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Bedrock geology interacts with altitude in affecting leaf growth and foliar nutrient status of mountain vascular plants

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Abstract

Aims
Altitude is often used as a proxy for ascertaining how warming affects plant growth and leaf level properties. However, we have a poor understanding of how the effects of altitude-related warming varies across geology. Therefore, this study examined the independent and interactive effects of altitude and geology and species on plant growth and foliar nutrient status.

Methods
We determined leaf growth rates and concentrations of major nutrients (nitrogen, N and phosphorus, P) in leaves of five species across two altitudinal gradients (1200–2200 m) in the Dolomites (south-eastern Alps, Italy). The two transects were located on carbonate bedrock and silicate bedrock, respectively. We also determined concentrations of inorganic and organic N and P forms in soils, and δ ^{15}N signature in leaves and soils.

Important Findings
Foliar N concentrations were unrelated to bedrock geology. The negative foliar δ ^{15}N signature suggested that organic N was the primary source of N supply across the gradients. Foliar P concentrations were strongly affected by bedrock geology and their altitudinal patterns depended on the concentrations of organic and inorganic P forms in the soil. Phosphates and organic P appeared to be the main sources of P supply. Leaf growth rates increased with higher altitude on silicate bedrock and decreased with higher altitude on carbonate bedrock and presented a significant positive correlation with foliar N:P. In conclusion, bedrock geology interacted with altitude in controlling the foliar nutrient status mainly owing to availability of soil P and its effect on foliar nutrient stoichiometry.

Key words: altitude; mycorrhiza; plant functional type; soil chemistry; ^{15}N

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INTRODUCTION

Decreasing temperature with increasing altitude can affect both growth rate and nutrient status of mountain vascular plants. Low temperature can limit plant growth by direct effects of cold limitation implying, e.g. insufficient photosynthesis (carbon gain limitation: Li *et al.* 2008), suppressed activity of meristems (sink limitation: Körner 1998; Streit *et al.* 2013) or both. Cold temperatures can also reduce soil nutrient availability because rates of nutrient mineralization are positively related to soil temperature (Salinas *et al.* 2011; Carbutt *et al.* 2013). It is difficult to disentangle direct effects of cold limitation from indirect effects of nutrient limitation to plant growth. For example, low soil temperatures depress root growth (Alvarez-Uria and Körner 2007) and also decrease nutrient uptake rates (Boczulak *et al.* 2014). The nutrient status of root tissues can trigger a feed-back mechanism for controlling nutrient uptake which indirectly regulates plant growth (Gessler *et al.* 2002). On the other hand, the nutrient status of plant tissues may depend on other sources besides root uptake of readily available, i.e. mineralized, nutrient compounds. Plants can acquire notable amounts of major nutrients through mycorrhizal fungi, that access organic nitrogen (N) compounds (Hyodo *et al.* 2013) or scavenge recalcitrant phosphorus (P) species in the soil (Lambers *et al.* 2008).

Although upslope reduction in air and soil temperature represents a general feature in mountains (Körner 2008), altitude represents a complex gradient (Körner 2007) in which individual factors can interact with each other in a non-linear way. For example, relationships between nutrient

mineralization and thermal regime can vary in relation to soil moisture content (Marrs *et al.* 1988). Mycorrhizal infection, as a putative adaptation to nutrient deficiency, tends to increase with increasing altitude. On the other hand, the activity of mycorrhizal fungi can be even stimulated by increased temperature (Kytöviita and Ruotsalainen 2007). Some studies reported increasing concentrations of foliar nutrients, especially N, as altitude increases (see, e.g. Morecroft *et al.* 1992; Peng *et al.* 2012). Such increases in nutrient concentrations with increasing altitude have been regarded as a side effect of reduced biomass production with increasing altitude. This would give support to direct effects of cold limitation as the main cause affecting foliar nutrient concentrations in mountains. However, cold limitation may largely depend on other factors such as plant functional type (PFT), soil water content (SWC) and soil nutrient availability. For example, Körner *et al.* (1986) found foliar N concentrations to increase with increasing altitude in herbaceous plants but not in evergreen woody plants in New Zealand, which suggests that plant responses to temperature and/or associated environmental factors may vary depending on PFT. Diaz *et al.* (1996) did not observe any significant effect of altitude on N concentrations in leaves of *Clusiaceae* in Venezuela probably because of interactions between nutrient availability and SWC. Similarly, foliar nutrient concentrations in an evergreen oak species in south-western China peak at intermediate altitude where the plants achieve maximum nutrient use efficiency without lowering water use efficiency (Li *et al.* 2009). Foliar nutrient concentrations can even decline with increasing altitude, e.g. in conifer trees in North America (Hultine and Marshall 2000) or in species belonging to different PFTs in tropical-montane forest in Ecuador (Soethe *et al.* 2008). These latter findings point to indirect effects of cold temperatures, possibly related to temperature limitations of soil microbial activity, on foliar nutrient concentrations at high altitude.

