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## Successional changes in soil nitrogen availability, non-symbiotic nitrogen fixation and carbon/nitrogen ratios in southern Chilean forest ecosystems

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**Abstract** Vast areas of southern Chile are now covered by second-growth forests because of fire and logging. To study successional patterns after moderate-intensity, anthropogenic fire disturbance, we assessed differences in soil properties and N fluxes across a chronosequence of seven successional stands (2–130 years old). We examined current predictions of successional theory concerning changes in the N cycle in forest ecosystems. Seasonal fluctuations of net N mineralization ( $N_{\min}$ ) in surface soil and N availability ( $N_a$ ;  $N_a = \text{NH}_4^+ - \text{N} + \text{NO}_3^- - \text{N}$ ) in upper and deep soil horizons were positively correlated with monthly precipitation. In accordance with theoretical predictions, stand age was positively, but weakly related to both  $N_a$  ( $r^2=0.282$ ,  $P<0.001$ ) and total N ( $N_{\text{tot}}$ ;  $r^2=0.192$ ,  $P<0.01$ ), and negatively related to soil C/N ratios ( $r^2=0.187$ ,  $P<0.01$ ) in surface soils. A weak linear increase in soil  $N_{\min}$  (upper plus deep soil horizons) was found across the chronosequence ( $r^2=0.124$ ,  $P<0.022$ ).  $N_{\min}$  occurred at modest rates in early successional stands, suggesting that soil disturbance did not impair microbial processes. The relationship between N fixation ( $N_{\text{fix}}$ ) in the litter layer and stand age best fitted a quadratic model ( $r^2=0.228$ ,  $P<0.01$ ). In contrast to documented successional trends for most temperate, tropical and Mediterranean forests, non-symbiotic  $N_{\text{fix}}$  in the litter layer is a steady N input to unpolluted southern temperate forests during mid and late succession, which may compensate for hydrological losses of organic N from old-growth ecosystems.

**Keywords** Nitrogen cycle · Net nitrogen mineralization · Southern temperate forests · Secondary succession

### Introduction

Compared to remote montane forests, lowland tropical and temperate forests have suffered greater destruction worldwide, because of land clearing for agriculture and human habitation. Before the Spanish conquest, evergreen rainforests, known as Valdivian and North Patagonian forests (Schmithüsen 1957; Veblen et al. 1983), were continuously distributed from 40° to 43°S in south-central Chile. During the second half of the nineteenth century, and especially in the twentieth century, these forests were cleared and degraded through the use of fire and logging (Donoso and Lara 1996; Wilson and Armesto 1996), leaving only scattered remnants. As an illustration of this process, when Charles Darwin visited Chiloé Island, southern Chile, in the summer of 1835, he reported a dark green forest covering the island from coast to coast except around small cottages (Darwin 1860). He also reported the occurrence of forest fires in the island during the dry summer period (Darwin 1860). Today, continuous old-growth forests are restricted to <30% of the island area (CONAF-CONAMA-BIRF 1999), predominantly due to land clearing for agriculture, by the use of fire and logging during the twentieth century. These changes are representative of those occurring over a large fraction of the land in south-central Chile, where degraded forests cover an estimated area of 3 million ha, according to a recent land cover survey (CONAF-CONAMA-BIRF 1999). As a result, patches of second-growth forest in various stages of succession cover vast areas of the regional landscape.

The aim of this work was to explore the successional patterns of lowland second growth forests in Chiloé Island, focussing particularly on ecosystem fluxes regulating soil N availability, such as net N mineralization and non-symbiotic N fixation and a key factor affecting these fluxes, namely soil C/N ratios. We studied successional processes across a chronosequence of remnant forest

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stands in a rural landscape in northern Chiloé Island. The chronosequence included young, pioneer forest stands (<11 years old), mid-successional (30–60 years old) and late-successional forests (130 years old). Pioneer and mid-successional stands were recovering from anthropogenic fire, as indicated by fire scars and visual clues. Disturbance to these stands was assumed to have been of moderate intensity, as indicated by the fact that a variable but small number of large trees within the stands survived the fire and soils were not severely degraded (Aravena et al. 2002).

We examined several hypotheses, based mainly on current knowledge of succession in northern hemisphere forests, regarding the patterns of change in soil properties and N fluxes across the successional chronosequence in Chiloé Island. These successional hypotheses were examined in a region free of industrial N deposition (Hedin et al. 1995), to help provide a complementary framework for successional theory. The following hypotheses were investigated:

1. Fire disturbance may produce important declines in N and C soil pools (Ewel et al. 1981; Jordan 1985; Matson et al. 1987; Kauffman et al. 1995). As stand biomass accumulates through succession, we predict that soil C and N pools also increase, accelerating the rates of net N mineralization and increasing available N pools in older forests.
2. In relation to changes in soil C/N ratios through succession, Goodale and Aber (2001) postulated that, in a high N deposition area of eastern USA, a successional trend towards a lower soil C/N ratio would be expected, because N accumulates faster than C. In contrast, in unpolluted regions subjected to lower N deposition, such as Chiloé Island, the relative accumulation rates of C and N could be the reverse, and consequently, we predict that soil C/N ratios should increase through succession.
3. Woody detritus provides a large number of microsites suitable for non-symbiotic N-fixers in forests (Gorham et al. 1979; Reiners 1981). Assuming an increase in detritus accumulation (Carmona et al. 2002) and a decrease in decomposition rates in late succession (Gorham et al. 1979; Reiners 1981), we predict that the accumulation of both fine litter and coarse woody debris (CWD) should produce an increase in non-symbiotic  $N_{fix}$  at the ecosystem level in old stands.

