Ecosystem development in short-term postglacial chronosequences: N and P limitation in glacier forelands from Santa Inés Island, Magellan Strait

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Abstract   Glacier foreland moraines provide an ideal model to examine the patterns of ecosystem development and the evolution of nitrogen and phosphorous limitation over successional time. In this paper, we focus on a 400-year soil chronosequence in the glacier forelands of Santa Inés Island in the Magellan Strait, southern Chile by examining forest development on phosphorus (P)-poor substrates in a uniquely unpolluted region of the world. Results show a steady increase in tree basal area and a humped trend in tree species richness over four centuries of stand development. The increase in basal area suggests that the late successional tree species were more efficient nutrient users than earlier successional ones. Total contents of carbon (C) and nitrogen (N) in soils increased during the chronosequence, reaching an asymptote in late succession. The net increases in soil C : N, C : P and N : P ratios observed over successional time suggest that nutrient limitation is maximal in 400-year-old substrates. Foliar C : N and C : P ratios also increased over time to reach an asymptote in old-growth stages, following soil stoichiometric relationships; however the foliar N-to-P ratio remained constant throughout the chronosequence. Biological N fixation was greater in early postglacial succession, associated with the presence of the symbiotic N-fixer *Gunnera magellanica*. Declining trends of $\delta^{15}N$ in surface soils through the 400-year chronosequence are evidence of decreasing N losses in old-growth forests. In synthesis, glacier foreland chronosequences at this high South American latitude provide evidence for increasing efficiency of N and P use in the ecosystem, with the replacement of shade-intolerant pioneers by more efficient, shade-tolerant tree species. This pattern of ecosystem development produces a constant foliar N : P ratio, regardless of variation in soil N-to-P ratio over four centuries.

Key words: C : N ratio, *Gunnera magellanica*, N : P ratio, *Nothofagus* forest, primary succession, sub-Antarctic forest.

INTRODUCTION

The long-term study of forest succession provides clues to understanding vegetation changes and the effects of nutrient limitation on tree species diversity and functional traits. A useful approach to study ecosystem and soil development during succession is the analysis of soil chronosequences, where sites of different ages are organized along a successional trajectory (Walker *et al*. 2010).

Short-term postglacial succession (<500 years) can be reconstructed from moraines left behind by receding glaciers. In glacier forelands such chronosequences are often characterized by a progressive accumulation of above-ground biomass up to an asymptote, along with a parallel trend for tree species richness (Walker & Del Moral 2003). Soil organic matter (originated mainly from senescent plant tissues) and elements such as carbon (C) and nitrogen (N) tend to accumulate during succession following a power function (Egli *et al*. 2001; He & Tang 2008; Mavris *et al*. 2010). After glacial retreat the main source of nitrogen to N-depleted soils is biological nitrogen fixation. In Glacier Bay, Alaska, actinorhizal N-fixation by symbiotic woody species, such as *Alnus* (Kohls *et al*. 2003), provides one of the initial N sources for succession. Increases in soil C-to-N ratio during succession suggest that nitrogen limitation might grow over time despite initial N accumulation in the ecosystem (Hobbie *et al*. 1998). In old-growth forests nitrogen limitation could eventually be relieved, as symbiotic cyanobacteria hosted in epiphytic mosses, liverworts and lichens become a significant nitrogen source to glacier foreland ecosystems (Menge & Hedin 2009).

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One helpful integrator of the nitrogen cycle over time is the natural abundance of stable isotopes of N. In temperate regions, ^{15}N isotope tends to be depleted during the course of succession, with a consistent decline of $\delta^{15}N$ in both green leaves and litter, probably because of increasing fractionation because of mycorrhizal N transfer (Hobbie *et al.* 1999) and a rather stable value in soils, suggesting low nitrogen losses along the chronosequence (Menge *et al.* 2011).

For other potentially limiting nutrients in short-term chronosequences, such as phosphorous (P), field studies have shown pronounced declines in total soil P content over time (Chapin *et al.* 1994). This successional drop in soil P is explained by the rapid depletion of apatite-derived minerals associated with bedrock weathering. Apatite minerals are the main source of P to pioneer species established on naked glacial substrates. Although not all short-term chronosequences in glacier forelands have shown the same trends for total soil P (Sigler *et al.* 2002; Tscherko *et al.* 2003), chronosequences studied have shown significant reductions of soil P and increasing N-to-P ratios in green foliage and litter over five centuries of soil development (Richardson *et al.* 2004; Parfitt *et al.* 2005). In P-limited ecosystems plants often overcome P limitation by developing a diversity of mycorrhizal associations, including arbuscular-, ecto- and ericoid-mycorrhiza, which have the ability to solubilize N and P that are strongly complexed with organic matter (Read & Pérez-Moreno 2003).

Much remains to be learned about changes in nutrient limitation during postglacial ecosystem development, especially from southern hemisphere locations where terrestrial ecosystems remain largely free from the effects of chronic industrial air pollution. Such effects, including enhanced N and P circulation, are pervasive in land-atmosphere interactions characterizing northern hemisphere ecosystem succession. The icecap of Santa Inés Island, in the Magellan Strait, is a relict of glacial advances that reached about 200 km east from its current position at the time of the last glacial maximum, about 20 000 BP (McCulloch *et al.* 2000). These glacial fluctuations left behind a landscape with moraine fields of varying ages. Successional changes that took place under the same general climate and on the same geological substrate can be reconstructed based on soil chronosequences (Aravena 2007; Walker *et al.* 2010). The study of postglacial chronosequences from Santa Inés Island represents a unique opportunity to examine the evolution of nutrient limitation in a rare scenario where industrial air pollution and human impacts are largely absent. Moreover, the parent material in these glacier forelands belongs to the Southern Patagonian batholith, which is composed of Cretaceous granitic minerals with average phosphorus contents of 0.04% (Hervé *et al.* 2007). Accordingly, such soils are classified as P-deficient primary minerals, which allow the analysis of postglacial successional trajectory under a clean atmosphere and on strongly P-poor substrates in southern Patagonia.

The following questions regarding ecosystem and soil development after deglaciation in Santa Inés Island were addressed in the present study:

1. Are there parallel increases in overall tree basal area (a proxy of above-ground biomass) and tree species richness during 400 years of succession?
2. Is above-ground biomass accumulation related to parallel increases in soil carbon, nitrogen and phosphorus concentrations during most of the chronosequence? Does the accumulation of these elements imply a relief of nutrient limitation over time as indicated by soil stoichiometric ratios?
3. Is soil carbon accumulation linked with an increase in biological N fixation? Are these trends reflected in soil $\delta^{15}N$ patterns along the chronosequence?
4. As above-ground biomass accumulates, do foliar C-to-P and C-to-N ratios evolve to reflect increasing efficiency in nutrient use relative to carbon gain? Are changes of foliar N-to-P ratios related to soil N-to-P ratios?

Answers to these basic questions can offer important clues to understanding how nutrient limitation develops during ecosystem succession, what are the rates of limitation over time, how do species and ecosystems respond to nutrient limitation over successional time, and particularly how do these patterns characterize unpolluted sub-Antarctic forests on P-poor geological substrates.

