result of tree

removal (Reynolds et al., 2000; Thibodeau et al., 2000; Heithecker and Halpern, 2006). However, other studies have found limited or no effects of selective logging on soil N transformations and litter decomposition (Berg and Edmonds, 1999; Brais et al., 2002; Kranabetter and Coates, 2004; Westbrook et al., 2006; Idol et al., 2006; Jerabkova et al., 2006) and no effects of logging on soil carbon and nitrogen storage (Johnson and Curtis, 2001). These conflictive results may be due to differences in site factors among the studied ecosystems, such as climate, vegetation, time since logging disturbance, type of machinery used in the selective logging and land use history. Changes in N availability following disturbance can alter other microbial processes in ecosystems such as non-symbiotic N fixation and denitrification, but there is less documentation of the effect of logging on these ecosystems processes (but see Shaffer et al., 2000; Ballard, 2000; Griffiths and Swanson, 2001).

Most studies of biogeochemistry in southern Chilean temperate forests have been conducted in montane rain forests. Lowland primary rain forests developed on highly productive, glacial soils are disappearing much faster than higher elevation forests, however, due to logging, fire and land use changes, especially in the last decades (Wilson and Armesto, 1996; Echeverría et al., 2007). Nowadays lowland primary rain forests in Chiloé Island occupy less than one-third of its original distribution. Chile belongs to the group of countries that have increased its overall deforestation rate from 1.02% during 1980s to 1.76% during the 1990s (Jha and Bawa, 2006), which has greatly altered the landscape of this temperate forest region. It has been estimated that only 5% of logging of native forests is based on controlled silvicultural practices (Lara, 1996). Depending on logging intensity, selective logging scenarios can substantially alter forest structure and tree species composition (Rüger et al., 2007), mainly because of the broad diversity of light requirements of different timber species (Donoso et al., 1999; Gutierrez et al., 2004; Figueroa and Lusk, 2001).

The main hypothesis of this work was that the removal of tree biomass by logging practices, generally consisting of selectively removing valuable timber species, should alter N transformations mediated by soil heterotrophic bacteria, which are highly dependent of organic matter quality in unpolluted forests. To test this hypothesis, we compared nitrogen cycling in a forest stand affected by industrial selective logging of trees for timber production (65% of the canopy removed 10 years ago), with a stand subjected to traditional selective logging by local people (continuously harvested at lower rate) and a reference unlogged, old-growth stand under similar climate and soils. For this purpose, we measured the following responses to logging treatments: (1) tree species composition and cover, (2) chemical quality (e.g. C/N ratio) and quantity of litterfall, (3) N return via litterfall from vegetation to soil, (4) chemical properties of soils and spring waters, (5) soil microbial N transformations (e.g. non-symbiotic nitrogen fixation, net N mineralization and denitrification), (6) litter decomposition rates, and (7) microclimate.

2. Materials and methods

2.1. Study sites

Study sites were located in Melleico (42°37′08″S, 73°46′06″W), 12 km west from Chonchi, Isla Grande de Chiloé, Chile (Fig. 1). Forest type in the study area is evergreen Valdivian rain forest dominated by broad-leaved tree species, such as *Laureliopsis philippiana* (Monimiaceae) and different Myrtaceae species (Armesto and Figueroa, 1987). Prevailing climate is wet-temperate with a strong oceanic influence (Di Castri and Hajek, 1976). Meteorological records (4 years) at Senda Darwin Biological Station, located about 100 km north of the study site, indicate an annual rainfall of 2090 mm and a mean annual temperature of 12 °C. Maximum monthly temperatures (January) are 16 °C and minimum monthly temperatures (July–August) are 5 °C. Rainfall occurs throughout the year, but 64% of the precipitation falls between April (austral fall) and September (austral spring). The forests studied are situated at the foothills of the Coastal Range at ca. 100–200 m above sea level. A full description of the flora, vegetation structure and dynamics is provided by Gutierrez et al. (2009) and Pérez et al. (in press).

Within an area of 2 km² (Fig. 1), relatively homogeneous regarding topography and soils, we selected forest stands subjected to two logging treatments: (1) forests continuously logged by small landowners for limited timber extraction and
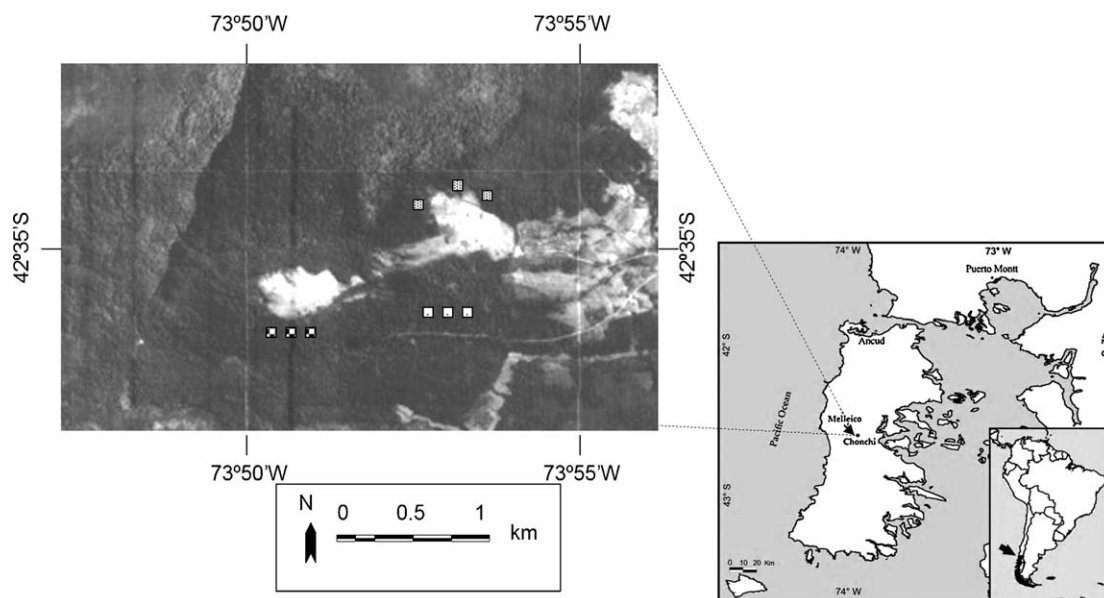
Fig. 1. Map of the study area in central Chiloé Island. Three reference spring waters in the OG forest: bottom left, three reference spring waters in ISL: bottom right, and three reference spring waters and plots in the TSL in the top. The orthophoto was taken in year 1993, so the ISL was still not applied.

harvest of firewood, the so-called traditional selective logging (TSL) and (2) forests industrially logged 10 years ago to extract timber for the production of wooden panels; known as industrial selective logging (ISL). In the second treatment, individuals >50 cm diameter at breast height (dbh) of the valuable timber species *L. philippiana* were extracted, leaving behind about 35% of the original mixed species canopy cover. An unlogged stand of old-growth forest (OG) ca. 300 years old was sampled as control, characterized by a fairly continuous canopy and regeneration dynamics associated with small tree-fall gaps. Estimates of canopy cover, with a spherical densitometer placed 1.30 m above the ground, indicated a 95.2% ground cover for the control forest, 94.4% for the stand subjected to ISL and 94% for the forest with TSL. Similar cover values among silvicultural treatments obtained with this method is due to the abundant colonization of the understory by species reaching more than 1.30 m height, such as the native bamboo *Chusquea quila* in ISL and abundant regeneration of shade-tolerant Myrtaceae tree species in the forest under TSL after opening of the canopy.