## Materials and methods

### Vegetation, soil and climate

The stands selected for this study were located in a rural area of northern Chiloé Island, southern Chile (42°30'S), in the vicinity of Senda Darwin Biological Station (SDBS, Fig. 1). The area is characterized by a mosaic of pastures, shrublands and forest patches in different successional stages. Seven forest stands were selected to represent a successional series of forest development following anthropogenic fire. Minimum stand ages, determined by tree-ring analysis (Aravena et al. 2002), ranged from 2 to >100 years since

last disturbance, constituting a century-old successional chronosequence. Forest patch areas varied from 10 to >100 ha, and patches were separated from one another by areas of open pastures and shrublands. Patch size was unrelated to stand age, and because forests were interconnected with other patches by strips of native vegetation, we did not consider patch size as a factor affecting soil processes (Aravena et al. 2002). All forests including late successional stands have been subjected to occasional grazing by cattle and limited selective harvesting of trees. Early and mid successional stands originated during the twentieth century after non-catastrophic, small scale anthropogenic fires and subsequent timber extraction, as indicated by the local presence of charcoal, woody detritus and stumps. All forests were dominated by broad-leaved evergreen trees, such as *Drimys winteri* (Winteraceae) and *Nothofagus nitida* (Nothofagaceae), but also included some narrow-leaf conifers (*Podocarpus nubigena* and *Saxegothaea conspicua*, both Podocarpaceae) (Aravena et al. 2002). Floristically, all remnant stands belong to the North Patagonian forest type (Veblen et al. 1983; Armesto et al. 1995) developing on relatively flat, glacio-fluvial deposits in lowland areas (<100 m). Soils were typically shallow cambisols, <1 m deep. Soil texture did not dramatically change among the successional stands, varying from loam to silt loam with a granular structure on well-drained and gentle slopes (5–10°). Abundant tree regeneration characterized all the stands (Aravena et al. 2002; Christie and Armesto 2003). Shrubs were generally rare or absent from all successional sites, except for thickets of the native bamboo *Chusquea* sp., under tree-fall gaps.

The prevailing climate is wet-temperate with a strong oceanic influence (Di Castri and Hajek 1976). Meteorological records (3 years) at SDBS (45°53'S, 73°40'W), within <10 km of all the study sites, indicate an annual rainfall of 2,035 mm and a mean annual temperature of 9.8°C. Summer (December–March) average temperature is 11.7°C and winter (June–September) average temperature is 8°C. Rainfall occurs throughout the year, but 64% of the annual precipitation falls between April and September, with a peak during winter (Fig. 1a). In some years, short droughts can occur during summer (January–March), with monthly precipitation below 40 mm (Fig. 2a).

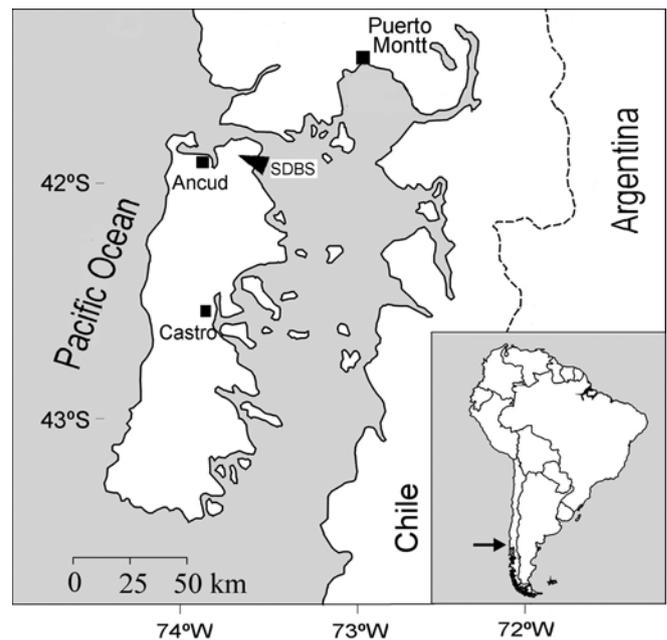


Fig. 1 Study area in Chiloé Island, southern Chile, indicating the location of Senda Darwin Biological Station (SDBS)

## Land use history

Land clearing for farming and agriculture greatly reduced the forest cover of Chiloé Island during the twentieth century. Because of the short dry season and wet overall climate, anthropogenic fires are often of moderate intensity and reduced spatial scale, resembling “slash and burn” practices (Ewel et al. 1981). At present, to clear land for pastures, authorized and controlled fires are applied to a small, previously logged, area during the dry season. However, in the past, large areas of forest were intentionally burnt to open a few hectares of pasture. In the latter case, above-ground vegetation is cleared, but the soil is not extremely degraded, allowing for the rapid regeneration of native vegetation in abandoned areas (Aravena et al. 2002). Frequently, scattered individuals, especially tall emergent trees, survive the fires, as confirmed by tree ages (Aravena et al. 2002), suggesting that crown fires are rare. Some woody detritus may be removed from the cleared sites by the landowners to be used as firewood (Carmona et al. 2002). Selective logging is a concurrent disturbance acting in many lowland secondary forests. This is considered a low intensity disturbance as it does not alter the survival of neighbouring trees. Once a tree is felled, timber is man-pulled out of the woods to the closest road or wide trail, and then transported by oxen. Machinery is not used in these traditional timber operations. As both fire and logging may occur at different degrees in most of these forests, no attempt was made to separate their effects in soil processes along the chronosequence.