METHODS

Study area

Santa Inés Island (53°45′S) is located in the western portion of the Magellan Strait (Fig. 1). The island, about 900 km², has an ice cap of about 50 km² descending from elevations above 1100 m. The climate is characterized by continuous frontal cyclonic activity resulting in a high number of cloudy days (86% of the year) and rainstorms distributed evenly over the year (322 days of precipitation annually; Carrasco *et al.* 2002). Mean annual precipitation is about 2777 mm (1913–2001 estimate from Evangelistas, about 100 km northwest of Santa Inés Island), with a mean annual temperature of 6.9°C. On the eastside of the island, the rather small and debris-free Alejandro Glacier (approx. 15 km²) drains the ice cap to about 20 m above sea level (Fig. 1). In front of the proglacial lake there is a relatively flat valley containing a series of moraines with well-developed forest cover. Adjacent to Alejandro glacier, the Beatriz Glacier is a smaller valley glacier (about 5 km²) draining to the northeast from the Santa Inés ice cap. The proglacial lake in front of the Beatriz glacier snout is located at about 40-m elevation,
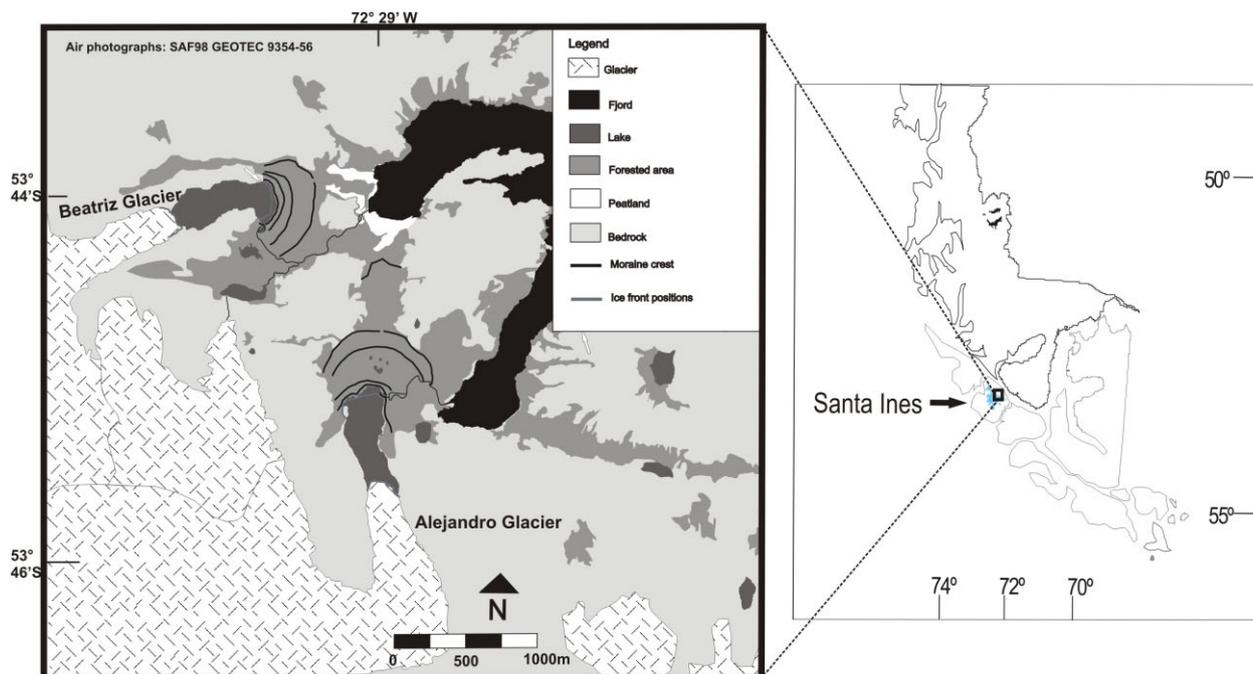
Fig. 1. Alejandro and Beatriz valley glaciers on Santa Inés Island, Magellan Strait, showing the moraines in grey lines. Map on the right shows the present Santa Inés ice cap.

followed by a downstream valley with a hilly topography and exposed bedrock.

Moraine ages and chronosequence definition

In each glacier foreland, minimum ages of moraine field were estimated using dendrochronological methods (Fritts 1976). In the years 2003 and 2008 increment cores were collected from the largest trees on each moraine to estimate minimum ages of pioneer tree establishment by counting the number of annual growth rings (Table 1). All trees were cored as close to the ground as possible. From these data, a minimum moraine age was obtained. Tree coring was conducted on moraine crests and on the distal (outer) slopes. As *Nothofagus betuloides* is one of the longest-living South American *Nothofagus* species (Veblen *et al.* 1996), all cored trees belonged to this species.

The similarity of vascular plant diversity and composition between both Alejandro and Beatriz glacier forelands was consistent with the assumption that they were part of a single chronosequence, which allowed for the unification of data from moraines in both glaciers for the analysis of successional trends (Walker *et al.* 2010) (Table 1).

Vegetation, soil sampling and chemical analysis

In each moraine, an area was selected on the top of the slope facing the glacier, where one circular 15-m radius plot (0.053 ha) was established. All trees >5 cm trunk diameter at breast height (dbh, at 1.3 m from the ground) within the plot were identified to species and their dbh measured. Basal areas per

Table 1. Dendrochronological parameters used for the determination of minimum moraine ages along a 400-year chronosequence in glacier forelands of Santa Ines Island, Magellan Strait, Chile

Site code	Total number of tree rings to year 2003 or 2008	Recruitment year for the oldest tree	Minimum moraine age until 2010
Bea4	26	1977	33
Bea3	55	1948	62
Ale4	76	1927	83
Ale3	128	1875	135
Ale2	178	1826	184
Bea1	198	1805	205
Ale1	213	1790	220
Bea0[†]	289	1721	291
Ale0[†]	380	1628	382

tree species and for the plot were calculated from dbh data. Additionally, relative cover of shrubs, herbs and non-vascular vegetation present within the plot were estimated using Braun-Blanquet's (1964) cover-abundance scale. One plot in each of nine of moraines were sampled (i.e. each of the successional sites defined by different moraine ages from tree-ring analysis) from the Alejandro and Beatriz glacier forelands.

During autumn and late spring of 2009 in both glacier forelands, and on the top of each moraine, one transect line was set up crossing the 15-m radius plot, and six random points separated by at least 10 m from one another along the line were sampled for litter and soil. At each sampling point along the transect, one sample from horizons O_l (recently

fallen litter) and A_h (surface organic-mineral horizon, first 10 cm) was removed with a shovel and deposited inside zip lock bags for transportation to the laboratory. Additional intact soil cores were taken from the same points with 80 cm^3 PVC tubes for bulk density determinations.

To assess foliar nutrient contents, samples of hand reachable fresh leaves were taken from three individuals of each species present in the plots (trees, shrubs and herbs) from each site in the chronosequence. Because of the high frequency of cloudy days and the short summer season in the study area, we assumed that there were no significant differences in nutrient concentration between 'sun' and 'shade' leaves. Soil and leaf samples were oven dried at 70°C for at least 2 days. Dry samples were ground for determination of total N and C, using flash combustion, with a NA2500 Carlo Erba Element Analyzer. Phosphorous content of plant leaves and soil was extracted in a concentrated sulfuric acid-water-peroxide solution in a Hach Digesdahl digestor, and determined by colorimetry with the molybdenum-blue method (Steubing & Fangmeier 1992). Element stoichiometric ratios (C-to-N, C-to-P, and N-to-P) were expressed as the mass ratio on a molar basis for comparative purposes. Foliage C : N and C : P ratios were used as 'proxies' of nutrient use efficiency, because highly significant correlations have been previously found between various N-use efficiency indices (i.e. litter C : N, %N retranslocated from leaves, and %N in the litterfall) and C : N ratio of green leaves in Chilean native trees (Pérez *et al*. 2003). The N-to-P ratio of green leaves has been used as an indirect indicator of nutrient limitation, because foliage N-to-P ratios >12.5 have been associated with P-limited ecosystems (Tessier & Raynal 2003).

Plant available soil nitrogen ($N_a = NH_4^+$-N + NO_3^--N) was extracted in a 0.021 mol L^{-1} $KAl(SO_4)_2$ solution (1 : 4) and ammonium and nitrate were determined by means of fractionated steam distillation (Pérez *et al*. 1998). Plant available P was extracted from soil through lactation by the CAL (Calcium-Acetate-Lactate) method and determined by the colorimetric molybdenum blue method (Steubing & Fangmeier 1992). Exchangeable calcium, sodium, potassium and magnesium in soil were extracted in a 1 M ammonium acetate solution (1 : 10) at pH 7 and determined in a Perkin Elmer 2380 atomic absorption spectrometer (Robertson *et al*. 1999). Soil pH was measured in a 1 : 2, soil to water suspension. All laboratory analyses were performed at the Biogeochemistry Laboratory, Pontificia Universidad Católica de Chile.

N fixation and δ¹⁵N determinations

To estimate N fixation rates, we measured acetylene reduction activity following Myrold *et al*. (1999) for three main ecosystem components: (i) fine litter (O_l horizon), (ii) epiphyte cover on tree bark, including mosses, hornworts, liverworts, and lichens known to host diazotrophic cyanobacteria, and (iii) green carpets covering the forest floor, made of different species of hornworts, liverworts, mosses, plus the perennial herb *Gunnera magellanica*, which is known to host nitrogen-fixing *Nostoc* colonies in their rhizomes. A mixed subsample of epiphytes taken from each tree (see below) and a fresh subsample of the forest floor vegetation were used for the acetylene reduction activity determinations.

Samples were incubated for up to 2 days in the laboratory at about 21°C at the field moisture (60–90% water content). Samples were placed inside hermetically closed 500 mL glass jars containing a mixture of air and acetylene at 10% v/v. An additional sample per substrate was incubated without acetylene as a control. One gas sample per jar was taken each day and analysed for ethylene production in a Schimadzu gas chromatograph equipped with a Porapack column and a flame ionization detector. To refer acetylene reduction rates to sample dry mass, substrates were dried at 70°C for at least two days and then weighed. Annual rates of non-symbiotic N fixation per ecosystem compartment were estimated assuming a theoretical stoichiometric conversion factor of 1/3 of the acetylene reduction rate, multiplied by their estimated biomass in the plots (Myrold *et al*. 1999).