Availability of mineral nutrients also depends on geochemical processes of mineral weathering. The latter are closely related to the nature of the underlying parent material, which controls important aspects of soil chemistry, especially pH. Therefore, the nature of the parent material plays a major role as a source of primarily rock-derived nutrients such as phosphorus (P) and potassium (Castle and Neff 2009; Porder and Ramachandran 2013). As an effect, complex patterns of foliar nutrient concentrations can take place across altitudinal gradients in mountains (Kitayama and Aiba 2002; Porder and Chadwick 2009; Anic *et al.* 2010) with possible additional inter-relations with ecological factors such as light and/or water (Luo *et al.* 2004). In this paper, we analyzed growth rates and nutrient concentrations in leaves of five species, each belonging to a different PFT, across altitudinal gradients on two different bedrock types. We focused our attention on N and P as the elements most commonly limiting plant growth in terrestrial ecosystems (Vitousek *et al.* 2010). Our objective was to test the following hypotheses.

(i) Foliar nutrient status is primarily determined by temperature-dependent growth limitation. If so, we expect foliar nutrient concentrations to increase and leaf growth rates to decline regularly across altitudinal gradients.

(ii) Foliar nutrient status is primarily determined by temperature-dependent nutrient limitation through reduced soil microbial activity. If so, we expect nutrient concentrations to decrease consistently across altitudinal gradients unrelated to growth rates and parent material.

(iii) Foliar nutrient status is primarily determined by (bio) geochemical controls on soil nutrient availability. If so, we expect nutrient concentrations to vary differently across altitudinal gradients depending on parent material. In this case, foliar growth rates may present varying relationships with foliar N or P concentrations depending on which of the two nutrients are most limiting to plant growth.

MATERIALS AND METHODS

Sampling design and sampling sites

The focal point of our study was to analyze altitudinal patterns of leaf growth and foliar nutrient status in plant species each belonging to a different PFT. As individual species within a PFT may differ considerably in foliar nutrient concentrations with additional variation being associated to local environmental factors (Bombonato *et al.* 2010), our sampling strategy was based on selecting species all occurring across the whole gradient on both parent materials at environmentally comparable sites. During preliminary surveys we realized that very few species can be actually found both on carbonate and silicate bedrocks across a rather wide altitudinal gradient as ours. We eventually chose five species all of them occurring with medium to high frequency over the whole gradient: *Vaccinium myrtillus* (*V. myrtillus*), a deciduous dwarf shrub; *Vaccinium vitis-idaea* (*V. vitis-idaea*), an evergreen shrub; *Picea excelsa* (*P. excelsa*), a conifer tree; *Homogyne alpina* (*H. alpina*), a wintergreen forb; and *Calamagrostis villosa* (*C. villosa*), a grass. All five species currently form mycorrhizal associations with different kinds of fungal partners: ericoid mycorrhizas (ErM) in *V. myrtillus* and *V. vitis-idaea*; ectomycorrhizal (ECM) in *P. excelsa*; arbuscular mycorrhiza (AM) in *H. alpina* and *C. villosa* (Harley and Harley 1987).

Sampling was carried out in two areas in the western part of the Dolomites (southern Alps, province of Trento, Italy). The first area was located in Val San Nicolò (46°25′N, 11°43′E) on carbonate bedrock (dolomite and limestone). The second area was located in Val Cadino (46°13′N, 11°27′E) on silicate bedrock (granitic porphyry). Six sampling sites were set up in each of the two areas. The six sites were located at *ca.* 200 m intervals, so that each of the two altitudinal transects spanned a *ca.* 1000 m wide altitudinal range (*ca.* 1200–2200 m). All sites had moderate slope angle (20° on average), northern aspect and no evident signs of disturbance. Mean air temperature during the period May–July 2011 ranged from 14°C

at 1000 m to 7.7°C at 2200 m, with a vertical temperature lapse of 0.52°C per 100 m altitude. Total precipitation during the same period was *ca.* 470 mm at the low-altitude sites and ca. 450 mm at the high-altitude sites (http://www.meteotrentino.it/dati-meteo/stazioni; http://www.arpa.veneto.it/dati-ambientali/open-data/clima). The soil temperatures during the same period ranged from *ca.* 9°C at 1200 m to *ca.* 5°C at 2200 m (see online supplementary material, Appendix 1). The vertical temperature lapse for soil temperatures was somewhat higher in the carbonate sites (0.44°C per 100 m altitude) than in the silicate sites (0.32°C). We did not determine SWC during sampling. However, we did measure SWC at the top of the growing season of 2016 when precipitation during May–July was almost the same compared with May–July 2011 (*ca.* 450 mm at the low-altitude sites and ca 460 mm at the high-altitude sites). The relative water content in the soil was *ca.* 23% on average and did not change either with altitude or with bedrock (see online supplementary material, Appendix 2).