## Chronosequence

Permanent plots (50×20 m) were set up in each of seven forest stands (Table 1), representing different successional stages after anthropogenic fire (see below). The plots were located at the centre of each forest fragment to eliminate edge effects. Between 1998 and 2000, we identified and tagged every tree with a trunk diameter of ≥5 cm at breast height (1.3 m) rooted within the plots. A full description of the flora and vegetation structure of stands is given by Aravena et al. (2002). The chronosequence was based on tree-ring counts for at least 30 canopy trees per stand (Aravena et al. 2002). The youngest successional stand was a forest accidentally burned during the dry summer of 1997. Most trees were standing dead at the beginning of the study with a few surviving trees that accumulated a basal area (BA) of 1.2 m<sup>2</sup>/ha (Table 1). For some analyses, the seven successional stands were grouped in three age classes, based on gaps in the chronosequence (Table 1): we distinguished early successional stands (ES; <11 years old, *n*=2), mid-successional stands (MS; 34–62 years old, *n*=3) and late successional stands (LS; ca.130 years old, *n*=2).

## Soil sampling and laboratory analyses

For soil sampling, we set up two 50-m-long parallel transects, 10 m apart, within each permanent plot. Every 12 m along each transect we established one sampling point, making a total of six sample points per plot and stand. In each sampling point, samples of both surface (0–10 cm) and deep (11–30 cm) soil horizons were collected using a shovel to obtain ca. 200–500 g per sample, making a total of 12 samples per plot and stand. Soil sampling was conducted seasonally (4 times a year) from July 1999 to January 2002.

## *N* availability and net *N* mineralization

Samples of surface (0–10 cm) and deep (11–30 cm) soil horizons were separately sieved through a 2-mm mesh to separate roots and the gross fraction of soil. Each soil sample was then divided into two subsamples. One subsample was taken to the laboratory to determine the initial ammonium and nitrate contents in the soil solution. The second subsample was placed inside a polyethylene

**Table 1** Structural characteristics and soil variables of lowland successional stands in Chiloé Island. BA Basal area of living trees, CWD coarse woody debris, *N<sub>a</sub>* soil pool of available N, *N<sub>min</sub>* field rates of net N mineralization, ARA potential acetylene reduction activity, %*W* soil moisture content

Successional group	Main type of disturbance	Minimum stand age	BA (m <sup>2</sup> /ha) <sup>a</sup>	CWD (ton/ha) <sup>a</sup>	<i>N<sub>a</sub></i> (kg/ha) <sup>b</sup>	<i>N<sub>min</sub></i> (kg/ha/year) <sup>c</sup>	ARA (nmol C <sub>2</sub> H <sub>4</sub> /g DW/day) <sup>d</sup>	<i>N<sub>fix</sub></i> (kg N /ha/year) <sup>b</sup>	Soil C/N	% <i>W</i> <sup>c</sup>
Early (ES-1)	Fire-logging	2±0	1.9	62	82.9	31.4	0.68	0.22	27.0	78.0
Early (ES-2)	Fire-logging	11±2	3.9	42	58.6	22.0	2.65	0.11	32.6	74.1
Mid (MS-1)	Fire-logging	34±6	16.6	9	76.7	15.4	32.98	0.64	30.3	81.7
Mid (MS-2)	Fire-logging	51±58	46.0	34	57.6	62.0	0.56	0.61	21.7	69.6
Mid (MS-3)	Fire-logging	62±44	49.7	36	57.7	38.0	1.28	1.66	28.2	72.4
Late (LS-1)	Selective logging only	133±46	65.5	47	86.6	52.4	0.87	1.41	21.8	76.9
Late (LS-2)	Selective logging only	134±78	85.7	65	67.7	33.2	1.3	0.23	28.3	74.7

<sup>a</sup>BA (from Aravena et al. 2002) and biomass of CWD (from Carmona et al. 2002) for each stand

<sup>b</sup>For the first 10 cm of soil

<sup>c</sup>For 0–30 cm soil depth

<sup>d</sup>Estimated for surface (0–10 cm) sieved mineral soil

zip lock bag and returned to the soil at the same location and depth of collection. Field incubated samples were retrieved after 30–35 days and taken to the laboratory to determine the final  $\text{NH}_4^+$  and  $\text{NO}_3^-$  contents. A 2%  $\text{KAl}(\text{SO}_4)_2$  solution was used for the extraction of available N ( $\text{N}_a$ ) from soil samples (1:4 soil/solution). The determination of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  contents was done by fractionated steam distillation (Pérez et al. 1998).  $\text{KAl}(\text{SO}_4)_2$  solution was more suitable than KCl for this procedure.  $\text{N}_a$  represents the sum of the  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N contents. Monthly rates of N production were estimated as the difference between the initial and final N contents of field-incubated soil samples. Seasonal rates were estimated by multiplying monthly rates by 3. The annual rate of net N mineralization ( $\text{N}_{\text{min}}$ ) was estimated as the sum of the four seasonal net values per 1 ha, based on estimates of the bulk density for each soil depth.