To better understand successional changes in N present in forest soils, the natural abundances of ¹⁵N isotope, expressed as δ¹⁵N (⁰/₀₀), were determined from three surface soil samples (A_h soil horizon, first 10 cm) taken from each moraine soil age in December 2009. Determinations were made in a Thermo Delta V Advantage Isotope Ratio mass spectrometer, at the Universität Trier, Germany.

Biomass estimates of nitrogen fixer hosts

During November 2010 (austral spring), we placed a 25 × 25 cm PVC frame on each of the six sample points established along transects set up on the summits of each moraine representing a different successional age. The aboveground herbaceous cover and litter within these areas was collected and placed inside zip-lock bags. In the laboratory the O_l horizon was separated from the remainder of the sample, consisting of mosses, hornworts and liverworts, and herbaceous *Gunnera magellanica*. The O_l horizon and the vegetation subsample of cryptogams and *Gunnera* were placed separately in a drying oven at 70°C for at least 48 h.

In each moraine representing a different successional age, two trees from each of three diameter classes (5–15 cm dbh, 15–25 cm dbh, >25 cm dbh) were sampled for epiphyte biomass. Epiphytes were collected from a 15-cm wide belt placed around the trunk at two heights in each of the six trees: 1.3 m (breast height) and near the tree base. Samples were taken to the laboratory and a subsample dried at 70°C for at least 2 days for dry mass determination. Dry mass was referred to the sampled belt area and averaged for both tree heights.

Statistical analyses

Soil and foliage element data for two sampling dates (May and December 2009) were averaged. Data for basal areas, tree species richness, soil and foliage nutrient contents and stoichiometric ratios, N fixation and δ¹⁵N isotopic ratio along the entire chronosequence of moraine sites are presented in scatter plots, with the estimated minimum stand age for each site as the independent variable (horizontal axis). In addition, overall tree basal areas for each stand were plotted against tree species richness. Statistical trends in these variables along the chronosequence of sites were

assessed by the minimum squares best fit line, determined using the program Curve Expert Professional 1.5.0, which compares the fit to the data of a series of linear and non-linear models set by the program (CurveExpert software, http://www.curveexpert.net, 2010). The model with the lowest AIC (Akaike Information Criterion) that significantly differed from the null model was selected as the best fit (Appendix S1) (Quinn & Keough 2002). ANOVAs were used to evaluate differences among sites in available soil nitrogen and phosphorus after box–cox transformations of data and *a posteriori* Tukey's tests (Quinn & Keough 2002). Differences among successional sites in pH and base cations were evaluated with Kruskal–Wallis and *a posteriori* Multiple Comparison tests for non-parametric variables. To evaluate the differences among plant species in foliar N and P concentrations, and C-to-N, C-to-P ratios across sites in the chronosequence, Kruskal–Wallis and *a posteriori* Multiple Comparison tests for non-parametric variables were used. Differences in foliar N-to-P ratios were evaluated with ANOVA after box–cox transformations and *a posteriori* Tukey's tests. Values in the results are averages of three individuals per species in each moraine site, and hence the total number of replicates depended on the number of sites where each species was present. Differences were considered statistically significant at $P < 0.05$. It was not possible to evaluate differences among species correcting for the co-variable stand age, because the assumption of co-linearity of both variables did not apply according to ANCOVA (Quinn & Keough 2002). The statistical program used for the analyses was R (R Development Core Team 2012).

To compare the accumulation rates of C, N and P in Santa Inés soils with other soil chronosequences of glacial forelands the C, N and P soil stocks (g m^{-2}) were estimated from their respective soil contents and referred to the amount of soil per unit area (from the intact soil cores; see above) and the depth of surface soil. Soil stocks of C, N and P at the final stage of the chronosequence were divided by 380 years of soil development to obtain an accumulation rate per annum.

RESULTS

Minimum stand ages in the chronosequence

Tree ring counts and cross dating provided an estimate of minimum ages for the postglacial stands developing on each moraine on Santa Inés Island that varied from 33 to 380 years old (Table 1). These minimum ages were used to establish a successional chronosequence.

Changes in tree basal area and species richness

Deciduous *Nothofagus antarctica* dominated the tree canopy in the early successional stands from ages 33 to 83 years (Fig. 2), whereas evergreen *N. betuloides*, became increasingly abundant in stands older than two centuries (Fig. 2). Both species presented ectomychorrizal associations. The evergreen tree *Drimys winteri* became important in the latest stages of succession over postglacial substrates. An understory layer dominated by the prostrate shrub *Gaultheria mucronata*, with ericoid mychorrizal associations, was present along the entire chronosequence. The only vascular plant with symbiotic N-fixers was the herbaceous *Gunnera magellanica*, which formed a stoloniferous carpet less than 5 cm tall, dominating at the onset of succession, but declining in late successional stages, when the forest floor became covered by a thick carpet of hornworts, liverworts, mosses and lichens (Fig. 2). Some of these cryptogams were also capable of fixing N through epiphytic and symbiotic associations with cyanobacteria. Other herbaceous species present in forest understories such as *Senecio acanthifolius* and *Marsipposermun grandiflorum* decreased in cover towards late succession (Fig. 2)

An increase in total tree basal area characterized the chronosequence studied for the first 380 years of postglacial succession, without reaching an asymptote (Fig. 3a). In contrast, a humped-type function was fitted to the relationship between tree species richness and stand age, as well as to the changes in basal area as a function of tree species richness (Figs 3b,c).

Soil carbon, nitrogen, and phosphorus contents and stoichiometric relationships

In young moraines, especially in the 33-year-old site, we found significantly lower values of plant available N and P, and exchangeable base cations than in mid-successional stages, with intermediate values of these elements characterizing in turn later stages of the chronosequence (Table 2). In contrast, soil pH showed a narrow range of variation throughout chronosequence, with values generally below 5.0 (Table 2).

A statistically significant logarithmic function was fitted to the increasing concentration of total soil C with moraine age (Fig. 4a, Appendix S1). Soil N concentration increased up to 135 years of stand age, followed by relatively constant values in older sites (Fig. 4b, Appendix S1). Soil P, in turn, showed a weak decline with increasing stand age (Fig. 4c, Appendix S1). On the other hand, litter N content showed a weak decline in early successional moraine sites until about 135 years, and then remained relatively constant in older ages of the chronosequence (Fig. 4d, Appendix S1). The C/N ratio in the upper soil horizon switched abruptly from low values in early and mid-succession stages to doubling the previous values after 184 years of stand development, keeping similar values thereafter (Fig. 5a, Appendix S1). Soil C/P ratio
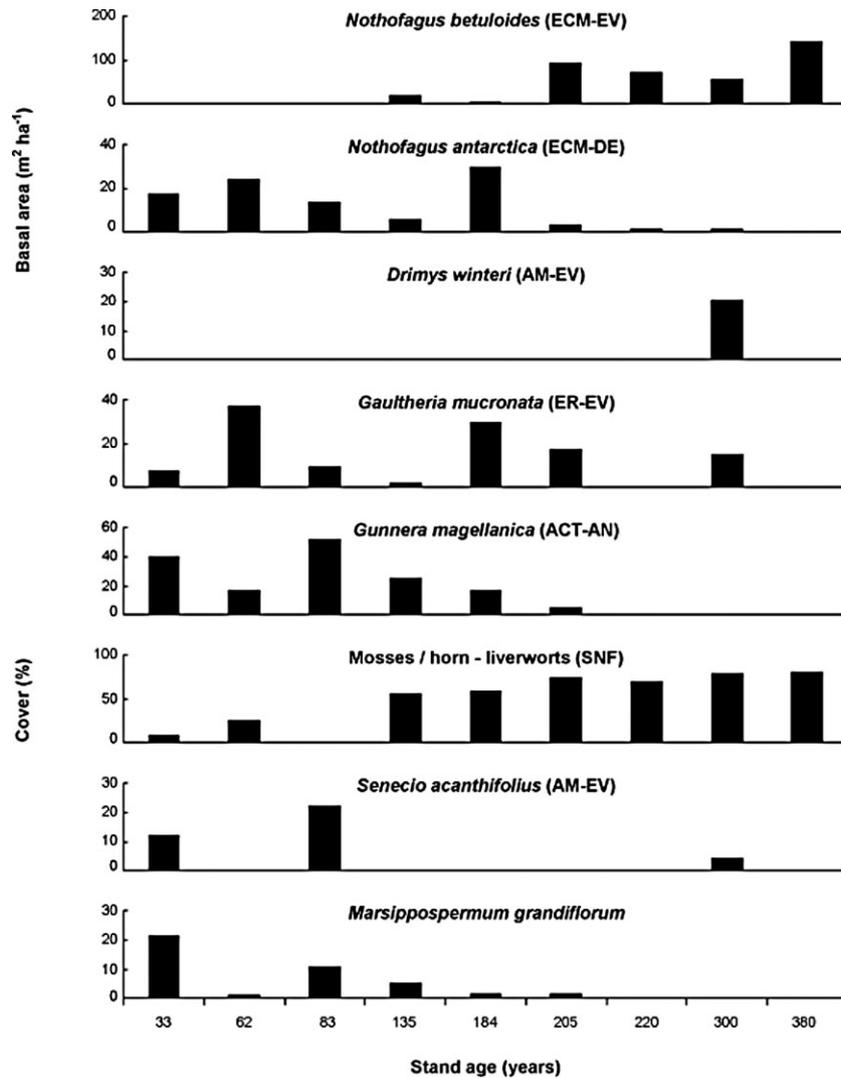
Fig. 2. Changes in basal area of tree species and cover of shrubby and herbaceous species for 400 years of succession, represented by a glacier foreland chronosequence on Santa Inés Island, Magellan Strait. ACT, with actinorrhiza; AM, with arbursular mycorrhiza; AN, annual herb, ECM, with ectomycorrhiza; ER, with ericoid mycorrhiza; EV, evergreen; DE, deciduous; SNF, symbiotic nitrogen fixation.