The sites from 1200 to 1800 m were located in closed spruce forests. The sites at 2000 m were located in more open spruce forests, close to the treeline. The sites at 2200 m were located above the treeline in subalpine scrubs with sparse trees. The soils were always characterized by a fine grained organic layer (mean depth *ca.* 10 cm) and an underlying mineral layer richer in coarser material. At all sites, the ground was covered by a well-developed moss layer (moss cover > 60%), with *Rhytidiadelphus triqueter*, *Hylocomium splendens* and *Pleurozium schreberi* as the dominant species. The sites had an area of *ca.* 1 ha. In each of them we randomly set up five 5 × 5 m plots, at least 15 m apart, for sampling.

Sampling

In spring 2011, we surveyed all sites at 2–3 days intervals in order to monitor the start of leaf growth for the five species in each site. Current-year leaves were sampled during two days (1–2 August 2011). On those dates, deciduous leaves had totally stopped growth while evergreen and wintergreen leaves probably still kept on growing especially at the high-altitude sites. Nonetheless, we decided to sample all leaves in that period because the leaves usually are heavily browsed by herbivores from early August onwards so that sampling later in the season would preclude measurements of both growth and nutrient concentrations (Kaarlejärvi *et al.* 2013). However, in order to account for possible underestimations of total growth, we adjusted all growth measurements by calculating daily growth rates based on the time elapsed between growth start and sampling (see online supplementary material, Appendix 3).

Sampling was carried out as follows in each of the five plots in the 12 sites. For *V. myrtillus* and *V. vitis-idaea*, we collected 10 fully expanded leaves, each taken from a different current-year shoot (usually the fourth leaf from the apex). For *P. excelsa*, we collected all leaves from four current-year shoots each taken at breast height, at different orientation, each from a different tree. For *H. alpina*, which has only leaves as aboveground organs, we collected 10 current-year leaves at least 50 cm apart. For *C. villosa*, we collected 10 fully expanded leaves each from a different culm (the third or the fourth leaf from the apex). On the same occasion, we collected soil samples in all plots. The soil was sampled from the top 5-cm organic layer, using a stainless steel cylindric corer (inner diameter 6.6 cm). In each plot, we took five subsamples that were then bulked in a composite sample.

Laboratory analyses

The leaf samples were oven-dried at 40°C for 48 h. A subsample of additional leaves was oven-dried at 105°C and weighed to determine the mass loss between 40°C and 105°C. Foliar growth was expressed as daily growth rate per individual leaf. Fifty milligrams of the leaves used for determining growth rates were powdered, extracted in 3 ml of selenous H_2SO_4 at 420°C and analyzed for total N concentrations by the salicylate method and total P concentration by the molybdenum blue method using a continuous flow autoanalyzer (FlowSys; Systea, Anagni, Italy).

The soil samples were air-dried prior to the analyses. A subsample of 5 g was used for determining soil pH in a 1:20 aqueous solution. A subsample of 0.1 g was extracted and analyzed for total N as for the leaves and the litter. A subsample of 20 g was extracted in 200 ml of 0.5 M K_2SO_4 and analyzed for NH_4 by the salicylate method and for NO_3 by the cadmium reduction method. A subsample of 1 g was extracted in 1:10 (soil to solution) 0.5 M K_2SO_4 for determining total dissolved nitrogen (TDN). TDN was analyzed by the cadmium reduction method after digestion with an oxidant reagent containing a buffer solution of boric acid (H_3BO_3), sodium hydroxide (NaOH) and potassium persulfate ($K_2S_2O_8$). Dissolved organic nitrogen (DON) was calculated by subtracting the sum of dissolved inorganic nitrogen fractions (NO_3 + NH_4) from TDN.

A subsample of 0.5 g was sequentially extracted for determining soil P fractions (Kitayama *et al.* 2000). Different solutions (30 ml each) were used in the following steps:

- 0.5 M $NaHCO_3$ adjusted to pH 8.5 with NaOH;
- 0.1 M NaOH;
- 1 M HCl;
- concentrated H_2SO_4.

The extracts of the $NaHCO_3$, NaOH and HCl solutions contained both inorganic (Pi) and organic (Po) P fractions. At each of the first three steps ($NaHCO_3$, NaOH and HCl), Pi was determined by the molybdenum blue method while total P was determined after digesting a subsample of the extract with acidified potassium persulfate ($K_2S_2O_8$) in order to convert Po into Pi. At each of these three steps, Po concentration was calculated by subtracting Pi from total P. Only total P was determined, as above, in the concentrated H_2SO_4 digest. Concentration of residual P (P res) was calculated by subtracting the sum of total P in the ($NaHCO_3$ + NaOH + HCl) solutions from total P in the concentrated H_2SO_4 digest.