#### Non-symbiotic N fixation

Non-symbiotic N fixation ( $\text{N}_{\text{fix}}$ ) rates were determined for three soil layers: litter ( $\text{O}_1$  horizon), surface ( $\text{A}_h$ ; 0–10 cm) and deep ( $\text{B}_v$ ; 11–30 cm) mineral soil, using the acetylene reduction activity technique (ARA; Myrold et al. 1999). The same six sample points within each permanent plot were used for field incubations of litter. Every season from August 2000 to January 2002, ca. 20–300 g of fine litter (leaves and twigs) was collected by hand (using clean rubber gloves) from an area adjacent to each sample point and placed inside a 1-l hermetic glass jar. Jars were incubated under field conditions for 2 days. Additionally, during summer 2001, nine small fragments of large CWD (ca. 70–300 g fresh mass) of different decay classes were collected from the forest floor in six stands (MS1 is missing, Table 1) and incubated in a 1-l hermetic glass jar in the field. Intact surface soil cores were taken from the six sample points using a 100- $\text{cm}^3$  steel cylinder. Then the soil core was carefully removed from the cylinder and deposited inside a 500-ml hermetic glass jar for field incubations. This experiment was done in two mid and one late successional forest during winter and spring 2001 and summer 2002. Litter, wood and soil samples were incubated inside hermetic jars containing a 10% v/v acetylene/air. An additional sample for each substrate was incubated without acetylene to serve as a control. A sample of air was taken from inside each jar daily and injected into 3-ml Venojets. Gas samples were frozen and analysed within 1 week for ethylene production using a Shimadzu gas chromatograph equipped with a Porapak column and an FID detector. The detection limit of this analysis was 0.01 nmol ethylene/ml.

In order to control for local site effects on ARA, we conducted laboratory assays for litter and surface and deep soil samples, representing potential rates of ARA. For this purpose six additional litter samples were taken to the laboratory for incubation under constant temperature (ca. 20°C). Samples of mineral soil collected each season were incubated under laboratory conditions, from July 2000 to December 2001. Six sieved (2-mm mesh size) samples of surface ( $\text{A}_h$ ) and deep soil ( $\text{B}_v$ ) were homogenized to one per soil depth, and then divided into four subsamples. Subsamples were placed inside 1-l hermetic glass jars for laboratory incubations at ca. 20°C. ARA was estimated from the slope of the ethylene accumulation curve in the headspace after 2 days of incubation. One subsample without acetylene was always used as control. Control samples showed zero or almost negligible ARA, so they were not considered in the final calculations.

Annual  $\text{N}_{\text{fix}}$  rates per soil compartment were estimated assuming the stoichiometric conversion factor of 1/3 of the ARA in field assays, multiplied by their respective dry biomasses per forest stand. Stand biomass of fine litter was estimated from the dry weight of six litter samples collected with a 20×25-cm metal frame in each sample point. Finally, we estimate an annual rate of  $\text{N}_{\text{fix}}$  in each stand by adding the rates of the most active substrates; mainly litter and CWD.

#### Total soil C and N

The total contents of C ( $\text{C}_{\text{tot}}$ ) and N ( $\text{N}_{\text{tot}}$ ) of surface soils were determined for three subsamples of sieved (2-mm mesh size) mineral soil per stand, collected each season. Soil samples were oven-dried at 70°C, ground and their C and N contents estimated by flash combustion method in a Carlo Erba NA 2500 elemental analyser.

#### Soil water content and bulk density

Water content (%W) of each of six soil samples per forest stand and depth was determined gravimetrically every season. Bulk density was determined only once at the beginning of the study in the six sample points per stand in both surface and deep soil. For these purposes, soil samples of 100  $\text{cm}^3$  (diameter=5.8 cm, height=3.8 cm) were collected with a steel cylinder at each soil depth.

#### Statistical analyses

We assessed the degree of association of soil variables with seasonal variation of precipitation and air temperature averaged monthly, using Pearson's correlation analysis ( $n=11$  sampling months for net  $\text{N}_{\text{min}}$  and  $\text{N}_a$ ,  $n=7$  sampling months for  $\text{N}_{\text{fix}}$ ). For this analyses, we used data for each soil variable averaged for three age groups of successional stands as described above (ES, MS, LS, Table 1).

For each of the six sample points in the seven successional stands, we calculated the averages over the four seasons and the 2 years of sampling. This was done for all soil variables, by considering data from July 1999 to January 2002 ( $n=42$ , six sample points per stand in each of seven stands). For  $\text{N}_{\text{fix}}$ , we averaged data from August 2000 to January 2002. The number of samples was  $n=42$  per stand for most soil variables, except for soil ARA in the laboratory, where three homogenized subsamples per stand were processed ( $n=21$ , three samples in each of seven stands), ARA of intact soil cores ( $n=18$ , six samples in three stands) and ARA of CWD in the field ( $n=54$ , nine samples in each of six stands). We used scatter plots and linear regressions to analyse the relationship between minimum stand age (as the independent variable, from Table 1) and the following soil variables; pools of  $\text{C}_{\text{tot}}$ ,  $\text{N}_{\text{tot}}$  and  $\text{N}_a$  and C/N ratios of surface soils, the in situ rates of  $\text{N}_{\text{fix}}$  and net  $\text{N}_{\text{min}}$  (adding values for surface and deep soil), bulk density and %W of surface soils. When linear models did not significantly fit the scatter plots, we searched for the best significant model; quadratic, exponential and logistic models were tested.