2.2. Vegetation, soil and litter sampling

Six permanent plots (50 m × 20 m) were set up in the study area; two in the OG forest, one in the ISL and three in the TSL. Plots were located at the centre of each forest stand (at least 200 m from forest margins) to eliminate the edge effect. Between year 2002 and 2005, all trees with a trunk diameter of >5 cm at breast height (1.3 m) and rooted within the plots were identified, tagged and their diameter at breast height (dbh) measured to the nearest centimetre.

Within the OG and logged forested watersheds, three spring waters were selected for sampling water chemistry and as references for soil sampling. Springs were located about 150–200 m from each other. In each spring, a first sample point was located 12 m away and perpendicular to the watercourse; the second sample point was located 12 m in the opposite direction of the first point. In total there were six soil-sampling points in each silvicultural treatment. Because the impacts of TSL on forests were more heterogeneous, three small watersheds were selected for sampling (Fig. 1), each of them had a spring crossing the forest. In each watershed a transect line was laid down across the mid point of the 0.1 ha permanent plot, selecting six sampling points, separated by about 12 m each other. In total there were 18 sampling points in the TSL. Litterfall was collected in 0.1 m^2 surface buckets covered with a nylon net, mesh size of 2 mm, one trap per sampling point. Litter samples were retrieved periodically and taken to the lab to obtain dry weights. The litter of three out of six traps per watershed was sorted by tree species and other components (leaves and fine woody debris) in each season and dry weighted. Litter collected was ground for the determination of total C and N. N return to the forest floor via litterfall was obtained by multiplying litter biomass input per season by their total N concentration.

Each of four seasons (spring, summer, fall and winter), from April 2005 to February 2008, mineral soil (A$_h$: 0–10 cm, B$_v$: 10–30 cm) and litter samples (O$_l$ horizon), were collected from the sample points in each forest stand or watershed and used for "in situ" experiments to assess N fluxes, and soil chemical properties as described below.

2.3. Litter decomposition experiment

The litterbag approach (Singh and Gupta, 1977) was used to estimate mass losses of fresh leaf litter through time in each plot. A 2-mm mesh size was used to allow the access of most of the mesofauna and all of the microfauna of decomposers. In August 2005, decomposition bags were filled with 5 g of dry material from the O$_l$ horizon consisting in recently fallen leaves and fine woody debris collected in July 2005. Eight litter decomposition bags were deposited on the forest floor under closed canopy in four sampling points per forest. Litter bags were removed 61, 94,152, 276, 450 and 822 days after the initiation of the experiment, taken to the lab, dried for 48 h at 70 °C, and then weighted to estimate mass loss. To obtain the decay constant (*k*) a negative exponential model was fitted to the mass loss trend over time (Olsen, 1963).

2.4. Spring water sampling

Each season, samples of running spring water were taken in acid washed 60 ml plastic bottles. Samples of running water were filtered on site using 0.45 μm pore size filter paper and sent for chemical analysis to the Institute of Ecosystem Studies, USA and to the Universität Trier, Germany. Spring water samples were analyzed to determine ammonium (colorimetric), nitrate (by ion chromatography) and total N (N$_t$; by catalytic combustion), including both dissolved organic and inorganic nitrogen concentrations.

2.5. Total C and N in soil and litterfall

Dry surface soil samples corresponding to the initial time for assessment of net N mineralization and litter collected from traps were ground for the determination of total N and C by means of flash combustion using a NA2500 Carlo Erba Element Analyzer at the Biogeochemistry Laboratory, Pontificia Universidad Católica de Chile (PUC). The C/N mass ratios of plant and soil samples were obtained in order to assess the chemical quality of organic matter. Litterfall and soil samples obtained in January 2007 were analyzed for the determination of natural abundances of δ^{15}N (‰) in a Thermo Delta V Advantage Isotope Ratio mass spectrometer, at the Universität Trier, Germany. These stable isotope ratios (^{15}N/^{14}N) indicate the amount of heavier in relation to lighter isotope. This type of measurement helps in the final interpretation of nitrogen dynamics in soils, in the sense that a higher amount of the heavier isotope in soils indicates faster turnover rates.

2.6. Exchangeable base cations, soil reaction, and water content of surface soils

The exchangeable base cations; calcium, magnesium potassium and sodium in soil, were extracted with in a 1 M ammonium acetate solution (1:10) and determined in Perkin Elmer 2380 AAS at the biogeochemistry laboratory, PUC. Soil reaction was determined with a pH electrode in a 1:5 soil:water suspension. Water content was determined gravimetrically.

2.7. In situ non-symbiotic N fixation rates

The acetylene reduction activity (ARA, Myrold et al., 1999) was used to estimate in situ non-symbiotic N fixation rates for different soil components: fine leaf litter (O$_l$ horizon), fine woody debris (FWD; twigs and branches < 5 cm diameter) and mineral soil (A$_h$ horizon). In each sampling point and for all forest stands, samples of O$_l$ horizon (composed mainly of leaves) and samples of FWD were taken and deposited inside 500 ml glass jars. Intact soil cores were taken from each sampling point using a 100 cm^3 steel cylinder. The soil core was carefully removed from the cylinder and deposited inside a glass jar. Immediately afterwards, the jars were hermetically closed. The samples were incubated in a mixture of air and acetylene at 10% (v/v) for up to 2 days. An additional sample per substrate type was incubated without acetylene as a control. Ethylene gas concentration at times 0, 1 and 2 days of incubation

was determined with a Schimadzu GC-8A gas chromatograph equipped with a FID detector, at the biogeochemistry laboratory, PUC. More details about methods are given by Pérez et al. (2004).

2.8. In situ net N mineralization rates

Soil samples from the surface (A_h) and deep (B_v) horizons were obtained and sieved in the field from each sampling point in control and logged stands. Each sample was divided in two subsamples, one of them was taken to the laboratory to determine the initial content of ammonium and nitrate in the soil solution (initial sample) and the second subsample was deposited inside polyethylene zip lock bags and returned to the soil at the same point; i.e. final sample (Eno, 1960). After 30–35 days of field incubation, final soil samples were recovered and taken to the laboratory for extraction of dissolved ammonium and nitrate in a 0.021 mol/L $KAl(SO_4)_2$ solution (1:4) and determination of concentration by fractionated steam distillation. More details about methods are given by Pérez et al. (1998).