A subsample of 0.5 g was extracted in concentrated H_2SO_4 and used for determining total Ca concentration by flame atomic absorption spectrophotometry (Solar 969, Unicam, Cambridge, UK). A subsample of 0.5 g was extracted in 0.2 M acid ammonium oxalate to pH 3, filtered (0.45 µm mesh) and used for determining concentrations of oxalate-extractable Fe and Al by ICP-MS (Thermo X-Series equipped with CCT^{ED}; Thermo Fisher Scientific, Bremen, Germany). A subsample of 1 g was oven-dried at 105°C and weighed to determine the mass loss between air-dried and oven-dried material. A subsample of 20 mg was used for analyzing total carbon (C) by a Shimadzu TOC-5000A (Shimadzu Corporation; Kyoto, Japan), connected with a solid sample module (Shimadzu SSM-5000A).

Nitrogen isotopic discrimination was assessed by determining ^{15}N content both in soils and leaves. The measurements were carried out by an elemental analyzer (EA 1110, Carlo Erba, Milan, Italy) coupled online with an isotope ratio mass spectrometer (delta Plus XP, ThermoFinnigan, Bremen, Germany). The values were expressed as $\delta\ ^{15}N$:

$$\delta^{15}N(‰) = ((R_{sample} / R_{standard}) - 1) \times 1000$$

where R_{sample} is the $^{15}N/^{14}N$ ratio in the sample and $R_{standard}$ the $^{15}N/^{14}N$ ratio in the standard (atmospheric N_2).

Statistics

The soil chemistry data were analyzed by a multivariate statistic method (principal component analysis; PCA). The analysis was based on the correlation matrix among the 15 soil chemistry variables which provided centred standardized scores for variables and plots, after scaling to unit variance all variable vectors.

The data on foliar chemistry were analyzed by three-way factorial analyses of variance with species, altitude, bedrock and their interactions as fixed factors. All data were log-transformed when not meeting the assumption of variance normality as assessed by the Kolmogorov–Smirnov test. Significance of differences among means were assessed using the Fishers's LSD post-hoc test. The data on leaf growth were analyzed by linear regressions of the mean values ($N = 5$) of each variable against altitude in the six sites across the gradients. Linear regressions were run for foliar chemistry variables as well. The regressions were run separately for the two transects and significance of differences between bedrock types was assessed by Student's *t* tests. The statistical computations were carried out using the package STATISTICA (Release 6; StatSoft Inc.©, Tulsa, OK, USA).

RESULTS

Soil chemistry

Bedrock geology strongly affected soil chemistry, with rather few soil chemical variables presenting consistent altitudinal patterns on the two parent materials (see online supplementary material, Appendix 4). The results of the PCA mirrored the relationships among soil chemistry variables (Fig. 1a). Carbon, total N, DON, NH_4, $NaHCO_3$-Pi and N:P presented positive correlations with each other (see online supplementary material, Appendix 5) and were hence grouped in the left-hand side of the PCA diagram (Fig. 1a). All other variables were located in the right part of the diagram as an effect of their mutual positive correlations (Fig. 1a). In particular, total P concentrations presented significant positive correlations with Po and with all Pi fractions except $NaHCO_3$-Pi (see online supplementary material, Appendix 5). NaOH-Pi was positively correlated with Al + Fe and with Po (see online supplementary material, Appendix 5). HCl-Pi was positively correlated with Ca and with Al + Fe (see online supplementary material, Appendix 5). Soil $\delta\ ^{15}N$ was negatively correlated with both soil total N concentration and soil C concentration but was positively correlated with all P fractions except $NaHCO_3$-Pi and with Ca and Al + Fe concentrations and pH as well (see online supplementary material, Appendix 5).

The ordination of the plot centroids in the PCA diagram indicated that soil chemistry changed regularly across the altitudinal gradient on carbonate bedrock (Fig. 1b). That gradient was principally characterized by increasing concentrations of all P fractions, with the only exception of $NaHCO_3$-Pi, and by increasing soil $\delta\ ^{15}N$ (Fig. 1b). In contrast, there were no consistent changes in soil chemistry across the altitudinal gradient on silicate bedrock (Fig. 1b).

Foliar chemistry

The five species differed strongly in terms of foliar N concentration. Species identity accounted for 83% of the explained variance in foliar N concentration, while altitude, bedrock and their interactions with species identity explained only a minor fraction of variance (Fig. 2a). Only in *P. excelsa* did foliar N concentrations increase with altitude on both parent materials while in the other species there was no consistent altitudinal trend of foliar N concentrations (Fig. 3a). Foliar P concentration also differed significantly among species but foliar P concentration was strongly affected by bedrock, which accounted for a larger fraction of the explained variation than species identity (40% vs. 15%, respectively; Fig. 2b). Altitude × bedrock interaction also contributed considerably (35%) to the explained variance, with modest interactions with species identity (Fig. 2b). In fact, foliar P concentrations in all species declined consistently with altitude on silicate bedrock and increased more or less regularly with altitude on carbonate bedrock (Fig. 3b).