increased during the first 200 years of stand development, but remained stable thereafter (Fig. 5b, Appendix S1). Soil N/P ratio showed a significant and steady increase during early succession, approaching an asymptote after 150 years of stand age (Fig. 5c, Appendix S1).

Biological nitrogen fixation

Non-symbiotic nitrogen fixation that took place in the litter layer showed a slow increase with stand age, followed by a slight decrease in the successional oldest stand (Fig. 6a, Appendix S1). Rates of symbiotic N fixation associated with cryptogamic epiphyte biomass on tree barks showed an increasing trend with stand age along the chronosequence (Fig. 6b, Appendix S1). On the other hand, symbiotic N fixation rates, associated primarily with forest understory cover of *Gunnera magellanica*, plus liverwort, hornwort and moss cover, were high in early succession, dropping to zero after 200 years of stand development (Fig. 6c, Appendix S1). This understory ecosystem compartment presented N fixation rates two orders of magnitude higher than cryptogams on tree bark, or free-living diazotrophs in the litter layer.

Natural ^{15}N abundance in soils

Natural abundance of ^{15}N isotope in surface soils, measured by δ^{15}N (‰), showed fluctuating values
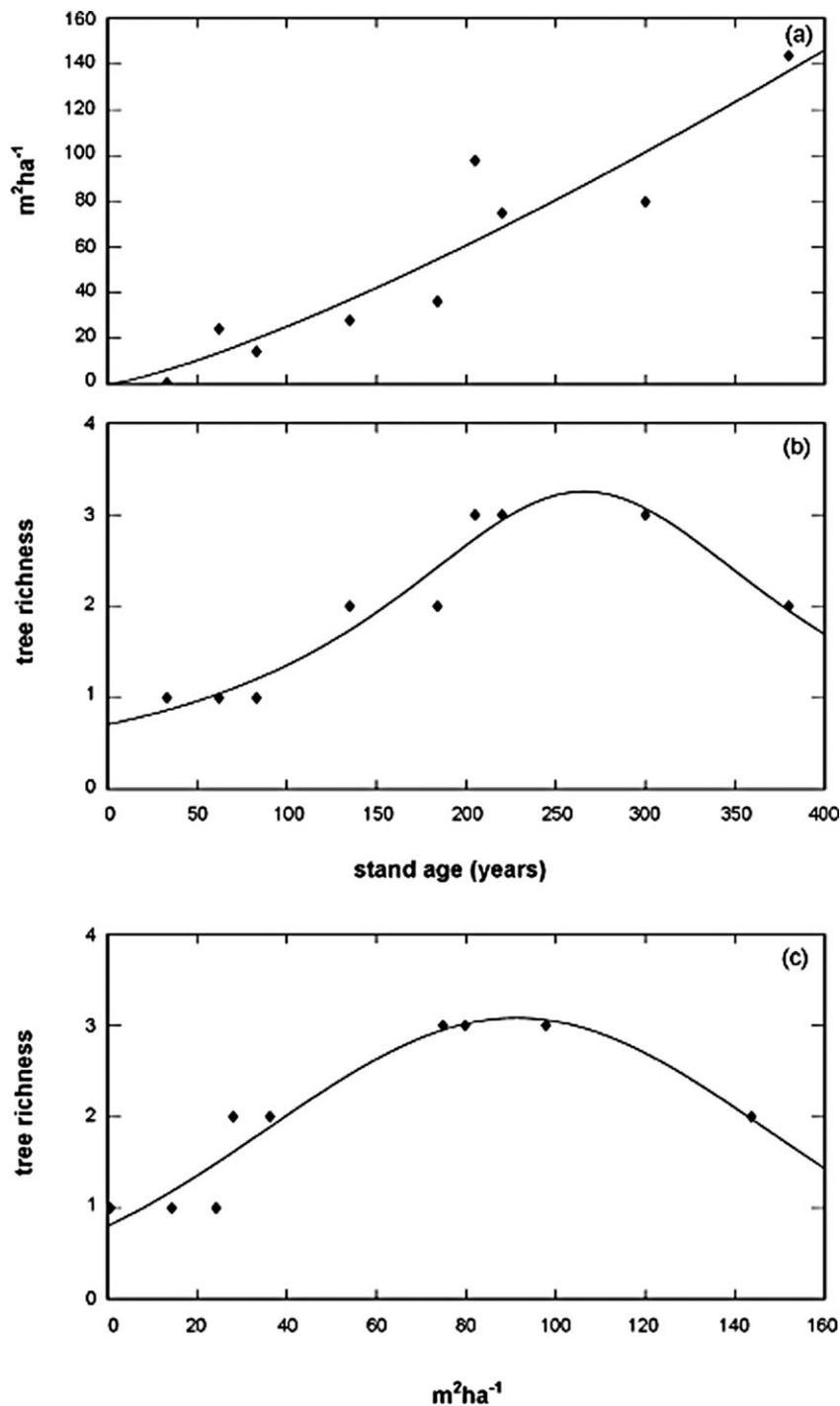
Fig. 3. Changes in (a) total tree basal area, (b) tree species richness, and (c) the relationship between tree basal area and tree species richness during a 400-year glacier foreland chronosequence on Santa Inés Island, Magellan Strait, Chile.

through the chronosequence, with a significant initial decline reversing in the oldest stand (Fig. 7, Appendix S1). Values of $\delta^{15}N$ ($^{\circ}/_{\circ\circ}$) varied from an average of -1 $^{\circ}/_{\circ\circ}$ in less than a century old moraines, to an average of -2.5 $^{\circ}/_{\circ\circ}$ in soil samples from old moraines after 300 years (Fig. 7).

Development of N and P limitation

Both N and P contents of fresh leaf samples were higher and more variable in plants from early successional stages (<100 years), declining to more stable values thereafter in the chronosequence (Fig. 8a,b,

Table 2. Ecological characteristics of study sites on moraines from two glacier forelands (Alejandro and Beatríz) assumed to form a chronosequence on Santa Inés Island, Magellan Strait, Chile

Site name	Minimum moraine age	Moraine successional stage	Altitude (m.a.s.l.)	pH (H$_2$O)	Base cations (cmol kg^{-1})	N$_a$ (mg kg^{-1})	P$_a$ (mg kg^{-1})
Bea 4	33	Early successional	47	5.5[ac] (0.4)	12.8[a] (0.7)	49.1[a] (7.1)	12.5[a] (1.3)
Bea 3	62	Early successional	63	4.6[ac] (0.1)	44.7[abc] (10.9)	89.4[ab] (17.3)	28.0[b] (6.1)
Ale 4	83	Early successional	24	5.1[ab] (0.1)	20.6[ab] (1.6)	64.6[ae] (6.0)	41.9[bc] (2.4)
Ale 3	135	Mid successional	43	4.9[ac] (0.1)	36.9[abc] (3.8)	339.1[d] (35.4)	76.0[d] (8.9)
Ale 2	184	Mid successional	29	4.7[ac] (0.1)	51.1[bc] (6.2)	139.7[bc] (23.1)	71.5[cd] (8.0)
Bea 1	205	Late successional	58	4.5[c] (0.1)	56.8[bc] (14.6)	140.7[bce] (40.6)	45.6[cd] (2.7)
Ale 1	220	Late successional	46	4.8[ac] (0.1)	26.9[abc] (1.4)	191.7[c] (17.9)	10.7[a] (1.1)
Bea 0	291	Late successional	41	4.6[ac] (0.1)	57.1[cd] (10.3)	154.4[cb] (34.4)	45.6[bc] (6.4)
Ale 0	382	Late successional	47	4.7[ac] (0.1)	88.4[cd] (13.4)	214.8[bcd] (26.0)	49.1[cd] (4.1)

Minimum moraine age based on tree ring count of largest pioneering tree. Figures for soil chemical characteristics are means with standard errors in brackets ($n = 6$ samples per site). N$_a$ = plant available N (NH$_4^+$-N + NO$_3^-$-N), P$_a$ = plant available P. Different letters indicate significant differences among sites ($P < 0.05$).