2.9. Potential denitrification rates

Denitrification rates were determined by the acetylene inhibition assay in intact soil cores (Groffman et al., 1999) taking into account that soil nitrate concentration was relatively high and therefore the effect of nitrate reductase inhibition by acetylene would be minimal. In each sampling point in logged and control stands one sample was taken with a 100 cm^3 steel cylinder and stored for up to 6 h before incubation at room temperature and field water content. Soil samples were placed inside 500 ml hermetic glass jars and incubated for 6 h at room temperature under a 10% (v/v) acetylene atmosphere. Gas samples were taken at 0, 2 and 6 h during the incubation and stored in 3 ml Venojets. Samples were frozen until analyzed. The N_2O concentration of gas samples was determined with a Shimadzu GC-8A gas chromatographer, equipped with a Porapack column Q 80/100 and electron capture detector, at the biogeochemistry laboratory, PUC. Calibration curves were prepared from dilutions of a standard gas of 1 ppm nitrous oxide balance in nitrogen of Scotty analyzed gases. The N_2O concentrations in the gas samples were determined from the lineal fit of the calibration curve. Denitrification rates were estimated from N_2O–N concentration difference estimated from an area basis between incubation times 6 and 2.

2.10. Microclimate

In August 2005 three Hobo data loggers per silvicultural treatment and control stands were installed within the forests, located about 1.3 m above the ground and set to monitor hourly air temperature and humidity. Data were averaged monthly during the study period.

2.11. Statistical analyses

In order to assess the effect of the silvicultural treatments (3 levels; ISL, TSL, and control) and substrates (2–3 levels; O_l, A_h and B_v soil horizons) on N transformations and availability in forest soil, the data collected during the entire study period (April 2005–January 2008) was analyzed by two-way analysis of variance (ANOVA) and a-posteriori Tukey's tests. For $\delta^{15}N$ analysis only the sampling period of January 2007, was used. One-way ANOVA and a-posteriori Tukey's tests was applied to test for the effect of ISL, TSL and control treatments on annual rates of N transformations, N return to the forest floor, litterfall, air temperature and humidity (i.e. microclimate), and chemical properties of surface soils and spring water. None statistics were performed in relation to total

basal area of stands, as only one stand of ISL was considered for vegetation analysis. Differences in decomposition rates were assessed with a Tukey's test for multiple comparisons of slopes (Zar, 1996). In cases when the assumption of variance homogeneity was not fulfilled, the data were either ranked or log-transformed. Weighted average of C/N ratio from dominant leaf litter components was estimated, in order to compare these values with the C/N ratio of the bulk leaf litter obtained from the litter traps. In order to test the effect of natural variation of soil moisture on net nitrogen mineralization rates using the buried bag method, a three factors ANOVA (silvicultural treatment, soil depth and time of incubation; i.e. initial and final samples) for repeated measurements (11 months) of soil water content was used.

3. Results

3.1. Vegetation structure and species contribution to litterfall

Total tree basal area tended to be lower in ISL than in TSL or in OG stands (Table 1). The canopy of unlogged forests was dominated by the evergreen, broad-leaved species L. philippiana (Fig. 2A). Basal are of this species decreased to less than one-third in both types of logged stands. Amomyrtus luma and Caldlcuvia paniculata increased their overall importance under TSL and under ISL, respectively (Fig. 2A). In TSL stands other tree species such as Nothofagus nitida and Drimys winteri also increased their relative importance.

We found no statistical differences in the total input of fine litter and litter-associated N return to the forest floor in both logged forests compared to the reference OG forest (Table 1). In both OG and TSL litter flux was co-dominated by L. philippiana and several Myrtaceae species, but in the stand subjected to ISL the understory became dominated by the shade-intolerant native C. quila (Fig. 2B). The woody liana Hydrangea serratifolia was an important component of litterfall in both logged and control stands. One of the dominant tree species in the stand subjected to TSL, A. luma, had a significantly higher litterfall C/N ratio (i.e. lower litter quality) than the bamboo C. quila which became overly abundant in the litterfall of ISL stands and also higher than the litterfall C/N ratio of L. philippiana, the canopy dominant in OG forests (Fig. 3).
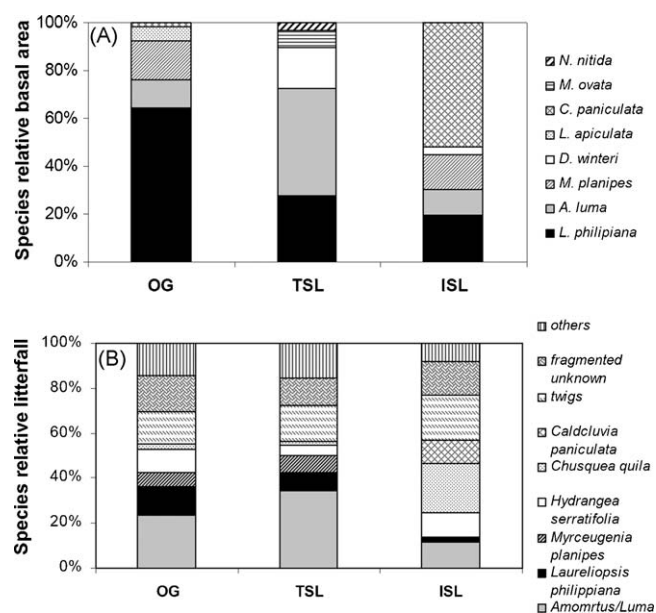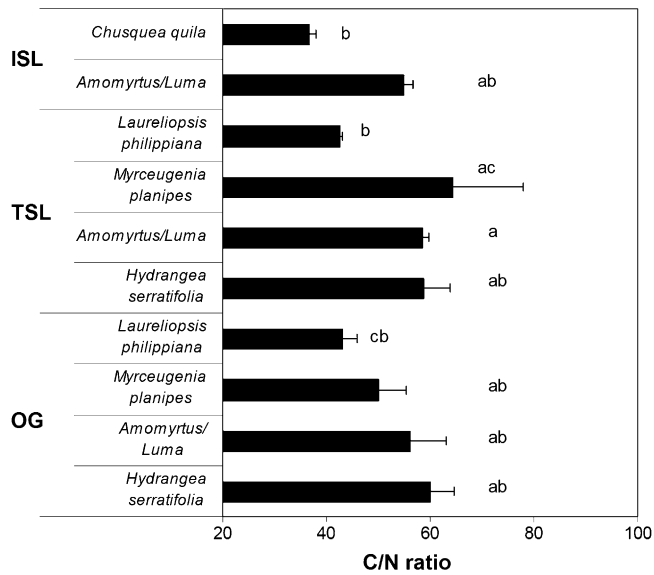

Fig. 2. Tree species contribution (%) to (A) stand basal area in unlogged old growth (OG), traditional selective logging (TSL) and industrial selective logging (ISL) and (B) litterfall biomass, in lowland evergreen rainforests of Chiloé Island.

Fig. 3. Average C/N ratio of senescent leaves based on 3-year seasonal data of dominant species in OG, TSL and ISL in lowland evergreen rainforests of Chiloé Island (average ± SE, $n = 6$ for OG and ISL and $n = 18$ for TSL). Different letters indicate significant differences among species, according to Tukey tests ($P < 0.05$).