The foliar N:P varied much with species. Indeed, species identity accounted for 69% of the explained variance (Fig. 2c). Bedrock and altitude × bedrock interaction also accounted for a relatively high fraction of the explained variance (25% in total), with poor interactions with species identity (Fig. 2c). Indeed, the foliar N:P decreased on carbonate bedrock and increased on silicate bedrock across the altitudinal gradient in all species, generally with a linear trend (Fig. 3c). The foliar $\delta\ ^{15}N$ was always negative (Table 1).

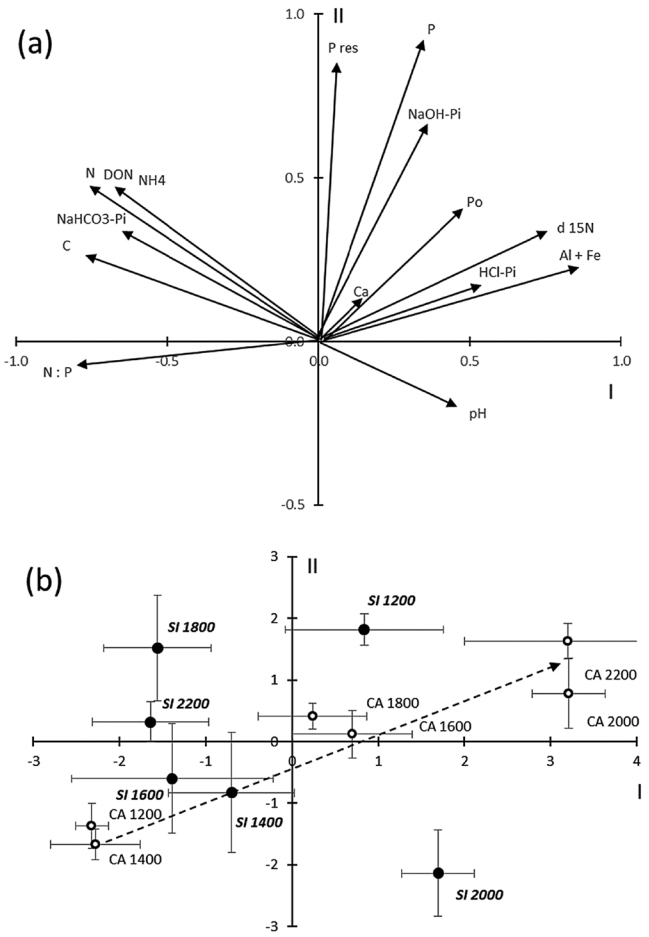

Figure 1: scores of soil chemistry variables and sampling plots on the first two PCA axes accounting for 36.5% (I axis) and 18.1% (II axis) of total variance, respectively. The scores of the soil chemistry variables are shown in (**a**) where the arrows indicate increasing values of each variable along the two PCA axes. The means (±SE) of the plot score centroids at different altitudes on the two bedrocks (CA = carbonate, empty symbols, normal characters; SI = silicate, full symbols, italics) are shown in (**b**) where the dashed arrow indicates ordination of the plot centroids along an altitudinal gradient on carbonate bedrock. Abbreviation: SE = standard error.

Altitude + altitude × bedrock interaction contributed more than species identity to the explained variance (50% vs. 42%, respectively) in foliar δ ^{15}N while interactions with species identity were overall poor (Fig. 2d). On carbonate bedrock, the foliar δ ^{15}N generally increased, i.e. became less negative, from 1200 to 1800–2000 m and decreased again at 2200. On silicate bedrock, the foliar δ ^{15}N decreased more or less regularly with increasing altitude in all species except *C. villosa* that presented an erratic trend across the gradient (Table 1). The δ $^{15}N_{(leaf–soil)}$ difference varied strongly with altitude in all species ($P < 0.001$). Conversely, there were no main effects of bedrock on the δ $^{15}N_{(leaf–soil)}$ difference for any species. The δ $^{15}N_{(leaf–soil)}$ difference was lowest at the highest sites (2000 and 2200 m) on both bedrock types for all species except *C. villosa* that presented an erratic trend in the δ $^{15}N_{(leaf–soil)}$ difference across the gradient on silicate bedrock (Table 1). This resulted in a significant ($P < 0.001$) altitude × bedrock interaction for *C. villosa*.

Leaf growth

Parent material exerted an overall poor main effect on leaf growth rates, as bedrock significantly influenced leaf growth only in *P. excelsa* that grew more ($P < 0.001$) on carbonate bedrock (Fig. 4e and f). Leaf growth in all species showed differing patterns across the gradient on the two parent materials. Leaf growth rates of all species increased with increasing altitude on silicate bedrock, although this trend was linear only for *V. vitis-idaea* and *C. villosa* (Fig. 4d and j). In contrast, leaf growth of all species except *C. villosa* (Fig. 4i and j) tended to decline, although rather erratically, with increasing altitude on carbonate bedrock (Fig. 4a, c, e and g).