Appendix S1). Foliar C/N ratios were low in early succession, but increased to nearly stable values in older stands (Fig. 8c, Appendix S1). Foliar C/P ratios nearly doubled during the first two centuries of succession, showing slight decline thereafter (Fig. 8d, Appendix S1). Finally, foliar N/P ratios fluctuated slightly (Fig. 8e, Appendix S1) across the successional chronosequence, but always remained above the critical stoichiometric ratio of 12.5 (horizontal line in Fig. 8e), which suggests that P-limitation occurred throughout the chronosequence.

The early successional herbaceous species *Gunnera magellanica*, presented significantly higher leaf N contents and lower foliar C/N and C/P ratios (Table 3) than other species that appeared later in the chronosequence. Later successional species in the chronosequence included the canopy trees *Nothofagus betuloides* and *Drimys winteri*, the understory heath species *Gaultheria mucronata*, and the vine *Lebetanthus myrsinites*. The early successional deciduous tree *N. antarctica* presented foliar contents of N and P similar to the evergreen *N. betuloides*, but with significantly lower C/N and C/P ratios. The sedge *Marsipospermum grandiflorum* (Juncaceae), in the herbaceous layer of early successional sites had similar element contents and stoichiometric ratios as late successional woody species. Foliar N/P ratios did not differ significantly among species in the successional chronosequence, with the tree *D. winteri* the only exception with higher N/P ratio than the shrub *Berberis ilicifolia* (Table 3).

DISCUSSION

### Postglacial development of vegetation and soils

1. Are there parallel trends in overall tree basal area and tree species richness during succession?

    As expected, we found increasing trends in both basal area and tree species richness, however the number of tree species saturated with few species, as 2–3 species remained in the older stages of succession, similar to the Glacier Bay chronosequence (Bormann & Sidle 1990).