3.2. Decomposition, litterfall, soil and spring water chemistry

The lowest decomposition rate, associated with significantly higher C/N ratios (i.e. lower litter quality) of both litterfall and surface soil, was measured in the stands subjected to TSL compared to OG forest and ISL stands (Table 1). Weighted average of C/N ratios are the following; OG = 53.0, ISL = 42.9, TSL = 56.8, which closely resemble the values obtained in the bulk litter (Table 1). Significantly lower C and N storage in surface soil were recorded in ISL stands compared to TSL treatment or OG forest. Litterfall was always depleted in $\delta^{15}N$ (‰) (i.e. negative values) and none significant differences were detected among silvicultural treatments (Table 1). A significantly higher NO_3–N/N_t ratio in the spring water was observed in forest under ISL than in the unlogged control forests (Table 1). The total concentrations of soil exchangeable base cations were significantly higher in both logged forests (TSL and ISL) than in OG forest. Soil pH was significantly lower under TSL than under ISL (Table 1).

Fig. 4. Average rates based on 3 years of seasonal data of (A) annual rates of non-symbiotic nitrogen fixation in the O_I and A_h horizons and fine woody debris (FWD), (B) N availability in A_h and B_v horizons, (C) annual rates of net N mineralization in A_h and B_v horizons and (D) annual rates of N_2O–N accumulation in surface soil in OG, TSL and ISL in lowland rainforests of Chiloé Island. Different letters indicate significant differences among silvicultural treatments and substrates, according to Tukey tests ($P < 0.05$) (average ± SE, $n = 6$ for OG and ISL and $n = 18$ for TSL).

3.3. Soil nitrogen transformations and natural abundance of $\delta^{15}N$

There was a significant effect of both silvicultural treatment and substrate on non-symbiotic N fixation (Fig. 4A and Table 2). OG
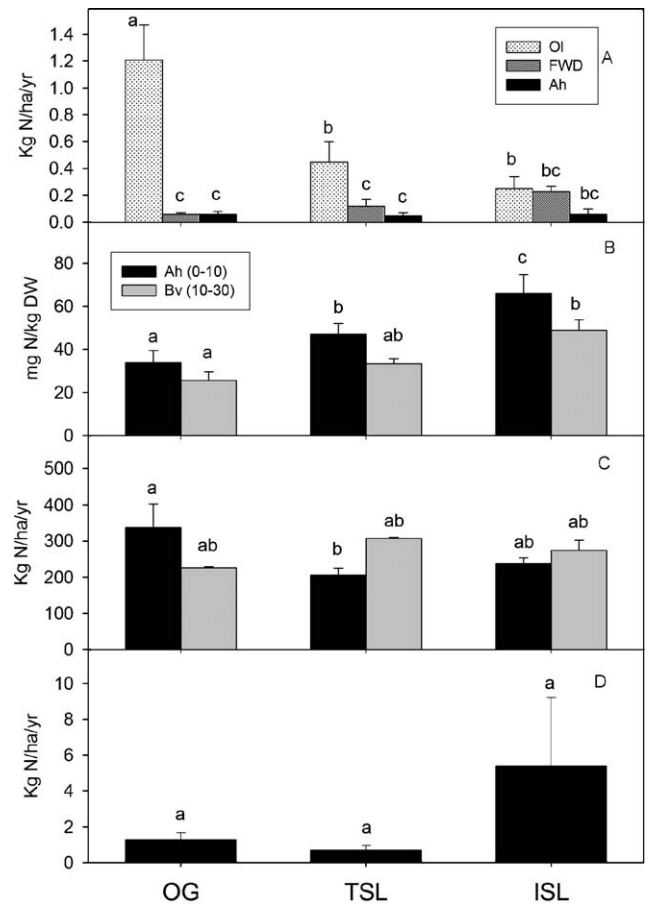
Table 1

Tree basal area of permanent plots, decomposition rate and averages (SE) of 3 years seasonal data of; litterfall flux, N return, net nitrification (nitrif.), and chemical properties of litterfall, surface soil and spring water, and carbon and nitrogen storage in surface soils of OG, ISL and TSL in lowland rainforests of Chiloé Island. F and P values belong to one-way ANOVAs. Different letters indicate significant differences among silvicultural treatments according to Tukey tests ($P < 0.05$) ($n = 6$ for OG and ISL and $n = 18$ for TSL).

	OG	ISL	TSL	F	P
Basal area (m²/0.1 ha)[a]	10 (4)	4	7 (3)	–	–
Litterfall (ton/ha/year)	5.5a (0.5)	4.8a (0.8)	5.4a (0.4)	0.3	0.7
N return (kg/ha/year)	57.8a (9.2)	63.7a (10.3)	51.4a (0.4)	0.6	0.6
Nitrif. (NO_3–N/ha/year)	110.0a (58.2)	77.3a (34.1)	41.7a (9.1)	0.743	0.485
k (year^{-1})	0.3a	0.3a	0.1b	9.5[b]	<0.001
C/N litterfall	44.6a (3.1)	39.1a (2.8)	53.6b (2.7)	9.9	0.001
C/N soil	15.0a (0.4)	16.5a (0.9)	19.6b (0.6)	10.7	<0.001
C pool (kg/ha)	7328ab (294)	6429a (287)	7358b (250)	3.7	0.039
N pool (kg/ha)	105.4ab (9)	99.1a (7)	123.6b (7.1)	3.9	0.33
$\delta^{15}N$ (‰) litterfall[c]	−2.1a (0.8)	−2.0a (0.5)	−2.6a (0.5)	0.3	0.718
NO_3^-–N/N_t spring water	0.1ac (0.0)	0.4b (0.2)	0.2bc (0.1)	8.7	0.017
Base cations (cmol/kg DW)	10.4a (1.8)	23.4b (4.9)	20.1b (5.9)	8.1	0.002
pH soil (H_2O)	4.8ab (0.1)	4.9b (0.1)	4.4a (0.2)	5.4	0.011

[a] $n = 2$ for OG, $n = 1$ for ISL, and $n = 3$ for TSL.

[b] Multiple slopes Tukey test.

[c] $n = 3$ for OG and ISL and $n = 9$ for TSL.

Table 2
Two-way ANOVAs of N transformations: ARA (acetylene reduction activity), N availability (Ndis), natural abundance of $\delta^{15}N$, Nmin (net N mineralization), nitrification and denitrification in soils of OG forests, TSL and ISL in lowland rainforests of Chiloé Island.