Leaf growth rate was negatively correlated with foliar N concentration in all species except *C. villosa* and even more strongly negatively correlated with foliar P concentration in all five species (Table 2). Consequently, leaf growth rate presented a significant positive correlation with foliar N:P in *V. myrtillus*, *P. excelsa*, *H. alpina* and *C. villosa* and a weakly ($P = 0.07$) significant positive correlation with foliar N:P in *V. vitis-idaea*. Leaf growth rate was negatively correlated with foliar δ ^{15}N in *V. vitis-idaea*, *P. excelsa* and *H. alpina* (Table 2).

DISCUSSION

Temperature-dependent leaf growth limitation (hypothesis 1)

There was no evidence of consistent changes in leaf growth rates of the five species across the altitudinal gradients. Only in the conifer tree *P. excelsa* did leaf growth rates decline at the uppermost sites especially on silicate bedrock. This suggests a threshold effect of temperature that hampers growth of high stature plants above the natural treeline (Paulsen and Körner 2014). In contrast, leaf growth of dwarf shrubs and herbs generally did not decrease, and in some cases even increased with increasing altitude because these species are less prone to temperature-dependent growth limitation thanks to their small stature (Körner 2008). Several studies reported high vegetative growth performances at high altitudes for low-stature plants unless growth was limited by environmental factors such as nutrient availability (Luo *et al.* 2004) or water availability (He *et al.* 2014a). In particular, leaf growth rates of *C. villosa* were highest at high-altitude sites in sparse forests (2000 m on silicate bedrock) or scrubs above treeline (2200 m on both bedrock types), compared with dense forests at lower altitudes where leaf growth of *C. villosa* was probably limited by insufficient light supply (Patsias and Bruelheide 2013).

In summary, our study did not reveal any consistent trend of leaf growth rates across altitudinal gradients on the two bedrock types. This leads us to exclude direct effects of cold temperature on leaf growth (hypothesis 1).

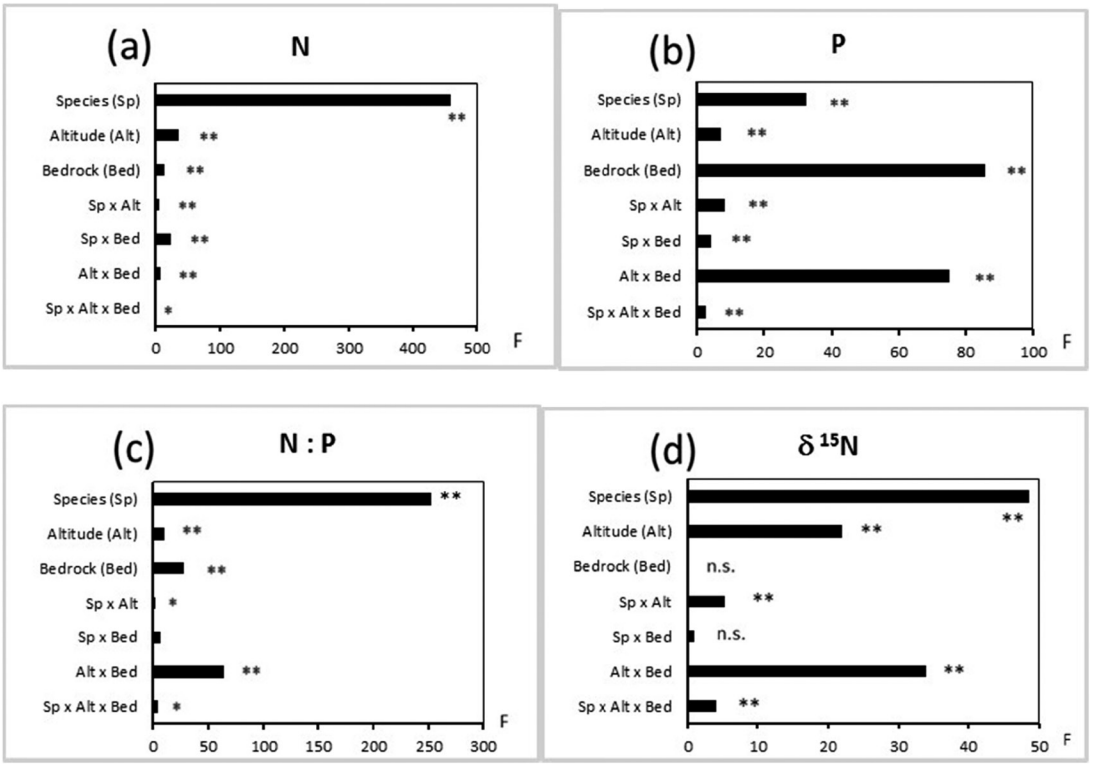

Figure 2: results of three-way factorial ANOVAs for N concentration (**a**), P concentration (**b**), N: P ratio (**c**) and δ ^{15}N (**d**) in leaves of five species across altitudinal gradients on carbonate bedrock and silicate bedrock. Abbreviations and d.o.f. are: species (Sp; 4,240); altitude (Alt; 5,240); bedrock (Bed; 1,240); sp × alt (20,240); sp × bed (4,240); alt × bed (5,240); sp × alt × bed (20,240). Abbreviation: ANOVA = analysis of variance.