    Tree basal areas of successional stands in Santa Inés Island showed a steady increase during 400 years despite evidence of P limitation in these ecosystems. Such a biomass development pattern suggests that the few late successional tree species, the evergreen *N. betuloides* and *D. winteri* are more efficient in exploiting limited resources made available by symbiotic mycorrhizal associations on P-poor substrates. Replacement of deciduous *Nothofagus antartica* by the broad-leaved evergreen *N. betuloides* and *D. winteri* in late stages of postglacial succession could be explained by the increasing shade tolerance and greater N use efficiency (see below) of both evergreen tree species. In the herbaceous layer, *Gunnera magellanica*, a pioneer N-fixing species, becomes replaced by shade tolerant bryophytes, which have different symbiotic associations capable of N fixation but at lower rates.
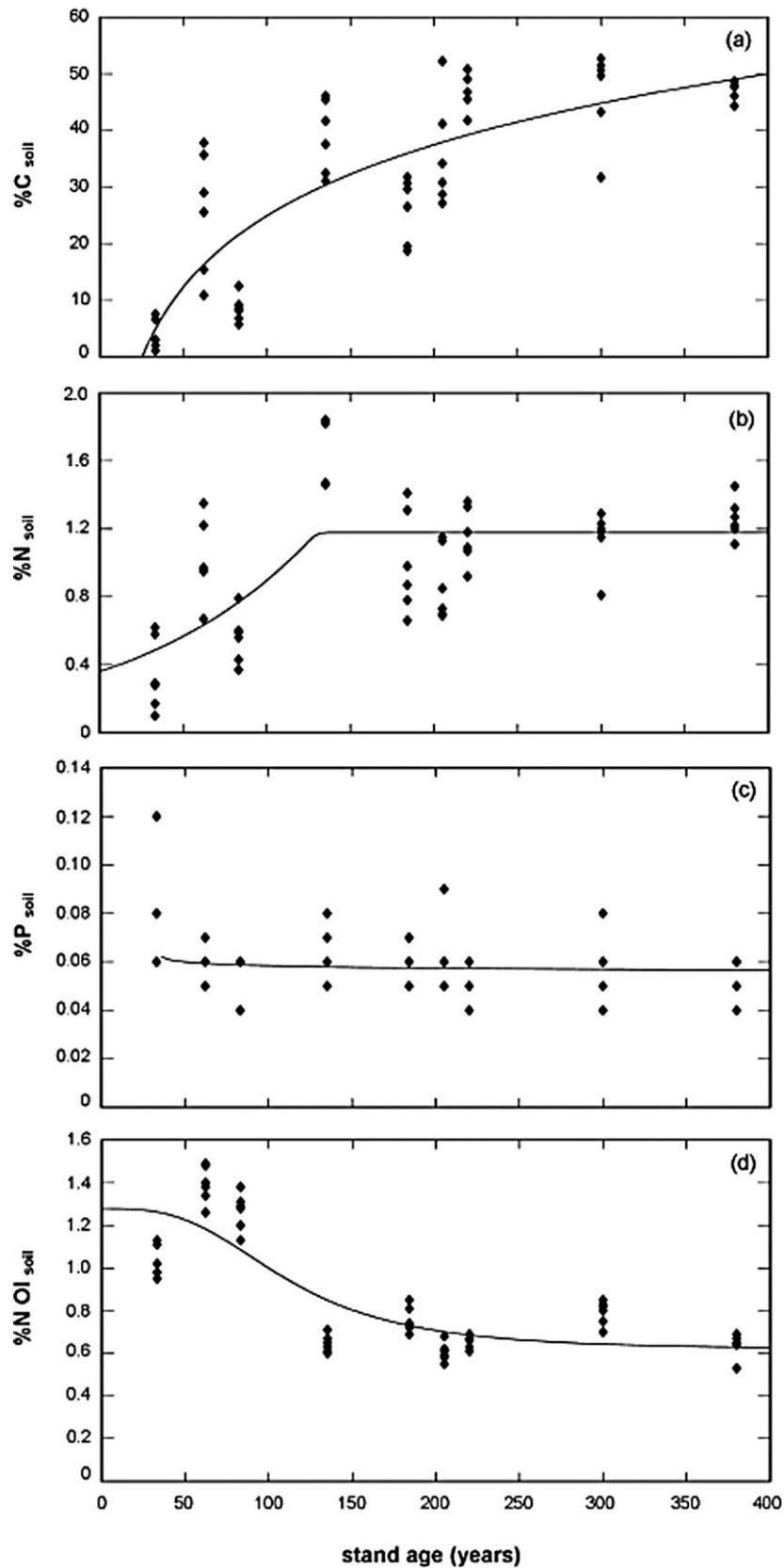
Fig. 4. Changes in (a) total carbon, (b) nitrogen and (c) phosphorus in surface soils and (d) N content in the litter layer O_l during a 400-year glacier foreland chronosequence on Santa Inés Island, Magellan Strait, Chile.
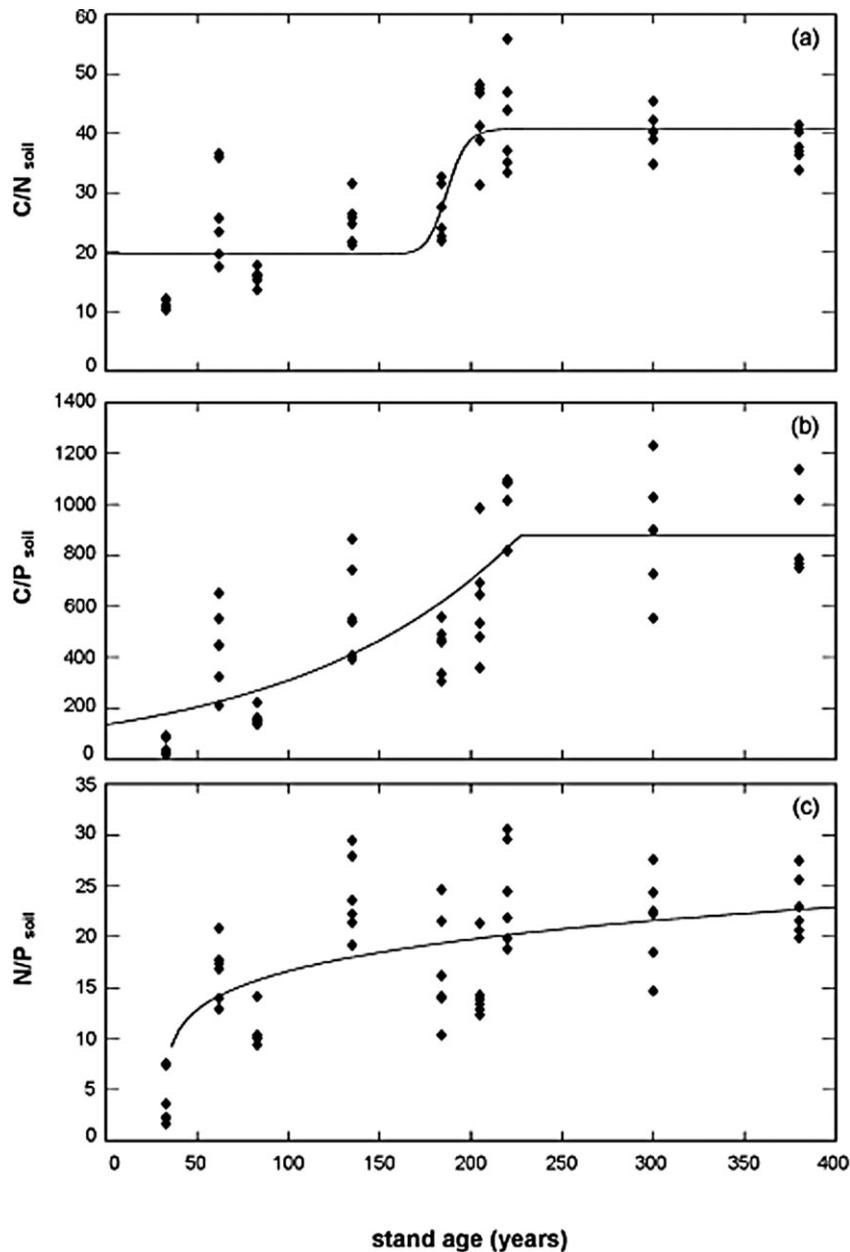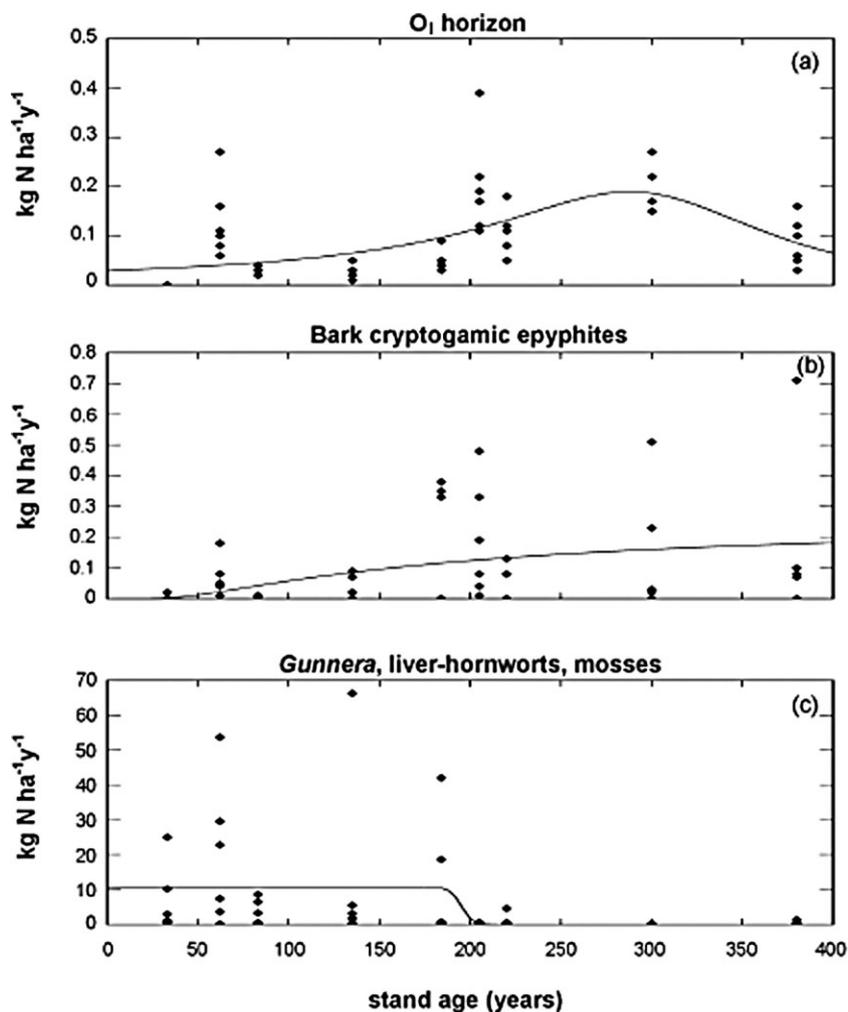
Fig. 5. Changes in (a) C/N, (b) C/P and (c) N/P element ratios in surface soils during a 400-year glacier foreland chronosequence on Santa Inés Island, Magellan Strait, Chile.

2. Is above-ground biomass accumulation related to parallel increases in soil C, N, and P during most of the chronosequence? Is there a relief of nutrient limitation over time as indicated by soil stoichiometric ratios?

In agreement with our expectations, total soil C and N show similar increasing trends, parallel to above-ground biomass, reaching saturation in older stages of succession. In contrast to our expectation, based on the initially P-poor bedrock and the observed accumulation of organic matter during succession, total P content showed a weak-declining trend. The saturation

of C/N, C/P, and N/P ratios in soils with stand age suggests that accumulation of soil N and losses of P lead to element limitation, rather than relief towards late succession.

Carbon and nitrogen accumulated in postglacial soils at average rates of 10.4 g C m^{-2} year^{-1} and 0.28 g N m^{-2} year^{-1} during the first 400 years of stand development. Trends of N accumulation in soils of Santa Inés Island were lower than those reported for a chronosequence in alpine glacier forelands in Switzerland, which was 0.66 g N m^{-2} year^{-1} (Mavris et al. 2010) and those reported for 70 years of postglacial