	F	P
ARA (nmol C_2H_4/g DW/day)		
Silvicultural treatment	6.2	0.003
Substrate	193.2	<0.001
Silvicultural treatment × substrate	6.4	<0.001
Ndis (mg N/kg DW)		
Silvicultural treatment	15.0	<0.001
Substrate	17.3	<0.001
Silvicultural treatment × substrate	1.4	0.247
$\delta^{15}N$ (‰)		
Silvicultural treatment	16.0	<0.001
Substrate	13.6	0.001
Silvicultural treatment × substrate	0.3	0.723
Nmin (kg/ha/year)		
Silvicultural treatment	1.7	0.186
Substrate	0.2	0.681
Silvicultural treatment × substrate	6.6	0.003
Nitrification (mg NO_3–N/kg DW/month)		
Silvicultural treatment	1.9	0.161
Substrate	2.3	0.132
Silvicultural treatment × substrate	0.9	0.433
Denitrification (μg N_2O–N/m^2/day)		
Silvicultural treatment	0.9	0.419

forest presented significantly higher non-symbiotic N-fixation rate in the O_l horizon than soils of both logging treatments, and the upper soil horizon was significantly more active than samples from the A_h horizon or FWD, except in ISL treatment where FWD presented similar rates to the O_l horizon (Fig. 4A). There was a significant effect of both silvicultural treatment and substrate on soil nitrogen availability (Fig. 4B and Table 2). The concentration of available nitrogen in surface soils was significantly higher in both logging treatments than in the unlogged old-growth forest (Fig. 4B). Under ISL the concentration of available N was significantly higher in the A_h mineral horizon than in the B_v. In the other forests we found no significant differences in available N among soil horizons (Fig. 4B). There was a significant effect of both silvicultural treatment and substrate on the natural abundance of $\delta^{15}N$ (Fig. 5 and Table 2). The natural abundance of $\delta^{15}N$ was always positive in soils and significantly higher in both soil horizons in the ISL than in both other forests. In TSL deeper soil horizon presented a significantly higher $\delta^{15}N$ than surface soil (Fig. 5). The significant interactive effect in net N mineralization
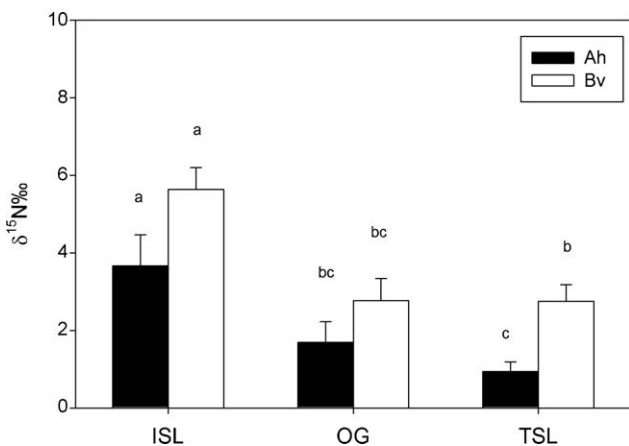
Fig. 5. Natural abundance of $\delta^{15}N$ in surface (A_h horizon) and deep soils (B_v horizon) in OG, TSL and ISL in lowland rainforests of Chiloé Island. Different letters indicate significant differences among silvicultural treatments and substrates, according to Tukey tests ($P < 0.05$) (average ± SE, $n = 3$ for OG and ISL and $n = 9$ for TSL).

rates (Table 2) indicate that only in the surface soil there are significant differences among silvicultural treatments as is the case of the reported higher rates in OG than in TSL (Fig. 4C). The water content of the in situ incubated soil samples within the buried bags did not differ from the initial samples ($F_{1,81} = 0.51$; $P = 0.47$). Net nitrification remained similar between logged and control forests and at both soil depths (Tables 1 and 2). There was a trend toward higher denitrification rates in the mineral soil horizon A_h of the ISL, however this difference was not statistically significant (Fig. 4D and Table 2).

3.4. Seasonal trends

Mean monthly air temperature and air humidity did not differ ($F_{2,87} = 1.234$; $P = 0.296$ and $F_{2,87} = 0.558$; $P = 0.574$, respectively) between stands subjected to different logging practices and did not differ from unlogged old-growth stands (Fig. 6A and B). The average annual temperature over 2 years of study was 9.9 °C in OG unlogged forest, 9.5 °C in ISL and 8.7 °C in TSL stands. Average air humidity remained most of the time near 100% in all forest stands compared. Soil moisture was significantly lower by about 10% in the OG forest than in both logging treatments during the study period ($F_{2,27} = 15.064$; $P < 0.0001$) (Fig. 6B). Non-symbiotic N fixation, measured by ARA, decreased significantly during the warmer and drier austral summer (Fig. 6C). Net N mineralization (Fig. 6D) and denitrification rates in forest soils (Fig. 6E) showed no seasonal trends. Net N mineralization rates declined during 2007, except for summer 2007–2008. N return via litterfall was similar in logged and unlogged forests (Fig. 6F and Table 1), with lower fluxes during austral spring (October).