Growth limitation through reduced mineralization rates (hypothesis 2)

NH_4 and $NaHCO_3$-Pi represent the most readily available forms of N and P, respectively. The NH_4 occurring in the soil solution results from N mineralization by soil microbes while $NaHCO_3$-Pi is comprised of both mineralized and easily mineralizable Pi forms (Vincent *et al.* 2014). NH_4 and $NaHCO_3$-Pi concentrations presented different altitudinal trends as $NaHCO_3$-Pi concentrations decreased with increasing altitude, especially at the high-altitude sites, while NH_4 concentrations did not. However, the altitudinal trends in NH_4 and $NaHCO_3$-Pi concentrations were much similar on the two parent materials. This suggests that the biogeochemical processes responsible for N and P mineralization possess different sensitivity to temperature, as observed in previous studies. Experimental warming of arctic (Aerts *et al.* 2012) or alpine (Rui *et al.* 2012) soils greatly enhanced P mineralization rates. Vincent *et al.* (2014) observed decreasing concentrations of labile P compounds across an altitudinal gradient in a subarctic mountainous region. Conversely, relationships between concentrations of mineral N compounds, especially NH_4, and temperature in soils of cold regions are not straightforward as higher temperatures can even result in negative net N mineralization rates because of increased competition for N with soil microorganisms (He *et al.* 2014b). Bonito *et al.* (2003) found increasing N mineralization rates across an altitudinal gradient in the southern Appalachians probably because of the larger N pools stored in high elevation soils. Interestingly, Bárta *et al.* (2014) observed lower temperature sensitivity for the enzyme aminopeptidase, responsible for N mineralization compared with phosphatase, responsible for P mineralization, in forest soils.

The overall negative correlations between leaf growth rates and foliar N and P concentrations mean that both nutrients were diluted in the leaf mass when the leaves grew more actively. However, P was diluted more than N which resulted in an overall positive correlations between leaf growth rates and foliar N:P. This contrasts with the assumption of the growth rate hypothesis, stating that N:P ratios tend to decrease in support of fast growth rates because of increased allocation to P-rich ribosomes and rRNA (Elser *et al.* 2000). Several studies did not find negative correlations between growth rates and foliar N:P ratios in different plant species and ecosystems. In particular, foliar N:P can be decoupled from growth when non-limiting nutrients are taken up in excess of plant demand for metabolic processes and protein synthesis (Elser *et al.* 2010). The positive relationships between leaf growth and foliar N:P suggest that leaf growth primarily was N-limited, as usually happens on young soils in cold ecosystems (Reich and Oleksyn 2004). However, should leaf growth be limited by insufficient availability of readily available mineral P compounds this would imply consistent trends of leaf growth and