Fig. 6. Annual rates of nitrogen fixation in (a) the O$_l$ soil layer, (b) the cryptogamic epiphyte layer on trees, and (c) the understory carpets of *Gunnera magellanica*, hornworts, liverworts and mosses during a 400-year glacier foreland chronosequence on Santa Inés Island, Magellan Strait, Chile.
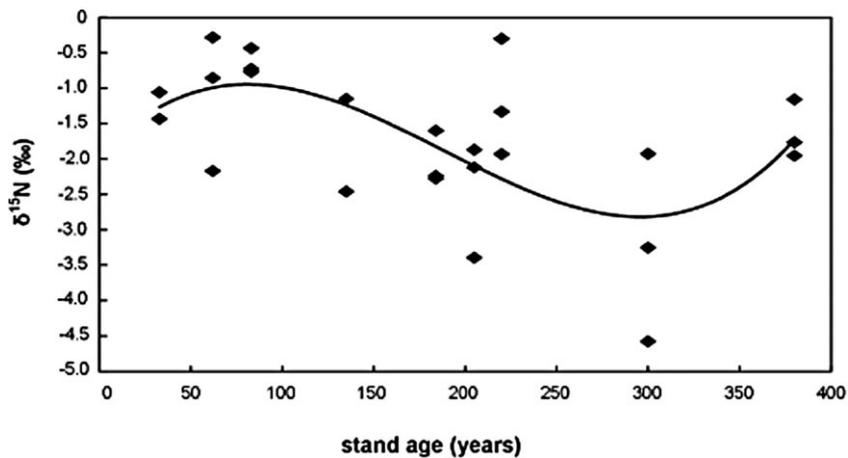
Fig. 7. δ^{15}N (‰) in surface soils for a 400-year glacier foreland chronosequence on Santa Inés Island, Magellan Strait, Chile.
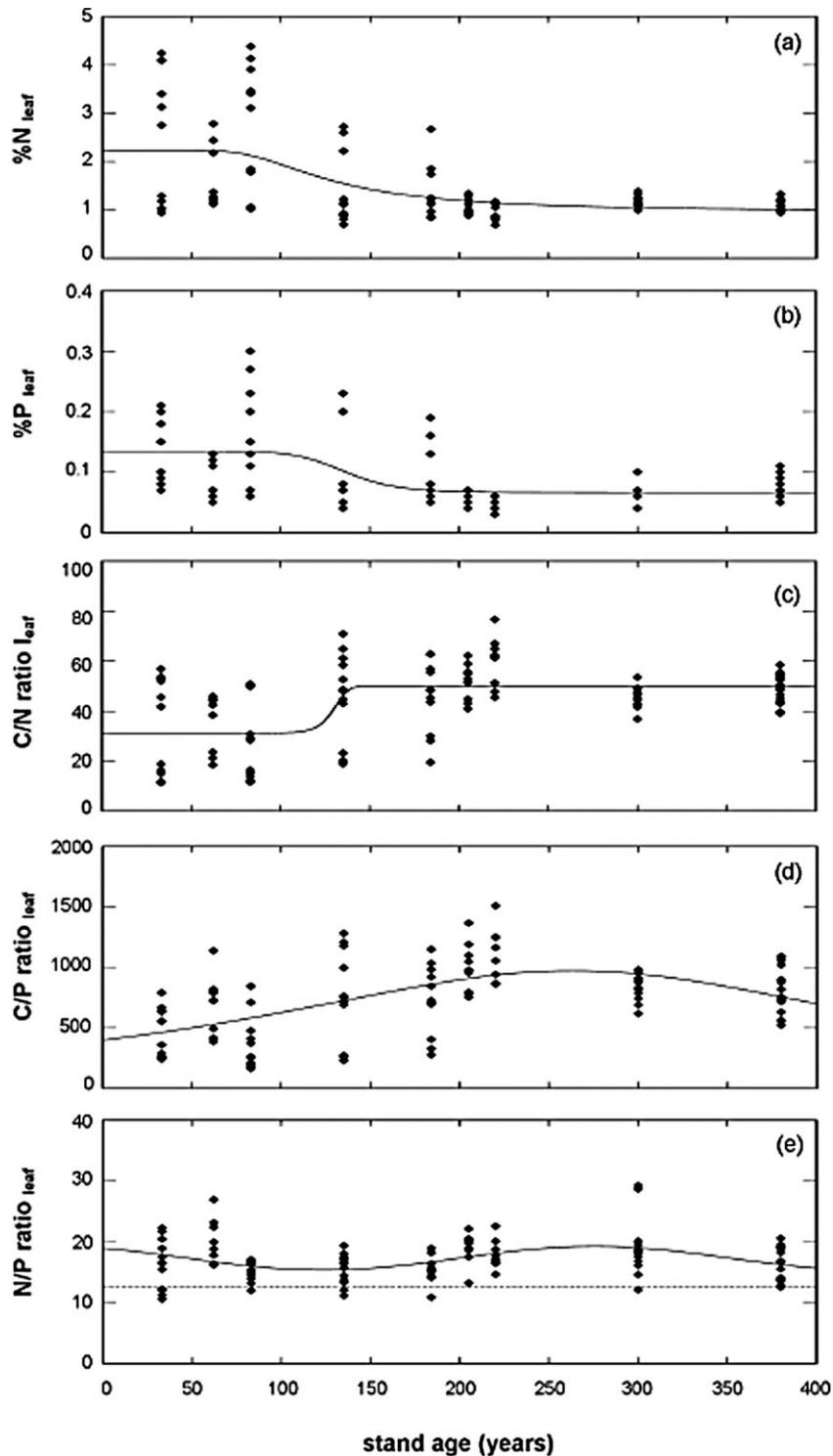
Fig. 8. Changes in (a) total nitrogen, (b) phosphorous, (c) C/N ratio, (d) C/P ratio and (e) N/P ratio for fresh leaves during a 400-year glacier foreland chronosequence on Santa Inés Island, Magellan Strait, Chile. In (e) the dotted line parallel to the x-axis indicate an N/P ratio of 12.5 above which P limitation is suggested.

succession dominated by N-fixing *Alnus sinuata* in Glacier Bay, Alaska, where rates of up to 0.8 g N m⁻² year⁻¹ were documented (Chapin *et al*. 1994). The lower rates of N accumulation in soils of Santa Inés glacier forelands may be a consequence of lower N inputs via precipitation than those characterizing the Swiss Alps, and the lack of symbiotic N-fixing woody species in early ecosystem development, as in

Table 3.   N and P contents of green leaves and stoichiometry of species across sites in glacier forelands of Santa Inés Island, Magellan Strait

Species	Family	Number of individuals	%N	%P	C/N	C/P	N/P
Early successional							
Gunnera magellanica	Gunneraceae	6	3.99[a] ± 0.28	0.23[a] ± 0.05	12.2[a] ± 1.0	216.9[a] ± 39.8	18.0[ab] ± 3.9
Nothofagus antarctica	Nothofagaceae	15	2.76[ab] ± 0.67	0.18[a] ± 0.04	19.9[a] ± 4.9	311.0[a] ± 82.8	16.0[ab] ± 3.6
Chiliotrichum diffusum	Compositae	3	1.82[abc] ± 0.03	0.13[ab] ± 0.02	29.5[ab] ± 1.3	419.4[ab] ± 53.1	14.3[ab] ± 2.2
Marsippospermum grandiflorum	Juncaceae	3	0.92[bc] ± 0.73	0.06[ab] ± 0.02	55.6[ab] ± 18.9	906.1[ab] ± 324.3	16.1[ab] ± 2.2
Late successional							
Nothofagus betuloides	Nothofagaceae	24	1.15[bc] ± 0.10	0.07[ab] ± 0.01	47.3[b] ± 4.0	756.3[b] ± 90.8	16.5[ab] ± 2.6
Drimys winteri	Winteraceae	15	1.01[c] ± 0.14	0.06[b] ± 0.01	54.4[b] ± 8.9	933.5[b] ± 226.1	19.9[a] ± 5.9
Gaultheria mucronata	Ericaceae	27	1.02[c] ± 0.16	0.06[b] ± 0.01	53.8[b] ± 8.0	959.81[b] ± 205.12	18.2[ab] ± 2.6
Lebetanthus myrsinites	Epacridaceae	3	1.00[c] ± 0.15	0.05[b] ± 0.00	54.9[b] ± 9.3	1078.0[b] ± 17.9	19.7[ab] ± 1.0
Berberis ilicifolia	Berberidaceae	4	1.28[abc] ± 0.08	0.09[ab] ± 0.01	40.8[ab] ± 2.5	719.8[ab] ± 207.3	14.0[b] ± 1.7

Different letters indicate significant differences among species ($P < 0.05$).

Glacier Bay. In contrast, soil carbon accumulation rates during postglacial succession in Santa Inés Island stands are similar to those measured in surface soils of other glacier foreland chronosequences. Values of carbon accumulation for similar alpine glacier forelands of Switzerland and China ranged from 6.7 to 36.0 g C m^{-2} year^{-1} (Egli *et al.* 2001; He & Tang 2008; Mavris *et al.* 2010).

An average molar N/P ratio of 22.1 ± 6.2 was found in organic soils from mid and late successional stands in the Santa Inés Island chronosequence. This value is greater than the average N/P value of 14.6 ± 1.8 reported for organic and mineral soils from several ($n = 47$) temperate and tropical forest sites (Cleveland & Lipzin 2007). The average molar ratios of C/N = 82.6 ± 21 and C/P = 1832.2 ± 704.7 calculated for the A$_h$ horizon of Santa Inés forest stands are close to the average values reported by Cleveland and Lipzin (2007) for the litter layer from different world forests (66.6 ± 6.3 for C/N; and 3144 ± 341, for C/P). Higher and relatively stable C/N and C/P ratios recorded in late successional soils (>200 years) from the Santa Inés chronosequence can be attributed to the expected development of recalcitrant pools of carbon during pedogenesis in high latitude soils.

The average soil pools of P in Santa Inés ecosystems (9.0 ± 5.6 kg P ha^{-1}) are not significantly different from those from P-limited tropical soils (4.3 ± 2.1 kg P ha^{-1}) but are much lower than P values reported by Johnson *et al.* (2003) for northern hemisphere temperate forest soils (50.3 ± 29 kg P ha^{-1}). This pronounced difference in soil P between Santa Inés Island postglacial soils and temperate forests studied in the northern hemisphere could be the product of the extremely low concentrations of apatite in the granitic rock that underlies the developing soils of Santa Inés Island. The average molar N/P ratio of fresh leaves from canopy trees in the postglacial chronosequence of

Santa Inés Island was 19.6 ± 4.8 ($n = 54$), a ratio that did not differ from other high latitude forests of the world, with foliage N/P ratios from about 10 to 50 (McGroddy *et al.* 2004; Reich & Oleskyn 2004).