4. Discussion

4.1. The effect of logging on vegetation and soil

According to remaining basal area, tree removal in the TSL stand was less intense than in forests subjected to ISL. The fact that the reduction in basal area with ISL was not reflected in a lower litter input in this forest can be explained by the additional contribution of the fast growing understory species of C. quila, which follows an intense canopy opening in ISL, by the reduction to 35% from the original canopy cover. Differences in understory species composition between logging treatments are due to the higher light requirement of the shade-intolerant bamboo C. quila compared to the shade-tolerant A. luma, which presents abundant advanced regeneration under the forest canopy (González et al., 2002; Lusk and Kelly, 2002). Ten years after the application of industrial selective logging, shade-intolerant species such as C. paniculata dominate the basal area in the ISL stands with an understory of C. quila that dominates litter flux. Under TSL, other shade-intolerant species co-dominate basal area, such as D. winteri and N. nitida, whereas C. quila is not an important component of the understory. Silvicultural practices did not affect N return via litterfall to the forest floor, but significantly changed the C/N ratio of falling litter. The higher litterfall C/N ratio of TSL stands is due to a shift in canopy dominance from Laureliopsis to other shade-tolerant canopy species such as A. luma and Myrceugenia planipes, which have a higher C/N ratio than the bamboo species C. quila in ISL and L. philippiana in the canopy of OG stands. Other co-dominant species in TSL stands such as N. nitida and D. winteri also have a higher litterfall C/N ratio (Pérez et al., 2003b), which collectively contribute to the higher C/N ratio of bulk litterfall in these forests compared to those subjected to ISL. The similar values of C/N ratios obtained in the bulk litter and the weighted average from leaf litter of dominant tree species confirms the fact that they are the main determinants to the bulk C/N ratio at ecosystem level.
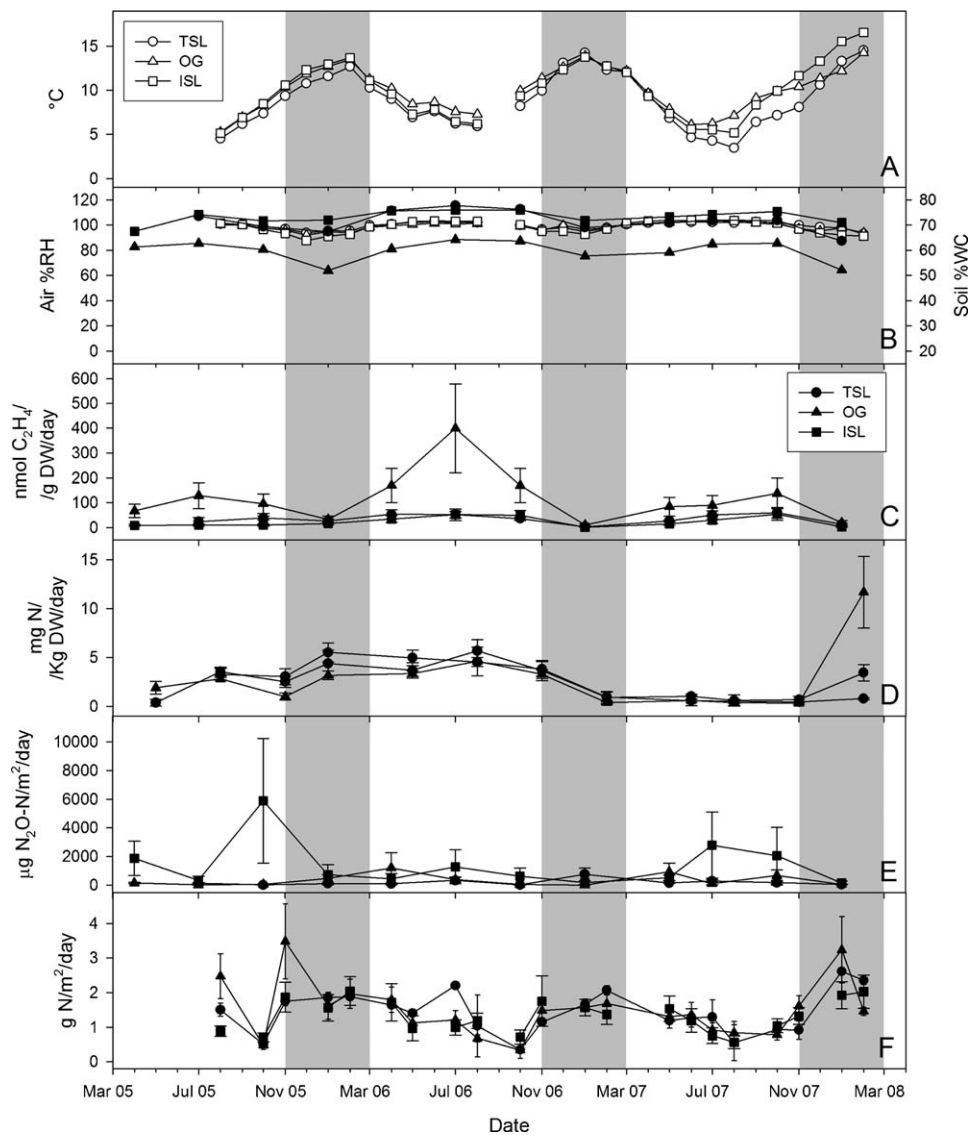
Fig. 6. Temporal variation of (A) air temperature, (B) air relative humidity (open symbols) and water content of soil (filled symbols), (C) acetylene reduction activity, (D) net N mineralization, (E) denitrification and (F) N return in the litterfall in OG, ISL and TSL in lowland rainforest of Chiloé Island. Shaded areas in the graph indicate the length of austral summer (average ± SE, $n = 6$ for OG and ISL and $n = 18$ for TSL).

Carbon and nitrogen storage and soil C/N ratio were differentially affected by logging type, so ISL had a lower carbon and nitrogen storage and lower soil C/N ratio than TSL. Increases in the content of exchangeable of base cations in soils with logging found in this study have also been reported in northern temperate forests due to a possible decreased vegetation uptake after the removal of tree biomass (Brais et al., 2004).

4.2. Seasonal trends and the effect of logging on microclimate

Logging did not significantly change microclimatic conditions within the forest stand about a decade after tree harvesting. Air temperature and humidity did not differ from OG, unlogged forest, however the lower soil moisture found in the OG forest than in logged stands may result from higher evapotranspiration associated with higher canopy leaf area in unlogged forests. Díaz et al. (2007) have shown that water table may rise in forest cleared stands of Chiloé Island due to reduced canopy interception and transpiration of rainfall.

The observed seasonal trends lead us to conclude that non-symbiotic N fixation by soil bacteria will be inhibited and litter N

flux enhanced during the warmer and drier summers of the study area. Such conditions may vary among years, presumably in direct relationship with climate variability driven by El Niño Southern Oscillation (ENSO) in the Pacific coast of southern South America (Montecinos and Aceituno, 2003).

4.3. The effect of logging on decomposition and N transformations

A higher litterfall C/N ratio in TSL, together with lower decomposition and net N mineralization rates in surface soils compared to OG forest resulted in greater soil storage of C and N. In contrast, lower C/N ratio of litterfall in ISL stands, together with enhanced denitrification and decomposition rates decreased soil C and N storage. Forest management had similar effects on litter C/N ratios and storage of these elements in organic soils of northern temperate forests (Klemedtsson et al., 2005). In both logged forests, observed increases of soil N availability were not associated with higher rates of soil N mineralization, but it is probably due to a decreased root sorption capacity associated to a decreased basal area in ISL. In other terms, N consumption by aboveground biomass would decrease in this logged forest,

increasing N availability on soils. In TSL the increase in N availability may be due to lower N requirements by the above-ground biomass as it is suggested by the high C/N ratio of litterfall.

Higher soil N availability could enhance a higher $NO_3–N/N_t$ ratio in the spring waters of ISL. N losses are manifested in the enhanced rates of denitrification measured in the ISL stands. High losses of N due to denitrification in soils are supported by the more positive $\delta^{15}N$ signal in stands subjected to ISL, because denitrification causes one of the highest fractionation of soil N (Robinson, 2001) by which light fraction of N gaseous products leave the ecosystems, leaving behind ^{15}N enriched soils. Higher denitrification rates, associated with increased soil N availability, have been measured in young stands of post-logging chronosequences in Douglas-fir forests from north western USA (Griffiths and Swanson, 2001). The fact that both logging practices significantly increase N availability in soils, but denitrification was higher only under ISL is related to the fact that C/N ratio was higher in TSL, suggesting a decrease in carbon lability for heterotrophic denitrifiers in the latter. Higher litterfall C/N ratio was also associated to lower net N mineralization in surface soils of TSL stands than in OG forest, suggesting an increase in N immobilization by soil bacteria under TSL.

The marked decrease in non-symbiotic, bacterial N fixation in the surface soil of both logged forests with respect to the unlogged stand was associated to decreases of net N mineralization in TSL. Studies in northern temperate forests have shown that clear cutting changed the species composition of leaf litter and, at the same time, the community composition of diazotrophic bacteria (Shaffer et al., 2000), which may alter microbial activity. Lower soil N mineralization, associated with reductions in soil microbial biomass and mesofauna, has also been documented in logged northern temperate forests (Lindo and Visser, 2003; Thibodeau et al., 2000). Hence, our results support the argument that logging may alter the rates of processes controlling N fluxes and recycling in forest stands.