## Results

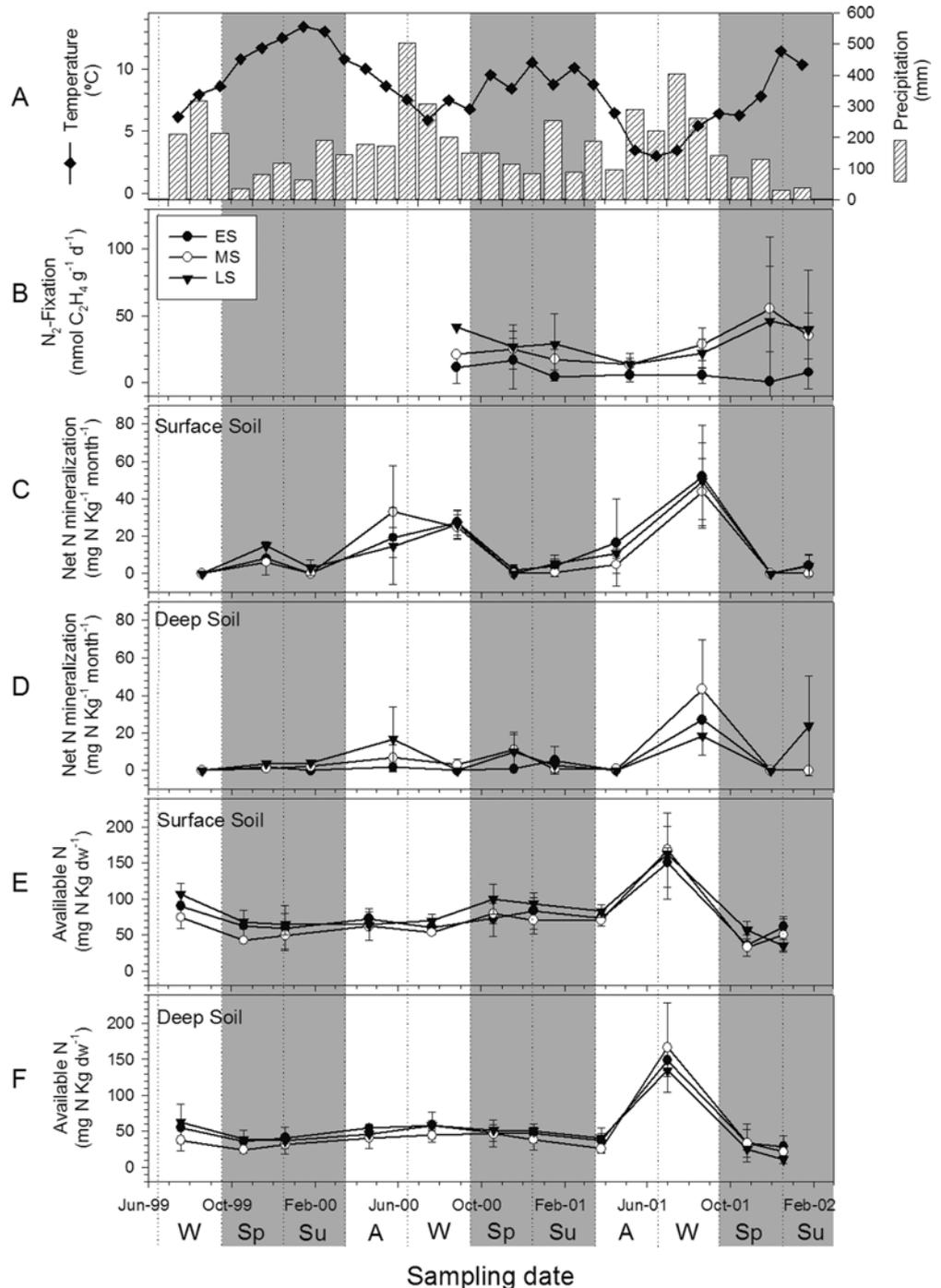
### Seasonal patterns

Most soil processes showed an association with seasonal variation in precipitation and average air temperature (Fig. 2a).  $\text{N}_{\text{fix}}$  estimated from field ARA in the litter layer was higher during the warmer months of spring and summer (November 2001 and January 2002, respectively, Fig. 2b) in both MS and LS stands; however, this variable was not significantly correlated with either precipitation or temperature ( $r^2=0.08$ ,  $r^2=0.187$ , respectively, both values not significant). In situ monthly net  $\text{N}_{\text{min}}$  in surface soils showed remarkable seasonal variation (Fig. 2C), which was positively correlated with precipitation ( $r^2=0.63$ ,  $P<0.01$ ), but not with air temperature ( $r^2=0.341$ ,

$P > 0.05$ ). Higher net  $N_{min}$  rates occurred during autumn and winter months of 2000 and 2001. During the warmer and drier periods of the year (<40 mm per month), internal flux of N from  $N_{min}$  declined to negligible values (Fig. 2c). The same seasonal trend was observed in the three age groups of successional stands. Deeper soil horizons were less variable (Fig. 2d), with positive net  $N_{min}$  rates during autumn and spring 2000, winter 2001 and summer 2002. Deeper soils presented a more depressed but more constant microbial activity than surface soils.  $N_a$  in surface (Fig. 2e) and deeper soil horizons (Fig. 2f) was positively correlated with precipitation ( $r^2=0.57$ ,  $P < 0.01$ , surface, and  $r^2=0.69$ ,

$P < 0.01$ , deep). Temperature showed no significant correlation with  $N_a$  in surface soil ( $r^2=0.341$ ,  $P > 0.05$ ). The peak of  $N_a$  recorded in July 2001, which lies outside the range of variability observed in the 2 years of study, was probably associated with heavy rains previous and during the sampling event, which according to meteorological records from SDBS more than doubled the precipitation recorded during July 2000.