The increase in soil C/P and N/P ratios (Fig. 5b,c) and declining P availability (Table 2) supports an enhancement of P limitation in late stages of succession, similar to that observed in the Franz Josef chronosequence of New Zealand during the retrogressive phase (Richardson *et al.* 2004). Both soil C/N and C/P ratios showed marked increases after 200 years of stand development in the Santa Inés Island chronosequence. Such ratios are well above the critical values of 25 for C/N and 300 for C/P, which are reported in the literature (Coleman *et al.* 2004) to favour immobilization of the respective elements by soil microbes rather than mineralization for plant uptake. These high stoichiometric ratios for the Santa Inés Island soils suggest that limitation of productivity by both elements should occur rapidly (within 150 years) in the postglacial chronosequence.

In contrast, in the case of the Franz Josef chronosequence, soil C/N ratios decreased from 45 to 18.2 during 500 years of soil development, whereas soil C/P and N/P increased from 2.3 to 312 and from 0.07 to 17.1, respectively (Turner *et al.* 2007). The high stoichiometric ratios of C/P = 800 and N/P = 20 found in soils from old stages of succession in our study and the reverse trend for soil C/N recorded during succession in Santa Inés Island sites are consistent with strong N and P limitation in these developing ecosystems.

3. Is soil carbon accumulation associated with an increase in biological N fixation? Are these trends reflected by soil δ^{15}N patterns in the chronosequence?

Heterotrophic nitrogen fixation in plant litter and symbiotic N fixation by cryptogamic epiphytes

growing on tree barks, although lower in magnitude, showed general increases towards late succession in this ecosystem, but reached saturation levels in the O_1 horizon. This pattern agrees with our prediction of increasing rates of N fixation with stand age, expected on the grounds of accumulating soil carbon sources in the case of litter and understory cryptogamic flora and of the greater provision of substrates in the case of tree bark epiphytes. Taking into account all the sources of new nitrogen to this developing ecosystem, an overall decline of biological nitrogen fixation rates during succession could be attributed exclusively to the diminishing cover of *Gunnera magellanica* along the chronosequence. The ^{15}N signal in mineral soil tends to become depleted with stand age, following an inverse pattern to the accumulation of C and heterotrophic N fixation, thus suggesting that as succession proceeds N-depleted products from mineralization accumulate rather than are lost from the ecosystem. Such losses could enrich ^{15}N signal in soils (Menge *et al*. 2011).

The Santa Ines Island chronosequence underwent a 60% depletion of the ^{15}N isotope over successional time. In contrast, in the Franz Josef chronosequence as well as in the short-term Glacier Bay chronosequence, values of δ^{15}N in organic soils were relatively stable and fluctuated around 0‰ along the entire chronosequence (Hobbie *et al*. 1999; Menge *et al*. 2011). We postulate that these contrasting patterns could be associated with stronger N retention in soils from sub-Antarctic glacier forelands. We suggest that during this phase of postglacial succession, N accumulates in the ecosystem because of more efficient retention of ammonium and nitrate from mineralization and nitrification of soil organic matter (Templer *et al*. 2007). Strong retention of available forms of nitrogen within forest ecosystems also characterizes soil organic matter accumulation and microbial communities from North-Patagonian forests in southern Chile (Perakis & Hedin 2001).

Heterotrophic N fixation rates reported in this study for late stages in the chronosequence (mean = 0.2 kg N ha^{-1} year^{-1}) fall below the range of values reported for boreal forests in the northern hemisphere (0.3–3.8 kg N ha^{-1} year^{-1}, Reed *et al*. 2011). In contrast, symbiotic N fixation during early stages of postglacial succession in Santa Inés Island (mean =10 kg N ha^{-1} year^{-1}) is higher than symbiotic N fixation rates reported for northern hemisphere boreal forests (0.3–6.6 kg N ha^{-1} year^{-1}, Reed *et al*. 2011). The herbaceous symbiotic N-fixer *Gunnera magellanica*, which dominates the understory in early succession in Santa Inés glacial forelands, seems to be the main source of new N to the developing ecosystem. This important N source declines rapidly after two centuries of stand development. *Nostoc* symbionts in the rhizomes of *Gunnera* apparently behave as in the *nitrostat* model of Menge and Hedin (2009), as lower nitrogen availability in the early postglacial sites should stimulate N fixation, leading to rapid N accumulation in the fresh leaves of *Gunnera*. N-rich litter derived from this perennial herb (Fig. 4d), which dies back every winter and decomposes on the forest floor, should enhance ecosystem N cycling, and hence the accumulation of N in surface soils from mid and late successional stands. This assumption is supported by the declining ^{15}N signal in surface soils along the chronosequence, which suggests the accumulation of N depleted products from decomposition and probably the increasing importance of mycorrhizal N products as succession proceeds (Hobbie *et al*. 1999).

Development of N and P limitation

4. As above-ground biomass accumulates, do foliar C-to-P and C-to-N ratios evolve to reflect increasing nutrient use efficiency? Are changes of foliar N : P ratios related to soil N : P ratios?

The nutrient use efficiency proxies C/N, C/P ratios in green leaves show a trend towards saturation in older stages of succession. In contrast to the patterns of change in foliar C/P and C/N ratios along the chronosequence of Santa Inés Island, we found no trend for foliar N/P ratio during postglacial succession. Values remained relatively stable across all study sites regardless of soil age and soil N : P ratio.

Average molar ratios of C/N = 88.6 ± 38.5 ($n = 54$) for green leaves from sub-Antarctic rain forest trees in our postglacial sites are two times greater than values reported for temperate and tropical forests from around the world (McGroddy *et al*. 2004), and the high foliar ratio of C/P = 1775 ± 728.2 ($n = 54$) is strongly suggestive of P limitation in these successional ecosystems. Average foliar C/P for the Santa Inés Island chronosequence is similar to values reported for P-limited tropical forests (McGroddy *et al*. 2004). Moreover foliar C/N ratios for late successional tree species in our chronosequence are much higher (Table 3) than those from the Glacier Bay chronosequence, with foliar C/N mass ratios ranging from 18 to 40 (Hobbie *et al*. 1998). These results suggest a stronger nitrogen and phosphorus use efficiency in relation to carbon gain in sub-Antarctic rain forests and also are evidence of the development of co-limitation by nitrogen and phosphorus in the soil chronosequence from Santa Inés Island.

Average declines from 0.13 to 0.07% in the total P content and from 2.35 to 1.11% in total N content in green leaves during four centuries of soil development in the Santa Inés Island chronosequence are also consistent with the above hypothesis. During a similar successional interval, in the Franz Josef chronosequence, concentrations of foliar P declined

less steeply from 0.2 to 0.15% while foliar N showed a similar decline from 2.2 to 1.4% (Richardson *et al.* 2004). Moreover, foliar P contents were 50% lower in the sub-Antarctic chronosequence. We propose that in the Santa Inés Island glacier forelands increasing N and P use efficiency by the plants during succession may represent a unique ecosystem response to the P-poor geological substrate in an extremely unpolluted environment, lacking significant atmospheric nutrient sources.

Along with the development of nutrient co-limitation during the Santa Inés postglacial chronosequence, we recorded a shift in forest dominance, from woody species with lower N and P use efficiency, such as the deciduous *Nothofagus antarctica*, and the understory N-fixer *Gunnera magellanica*, to increasingly nutrient-use efficient species, such as the evergreen *Nothofagus betuloides* and *Drimys winteri* in the canopy, and mosses, hornworts and liverworts on the forest floor. Widespread presence of mychorrizal associations along the Santa Inés Island chronosequence would allow P mining from P-poor postglacial substrates.

We hypothesize that foliar N/P ratios in the chronosequence of Santa Inés Island represent a homeostatic successional pattern, in the sense that foliar N/P ratios become largely independent of temporal fluctuations in soil N/P (Sterner & Elser 2002). Such a pattern is characteristic of ecosystems where plants tend to be co-limited by both N and P (Sterner & Elser 2002), such as we suggest occurs in the Santa Inés Island chronosequence in which forests develop over a P-poor substrate and in the absence of significant inputs of reactive nitrogen from industrial sources.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Models developed to explore regressions of stand age against different dependent variables.