Denitrification rates reported here for Chilean unlogged, old-growth forests are similar to values of 1.9 kg N/ha/year reported for northern temperate forest soils (Barton et al., 1999). Because the denitrification rates measured in the unlogged evergreen rain forests studied in Chiloé Island are very similar to non-symbiotic N fixation rates per unit area of soil, it can be suggested that both gaseous inputs and outputs of N are closely balanced. A study of soil microbial diversity using molecular markers in a similar old-growth, lowland rain forest of Chiloé Island reported abundant representation of different clones of N-fixing Rhizobiales, Azospirillum and Flavobacterium (Guevara, 2007). These diazotrophs are also capable of denitrification (Paul and Clark, 1989). The particular bacterial assemblage present in OG forest soils, which perform two opposite ecosystem functions, may explain the tight annual balance between N-fixation and denitrification reported in this paper.

5. Conclusions

The higher C/N ratio of litterfall and soil found in TSL is due to the dominance of shade-tolerant tree species with higher C/N ratio. This affected in lower decomposition rates, higher carbon and nitrogen pools and lower soil pH under TSL. Exchangeable base cations and nitrogen availability was higher in both logged forests than in OG. A higher $NO_3–N/N_t$ ratio in spring waters and a trend toward higher denitrification rates was found in ISL than in OG forests. Non-symbiotic N fixation was drastically reduced in both logging treatments. In summary ISL showed a more leaky N cycle than OG and TSL as it is reflected in the more positive ^{15}N signal in its soils. The fact that TSL evidenced a tighter N cycle than ISL can be attributed to the less intensive canopy removal under TSL,

which allowed the rapid colonization of the understory by shade-tolerant tree species, such as several species of Myrtaceae. This response contrasts with the massive occupation of the understory of the ISL stand by the shade-intolerant bamboo C. quila. This invasive native bamboo has lower C/N ratio of litterfall, i.e. a better litter quality and dominates post-logging litter flux in ISL. Therefore, sustainable logging practices in lowland southern temperate forest should consider leaving behind a higher proportion of basal area, such as to decrease the area of canopy openings that favours bamboo invasion. Smaller openings would favour regeneration by intermediate tolerance and shade-intolerant tree species, which make the majority of the successional tree species assemblage in these rain forests (Figueroa and Lusk, 2001), hence maintaining the tightness of the nitrogen cycle.

References

Armesto, J.J., Figueroa, J., 1987. Stand structure and dynamics in the temperate rain forests of Chiloé Archipielago, Chile. J. Biogeogr. 14, 367–376.

Ballard, T.M., 2000. Impacts of forests management on northern forest soils. Forest Ecol. Manage. 133, 37–42.

Barton, L., McLay, C.D., Schipper, L.A., Smith, C.T., 1999. Annual denitrification rates in agricultural and forest soils: a review. Aust. J. Soil Res. 37, 1073–1093.

Berg, A.K., Edmonds, R.L., 1999. Influence of partial cutting on site microclimate, soil nitrogen dynamics, and microbial biomass in Douglas-fir stands in western Washington. Can. J. Forest Res. 29, 705–713.

Brais, S., Paré, D., Camiré, C., Rochon, P., Vasseur, C., 2002. Nitrogen net mineralization and dynamics following whole-tree harvesting and winter windrowing on clayed sites on northwestern Quebec. Forest Ecol. Manage. 157, 119–130.

Brais, S., Harvey, B.H., Bergeron, Y., Messier, C., Green, D., Belleau, A., Paré, D., 2004. Testing forest ecosystem management in boreal mixedwoods of northwestern Quebec: initial response of aspen stands to different levels of harvesting. Can. J. Forest Res. 34, 431–446.

Díaz, F., Bigelow, S., Armesto, J.J., 2007. Alteration of the hydrologic cycle due to forest clearing and its consequences for rainforest succession. Forest Ecol. Manage. 244, 32–40.

Di Castri, F., Hajek, E.R., 1976. Bioclimatología de Chile. Vicerrectoría de Comunicaciones. Universidad Católica de Chile, Santiago, pp. 128.

Diehl, P., Mazzarino, M.J., Fontela, S., 2008. Plant limiting nutrients in Andean–Patagonian woody species: effects of interannual rainfall variation, soil fertility and mycorrhizal infection. Forest Ecol. Manage. 255, 2973–2980.

Donoso, C., Donoso, P., González, M., Sandoval, V., 1999. Los bosques siempreverdes. In: Donoso, C., Lara, A. (Eds.), Silvicultura de los bosques nativos de Chile. Editorial Universitaria, Santiago, pp. 297–337.

Echeverría, C., Newton, A.C., Lara, A., Benayas, J.M.R., Coomes, D.A., 2007. Impacts of forest fragmentation on species composition and forest structure in the temperate landscape of southern Chile. Global Ecol. Biogeogr. 16, 426–439.

Eno, C.F., 1960. Nitrate production in the field by incubating the soil in polyethylene bags. Soil Sci. Soc. Am. Proc. 24, 277–299.

Figueroa, J.A., Lusk, C.H., 2001. Germination requirements and seedling shade tolerance are not correlated in a Chilean temperate rain forest. New Phytol. 152, 483–489.

González, M.E., Veblen, T.T., Donoso, C., Valeria, L., 2002. Tree regeneration responses in a lowland Nothofagus-dominated forest after bamboo dieback in South-Central Chile. Plant Ecol. 161, 59–73.

Griffiths, R.P., Swanson, A.K., 2001. Forest soil characteristics in a chronosequence of harvested Douglas-fir forests. Can. J. Forest Res. 31, 1871–1879.

Groffman, P.M., Holland, E.A., Myrold, D.D., Robertson, G.P., Zou, X., 1999. Denitrification. In: Robertson, G.P., Coleman, D.C., Bledsoe, C.S., Sollins, P. (Eds.), Standard Soil Methods for Long Term Ecological Research. Oxford University Press, New York, pp. 272–288.

Guevara, R., 2007. Diversidad genética y funcional de grupos bacterianos en suelos con diferente tipo de cobertura vegetal: Efectos biogeográficos y perturbación humana. PhD Thesis. Universidad de Chile, Santiago, pp. 170.

Gutierrez, A., Armesto, J.J., Aravena, J.C., 2004. Disturbance and regeneration dynamics of an old-growth Nord-Patagonian rainforest in Chiloé Island, Chile. J. Ecol. 92, 598–608.

Gutierrez, A., Armesto, J.J., Aravena, J.C., Carmona, M., Carrasco, N., Christie, D., Peña, M.P., Pérez, C., Huth, A., 2009. Structural and environmental characterization of old-growth temperate rainforests of northern Chilé Island, Chile: regional and global relevance. Forest Ecol. Manage. 258, 376–388.

Hedin, L.O., Armesto, J.J., Johnson, A.H., 1995. Patterns of nutrient loss from unpolluted old-growth temperate forests: evaluation of biogeochemical theory. Ecology 76, 493–509.

Heithecker, T.D., Halpern, C.B., 2006. Variation in microclimate associated with dispersed-retention harvests in coniferous forests of western Washington. Forest Ecol. Manage. 226, 60–71.

Hope, G.D., Prescott, C.E., Blevins, L.L., 2003. Responses of available soil nitrogen and litter decomposition to openings of different sizes in dry interior Douglas-fir forests in British Columbia. Forest Ecol. Manage. 186, 33–46.