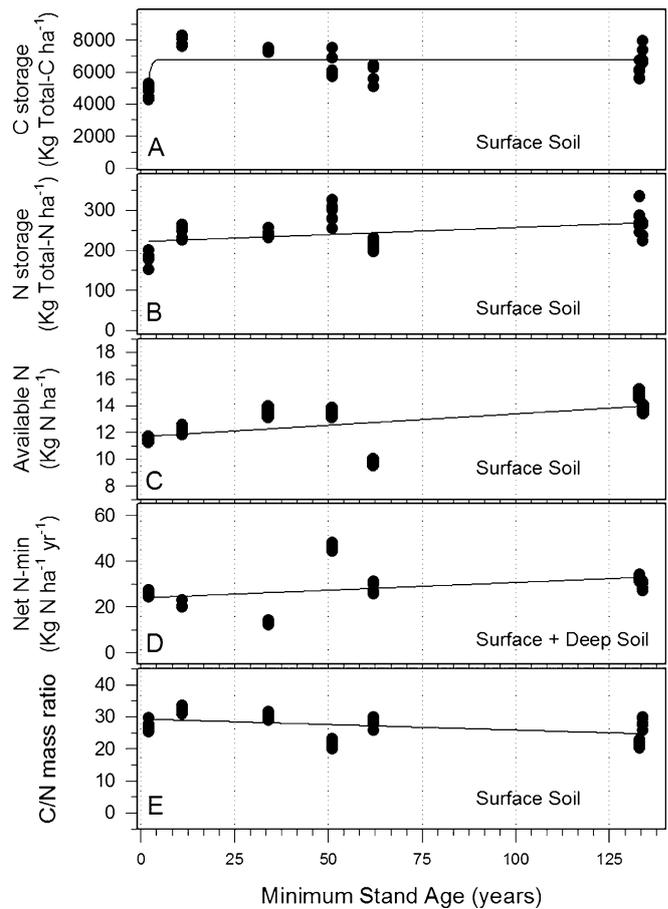
**Fig. 2a-f** Seasonal variation of soil variables from July 1999 to January 2002, for the group of early (ES; filled circles,  $n=12$ ), mid (MS; open circles,  $n=18$ ) and late (LS; filled triangles,  $n=12$ ) successional forests. Monthly mean air temperature and total monthly precipitation (a) based on meteorological data from SDBS, Chiloé Island, during the sampling period, are also presented. Soil variables shown are: field acetylene reduction activity (ARA) in the forest litter layer (b), in situ monthly net N mineralization in upper (c) and deep soil horizons (d), pools of available N for upper (e) and deep soil horizons (f). Shaded areas in the graph indicate the length of spring-summer season. W Winter, Sp spring, Su summer, A autumn, Jun June, Oct October, Feb February



## Overall trends with stand age

Considering all stands sampled, surface soil pools of  $C_{tot}$  showed a pronounced increase during the first decade of stand development and then remained constant with stand age (Fig. 3a, Table 2). Surface soil pools of both  $N_{tot}$  and  $N_a$  increased slightly with stand age (Fig. 3b, c, Table 2). Annual  $N_{min}$  rates in surface plus deep soils showed a slight but significant increase with stand age (Fig. 3d, Table 2). C/N ratios showed a weak declining trend with increasing stand age (Fig. 3e, Table 2). Field  $N_{fix}$  in the litter layer was significantly and positively related to stand age according to a quadratic function (Fig. 4a, Table 2), describing a trend towards greater  $N_{fix}$  from early to mid successional stages, and then a slight decrease in late successional stages but remaining higher than in pioneer stages.  $N_{fix}$  of CWD showed an exponential decrease with stand age (Fig. 4b, Table 2) with a strong decline from stand ages two to 11. The addition of  $N_{fix}$  in the litter layer plus woody debris showed no trend with stand age (Fig. 4c, Table 2), mainly because the recently burned stand presented similar values to the LS stands, but  $N_{fix}$  rates increased from MS to LS stands. Field estimates of ARA in intact soil cores of surface soil were  $2.68 \pm 1.63$  nmol  $C_4H_4/g$  dry weight (DW) per day in MS1 and  $0.08 \pm 0.12$  nmol  $C_4H_4/g$  DW per day in LS2. Potential rates in mineral soil were also low except for the value in MS1 (Table 1). These values were much lower than potential rates in the litter layer. Litter ARA varied from  $13.9 \pm 2.2$  nmol  $C_4H_4/g$  DW per day in ES2 to  $112.1 \pm 23.7$  nmol  $C_4H_4/g$  DW per day in MS3.

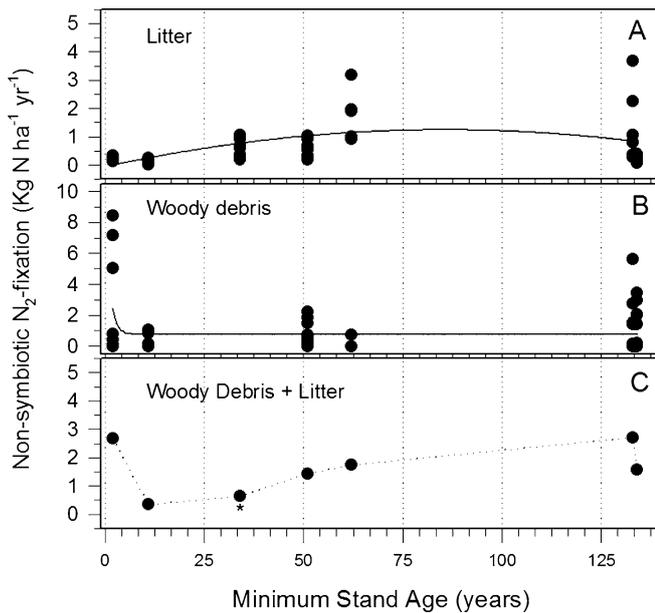
We found no significant relationship between stand age and either bulk soil density, that averaged  $0.22 \text{ g/cm}^3$  at both depths, or %W ( $r^2 < 0.1$ ,  $P > 0.05$ , Table 1).



**Fig. 3a–e** Statistically significant ( $r^2=0.124\text{--}0.43$ ,  $P<0.05$ ) trends of soil variables across the successional chronosequence of lowland rainforest stands in Chiloé Island. Data are shown for pools of total C (a), total N (b), available N ( $NH_4^+ - N + NO_3^- - N$ ) in surface soil (c), annual field rates of net N mineralization ( $NH_4^+ - N + NO_3^- - N$ ) in surface plus deep soils (d), soil C/N mass ratio (e). Values are 2-year averages of seasonal data for six sample points per stand ( $n=42$ ). yr Year

**Table 2** Best fit regression models for the relationship between stand age (independent variable,  $x$ ) and soil variables ( $y$ ) in a the successional chronosequence of Chiloé Island

Soil variable	Model	$r^2$	$F$	$P$
<b>Figure 3</b>				
C storage (a)	Exponential rise to a maximum $Y=y_0+a(1-\exp(-bx))$	0.430	$F_{(2,39)}=14.725$	<0.0001
N storage (b)	Linear $Y=y_0+bx$	0.192	$F_{(1,40)}=9.512$	0.0037
Available N (c)	Linear $Y=y_0+bx$	0.282	$F_{(1,40)}=15.711$	0.0003
Net N-min surface+deep soil (d)	Linear $Y=y_0+bx$	0.124	$F_{(1,40)}=5.662$	0.022
C/N mass ratio (e)	Linear $Y=y_0+bx$	0.187	$F_{(1,40)}=9.173$	0.0043
<b>Figure 4</b>				
$N_{fix}$ litter (a)		0.228	$F_{(1,40)}=9.173$	0.01
$N_{fix}$ CWD (b)	Exponential decay to a minimum $Y=y_0+a \exp(-bx)$	0.121	$F_{(2,51)}=3.506$	0.0374



**Fig. 4** Field annual rates of non-symbiotic N fixation in litter layer (a), in CWD (b), and litter layer+CWD (c) for each stand in the chronosequence. Litter estimates are 2-year averages of seasonal data for six sample points per stand ( $n=42$ ). Estimates for woody residues are for nine samples per stand during the summer season for six stands (no data for MS1). The asterisk in c indicates missing data

## Discussion

### Seasonality of soil processes

Field estimates of net  $N_{\min}$  and  $N_a$  showed marked seasonality in these lowland rainforests, with a higher internal N flux during the winter months. Seasonal trends for lowland forests thus differ from those previously reported for montane conifer forests at 600 m elevation in Chiloé Island that presented higher  $N_{\min}$  rates during spring and summer (Pérez et al. 1998). Moreover, in lowland secondary forests, net  $N_{\min}$  and  $N_a$  in surface soils correlated positively and significantly with monthly precipitation, but not with temperature. This difference between coastal montane and lowland forests in Chiloé Island may be attributed to the much lower total annual precipitation in lowland forests (2,035 mm in lowland vs. ~5,500 mm in montane forests) and the fact that the former are occasionally subjected to short summer droughts. Less than 40 mm of rain per month during summer months were reported for two growing seasons in the present study (Fig. 2a), which is well below the seasonal average of 122 mm per month reported by López and Aceituno (1998) based on ca. 40 years of records in this area. These short dry spells can decrease soil microbial activity, affecting net N mineralization (Evans et al. 1998). Winter months are generally warmer in lowland forests than in montane forests and snowfalls are not recorded. Higher winter temperatures (average 8°C) in lowland forests compared to montane forests (average 4.2°C) account for a positive N flux from mineralization during the wet season. Occasionally, positive N fluxes may also occur during

summer in deeper soil layers (Fig. 2c), allowing some relief from N immobilization in the upper soil horizon. Old-growth lowland forests in northern Chiloé Island also presented higher annual rates of  $N_{\min}$  (Table 1) than previously reported for montane forests (Pérez et al. 1998, 2003).

The activity of heterotrophic N fixers in litter, in turn, was not depressed during the spring and summer months, thus suggesting that microbial N fixers are more responsive to warmer temperature and less sensitive to seasonal drought than N mineralizers and nitrifiers.

### Major trends with stand age

The slight linear increase in the  $N_{\text{tot}}$  pool with stand age is associated with a linear decrease in soil C/N ratio, indicating that a successional change of C/N ratios is mainly due to changes in  $N_{\text{tot}}$  but not in  $C_{\text{tot}}$ . The soil pool of  $C_{\text{tot}}$  was initially low in the recently disturbed stand, but increased and remained constant from mid to late succession. The high soil C/N ratios in early successional stands may result from the recalcitrant organic matter, derived from fire disturbance, particularly woody detritus which is not consumed by fire (Carmona et al. 2002). Associated with their higher soil C/N ratios in the chronosequence, early successional stands had lower soil  $N_a$ , reduced  $N_{\text{tot}}$ , and lower rates of net  $N_{\min}$  in surface plus deep soils compared to older stands, which are presumably due to losses of N by volatilization during the fire (Matson et al. 1987).

Contrasting successional patterns of soil C/N ratios have been reported for northern hemisphere temperate forests. In an old field succession in central Massachusetts, Compton et al. (1998) reported increasing soil C/N ratios with stand age. The opposite trend, however, was found by Goodale and Aber (2001). These authors showed a decreasing soil C/N ratio through a successional chronosequence in hardwood forests located in high deposition areas of eastern USA. The latter pattern is consistent with our case in Chiloé forests. However, the mechanism postulated for the decline in soil C/N ratio in high N deposition areas of North America does not apply to unpolluted Chiloé forests (see below).