Idol, T.W., Pope, P.E., Ponder Jr., F., 2006. N mineralization, nitrification, and N uptake across a 100-year chronosequence of upland hardwood forests. Forest Ecol. Manage. 176, 509–518.

Inagaki, Y., Kuramoto, S., Torii, A., Shinomiya, Y., Fukata, H., 2008. Effects of thinning on leaf-fall and leaf-litter nitrogen concentration in hinoki cypress (*Chamaecyparis obtusa* Endlicher) plantations stands in Japan. Forest Ecol. Manage. 255, 1859–1867.

Jerabkova, L., Prescott, C., Kishchuk, B.E., 2006. Effect of variable-retention harvesting on soil nitrogen availability in boreal mixed forests. Can. J. Forest Res. 36, 3029–3038.

Jha, S., Bawa, K.S., 2006. Population growth, human development, and deforestation in biodiversity hotspots. Conserv. Biol. 20, 906–912.

Johnson, D.W., Curtis, P.S., 2001. Effects of forest management on soil C and N storage: meta analysis. Forest Ecol. Manage. 140, 227–238.

Klemedtsson, L., Von Arnold, K., Weslien, P., Gundersen, P., 2005. Soil C/N ratio as a scalar parameter to predict nitrous oxide emissions. Global Change Biol. 11, 1142–1147.

Kranabetter, J.M., Coates, K.D., 2004. Ten-year postharvest effects of silviculture systems on soil-resource availability and conifer nutrition in a northern temperate forest. Can. J. Forest Res. 34, 800–809.

Lara, A., 1996. Una propuesta general de silvicultural para Chile. Ambiente y Desarrollo 12, 31–40.

Lindo, Z., Visser, S., 2003. Microbial biomass, nitrogen and phosphorus mineralization, and mesofauna in boreal conifer and deciduous forest floors following partial and clear-cut harvesting. Can. J. Forest Res. 33, 1610–1620.

Lusk, C.H., Kelly, C.K., 2002. Interspecific variation in seed size and safe sites in a temperate rain forest. New Phytol. 158, 535–541.

Montecinos, A., Aceituno, P., 2003. Seasonality of the ENSO-related rainfall variability in central Chile and associated circulation anomalies. J. Climate 16, 281–296.

Myrold, D.D., Ruess, R.R., Klug, M.J., 1999. Dinitrogen fixation. In: Robertson, G.P., Coleman, D.C., Bledsoe, C.S., Sollins, P. (Eds.), Standard Soil Methods for Long Term Ecological Research. Oxford University Press, New York, pp. 241–257.

Olsen, J.S., 1963. Energy storage and the balance of producers and decomposers in ecological systems. Ecology 44, 322–331.

Paul, E.A., Clark, F.E., 1989. Soil Microbiology and Biochemistry. Academic Press, USA, pp. 275.

Perakis, S., Hedin, L., 2001. Fluxes and fates of nitrogen in soil of an unpolluted old-growth temperate forest, southern Chile. Ecology 82, 2245–2260.

Pérez, C.A., Hedin, L.O., Armesto, J.J., 1998. Nitrogen mineralization in two unpolluted old-growth forests of contrasting biodiversity and dynamics. Ecosystems 1, 361–373.

Pérez, C.A., Carmona, M.R., Armesto, J.J., 2003a. Non-symbiotic nitrogen fixation, net nitrogen mineralization, and denitrification in evergreen forest of Chiloé Island, Chile: a comparison with other temperate forests. Gayana 60, 25–33.

Pérez, C.A., Armesto, J.J., Torrealba, C., Carmona, M.R., 2003b. Litterfall dynamics and nutrient use efficiency in two evergreen temperate rain forests of southern Chile. Aust. Ecol. 28, 291–600.

Pérez, C.A., Carmona, M.R., Aravena, J.C., Armesto, J.J., 2004. Successional changes in soil nitrogen availability, non symbiotic nitrogen fixation and C/N ratios in southern chilean forest ecosystems. Oecologia 140, 617–625.

Pérez, C.A., Carmona, M.R., Aravena, J.C., Fariña, J.M., Armesto, J.J. Land Cover Change from Primary to Secondary Lowland Forests: Effects on Tree Species Composition and C/N Ratio of Litter and Soil. Academia Press, Belgium, in press.

Prescott, C., 1997. Effects of clearcutting and alternative silvicultural systems on rates of decomposition and nitrogen mineralization in a coastal montane coniferous forest. Forest Ecol. Manage. 95, 253–260.

Reynolds, P.E., Thevathasan, N.V., Simpson, J., Gordon, A., Lautenschlager, A.M., Bell, R.A., Gresch, W.F., Buckley, D.A., 2000. Alternative forest release treatments affect microclimate and soil nitrogen mineralization. Forest Ecol. Manage. 133, 115–125.

Robinson, D., 2001. δ15N as an integrator of the nitrogen cycle. Trends Ecol. Evol. 16, 153–162.

Rüger, N., Gutiérrez, A.G., Kissling, W.D., Armesto, J.J., Huth, A., 2007. Ecological impacts of different harvesting scenarios for temperate evergreen rain forest in southern Chile: a simulation experiment. Forest Ecol. Manage. 252, 52–66.

Rütting, T., Huygens, D., Müller, C., Van Cleemput, O., Godoy, R., Boeckx, P., 2008. Functional role of DNRA and nitrite reduction in a pristine south Chilean Nothofagus forest. Biogeochemistry 90, 243–258.

Satti, P., Mazzarino, M.J., Gobbi, M., Funes, F., Roselli, L., Fernandez, H., 2003. Soil N dynamics in relation to leaf litter quality and soil fertility in north-western Patagonian forests. J. Ecol. 91, 173–181.

Shaffer, B.T., Widmer, F., Porteus, L.A., Seidler, R.J., 2000. Temporal and spatial distribution of the nifH gene of N_2 fixing bacteria in forests and clearcluts in western Oregon. Microb. Ecol. 39, 12–21.

Singh, A.P., Gupta, S.R., 1977. Plant decomposition and soil respiration in terrestrial ecosystems. Bot. Rev. 43, 449–528.

Thibodeau, L., Raymond, P., Camiré, C., Munson, A.D., 2000. Impact of precomercial thinning in balsam fir stands on soil nitrogen dynamics, microbial biomass, decomposition and foliar nutrition. Can. J. Forest Res. 30, 229–238.

Vann, D.R., Joshi, A., Pérez, C., Johnson, A.H., Frizano, J., Zarin, D.J., Armesto, J.J., 2002. Distribution and cycling of C, N, Ca, Mg, K and P in three pristine, old-growth forests in the Cordillera de Piuchué, Chile. Biogeochemistry 60, 25–47.

Westbrook, C., Devito, K.J., Allan, C.J., 2006. Soil N cycling in harvested and pristine boreal forests and peatlands. Forest Ecol. Manage. 234, 227–237.

Wilson, M. F., Armesto, J. J., 1996. The natural history of Chiloé: on Darwin's trail. Revista Chilena de Historia Natural 69, 149-161.

Zar, J.H., 1996. Biostatistical Analysis, third edition. Prentice Hall, Upper Saddle River, pp. 622.