We found only a weak overall trend of increasing net  $N_{\min}$  rates across the chronosequence of stands, suggesting that microbial activity in the soil was not seriously disrupted by the moderate anthropogenic fire initiating succession. This observation contrasts with results from managed forests where fire is often used to eliminate residues, with negative impacts on soil microbial processes (Vitousek and Matson 1984). Our result supports the idea that anthropogenic fire disturbances in lowland Chiloé forests have only moderate effects on the soil biota and do not drastically alter soil processes, thus allowing for the rapid recovery of forest cover (Aravena et al. 2002). These effects may be stronger, however, after repeated fires.

The ability of litter heterotrophic bacteria to fix atmospheric N in situ tends to increase from early to mid succession (during the first 100 years according to our chronosequence), and just slightly decreases in older stands. Excluding the recently burned site (ES1, Table 1), which has large amounts of woody residues that should enhance non-symbiotic N fixation associated with this compartment,  $N_{\text{fix}}$  (litter+CWD substrates) increased with stand age (Fig. 4c). This increased N input should allow the build up of N pools in lowland forests from mid up to and including late succession, and may represent a sustained flux of new N to older forests, which may offset substantial hydrological losses of dissolved organic N measured in these ecosystems (Hedin et al. 1995).

With respect to N fixation, successional trends found in temperate rainforests in Chiloé are unusual with respect to most studies of succession in temperate, tropical and Mediterranean forests. Dawson (1983), Skujins et al. (1987), and Heath et al. (1987) have documented that maximum  $N_{\text{fix}}$  occurred in recently disturbed stands and then declined through mid and late succession. In Douglas-fir forests in the Pacific Northwest, Hope and Li (1997) also found higher ARA in the litter layer of younger stands (70–80 years old) than in old-growth stands (450 years old). These authors attributed this successional trend of declining  $N_{\text{fix}}$  to changes in soil microbial composition, particularly a drop in non-symbiotic N fixers, through succession. In addition, symbiotic N-fixers present in soils, which are largely absent from southern temperate rainforests, such as bacteria associated with the rhizosphere of alder (*Alnus* spp.) and with a number of pioneer woody legumes in tropical forests (Perry 1994), also produce high rates of N fixation in early succession. N fixation will decline later in succession as these pioneer species disappear from the forests. Likewise, in Mediterranean ecosystems, associative N-fixers facilitate early successional development, contributing to N recovery after forest fires. For example, in Australia, *Eucalyptus regnas* forests affected by fire are replaced by *Acacia*-dominated pioneer stands, which present associative N-fixers (Adams and Attiwill 1984). N losses from the ecosystem caused by fire may be compensated within 3 years. In California, *Ceanothus velutinus* with associated symbiotic N fixers, colonizes burned areas, originally dominated by pines (Johnson and Susfalk 1997). In the Mediterranean zone of Chile, however, facilitation by N-fixers is not considered as a mechanism of post-fire succession (Armesto and Pickett 1985).

Native associative N-fixers are entirely absent from the chronosequence of forests in Chiloé Island, and are rare in southern Chilean forests, and hence the contrasting successional trend for N fixation found in Chilean forests can be entirely accounted for by changes in the abundance and activity of non-symbiotic N fixers. In managed forests of eastern USA and Sweden (cf. Dawson 1983), a cyanobacterial crust develops after clearcutting, increasing autotrophic N fixation in pioneer stands. We found no evidence of such a cyanobacterial crust in southern temperate rainforests. It may be possible that other

functional types of non-symbiotic N fixers, presumably heterotrophic bacteria associated with mature successional stages, contribute to the successional trend reported for lowland Chiloé forests. Heterotrophic bacteria are often associated with woody residues that tend to accumulate in late succession (Harmon et al. 1986; Carmona et al. 2002). Some studies in North American conifer forests reported that as woody debris biomass increased in older forests, ARA, and therefore  $N_{\text{fix}}$ , also increased (Roskoski 1980; Jurgensen et al. 1987).

## Conclusions

N-cycle changes during succession, following biomass burning and low intensity selective logging in lowland forests of northern Chiloé island, can be briefly synthesized as follows:

1. Consistent with current predictions of biogeochemical theory, soil pools of  $N_a$  and  $N_{\text{tot}}$  are lower in recently disturbed stands, as a consequence of disturbance-related losses, and tend to increase in later stages of succession. In addition, we observed a steady, but weak, increase of  $N_{\text{min}}$  through succession in lowland Chiloé forests. Possible differences in the initial conditions among stands included in the chronosequence, due to both the intensity of fire and previous selective logging, may account for some of the unexplained variability of these relationships. This is an inherent limitation of the chronosequence approach (Pickett 1989).
2. Rates of heterotrophic  $N_{\text{fix}}$  in litter layer of lowland forests were higher in mid successional stands, with the exception of  $N_{\text{fix}}$  associated with woody residues in the recently burned stand. Overall,  $N_{\text{fix}}$  was higher in mid and late successional forests, in contrast to reports for succession in most tropical, temperate and Mediterranean forests. Heterotrophic  $N_{\text{fix}}$  may be an important and sustained source of N to unpolluted old-growth forests in southern Chile.
3. Contrary to expectations from successional theory (Goodale and Aber 2001), soil C/N ratio decreased with stand age, associated with increased N inputs from non-symbiotic N fixation and constant soil C contents in mid and late successional stands.
4. In summary, we argue that successional trends in the biogeochemical processes associated with the N cycle are strongly dependent on how the particular disturbance regimes and the local biotic assemblage affect C and N losses and biological N inputs.

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