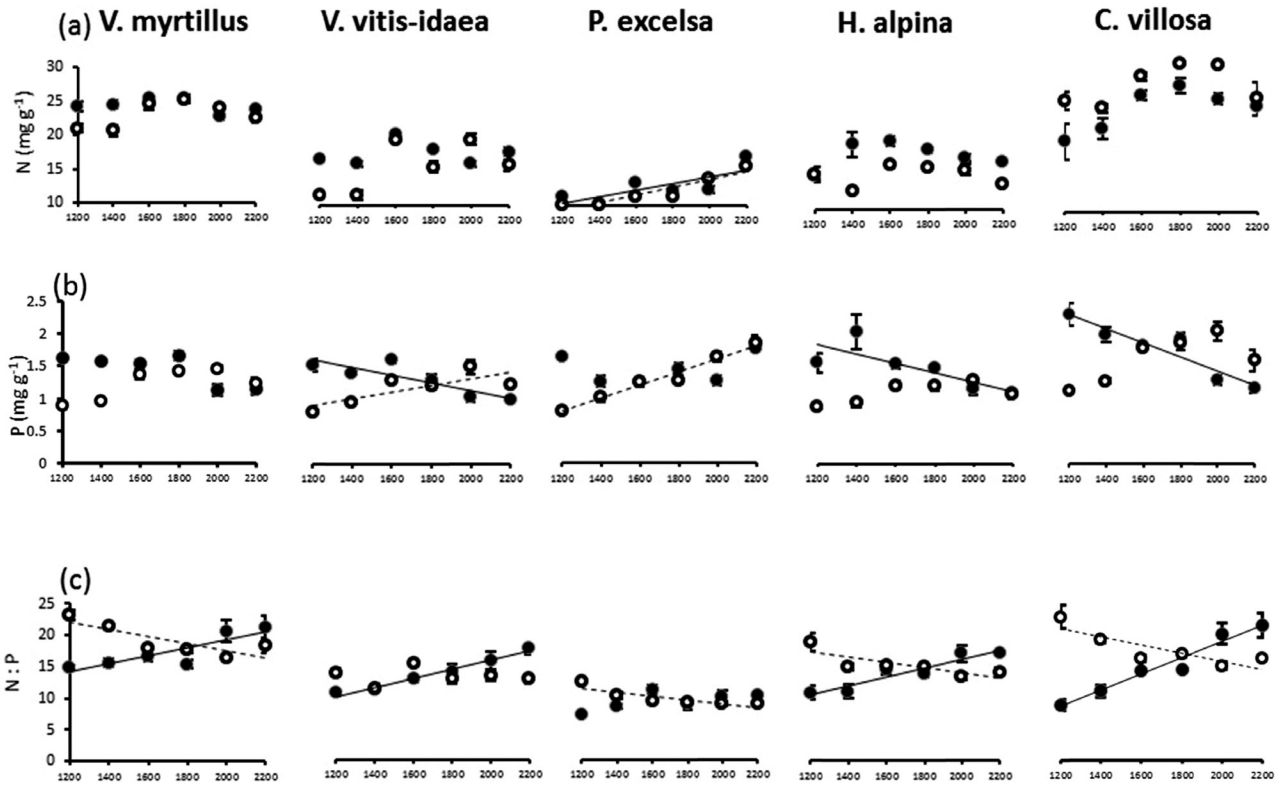

Figure 3: mean (±1SE) values of N concentration (**a**), P concentration (**b**) and N: P (**c**) in leaves of five species across altitudinal gradients on carbonate bedrock (empty symbols, dashed arrows) and silicate bedrock (full symbols, full arrows). Significant ($P < 0.05$) linear regressions of the means against altitude are: N—*P. excelsa* on carbonate bedrock (N = 2.29 + 0.0058 × Altitude; $r^2 = 0.87$); *P. excelsa* on silicate bedrock (N = 4.12 + 0.0051 × Altitude; $r^2 = 0.59$). P—*V. vitis-idaea* on carbonate bedrock (P = 0.31 + 0.0006 × Altitude; $r^2 = 0.61$); *V. vitis-idaea* on silicate bedrock (P = 2.56 − 0.0007 × Altitude; $r^2 = 0.75$); *P. excelsa* on carbonate bedrock (P = −0.44 + 0.0011 × Altitude; $r^2 = 0.97$); *H. alpina* on silicate bedrock (P = 2.74 − 0.0007 × Altitude; $r^2 = 0.62$); *C. villosa* on silicate bedrock (P = 3.55 − 0.0011 × Altitude; $r^2 = 0.89$). N: P—*V. myrtillus* on carbonate bedrock (N:P = 28.79 − 0.0056 × Altitude; $r^2 = 0.66$); *V. myrtillus* on silicate bedrock (N:P = 6.22 + 0.0066 × Altitude; $r^2 = 0.75$); *V. vitis-idaea* on silicate bedrock (N:P = 1.47 + 0.0068 × Altitude; $r^2 = 0.98$); *P. excelsa* on carbonate bedrock (N:P = 13.33 − 0.0027 × Altitude; $r^2 = 0.71$); *H. alpina* on carbonate bedrock (N:P = 20.49 − 0.0039 × Altitude; $r^2 = 0.67$); *H. alpina* on silicate bedrock (N:P = 2.08 + 0.0063 × Altitude; $r^2 = 0.89$); *C. villosa* on carbonate bedrock (N:P = 28.19 − 0.0063 × Altitude; $r^2 = 0.71$); *C. villosa* on silicate bedrock (N:P = −6.93 + 0.0128 × Altitude; $r^2 = 0.95$). Abbreviation: SE = standard error.

foliar nutrient concentrations independent of plant material. This was not our case, so that we exclude indirect effects of temperature-dependent nutrient limitation on leaf growth (hypothesis 2).

Effects of bedrock geology across the altitudinal gradient (hypothesis 3)

Labile inorganic forms of N and P (NH_4 and $NaHCO_3$-Pi, respectively), readily available for plant uptake, represented a minor fraction of the soil nutrient pools on both parent materials. There is indirect evidence that the plants could access more recalcitrant, organic or inorganic nutrient compounds, mainly owing to the activity of their mycorrhizal associates. On one hand, the foliar $\delta\ ^{15}N$ signatures of all species generally were considerably more negative than the soil $\delta\ ^{15}N$ signature, thus indicating isotopic discrimination during N absorption by plants. Poor, if any, isotopic discrimination occurs during NH_4 absorption by plant roots (Högberg 1997) while absorption of soluble organic N compounds, especially through associated mycorrhizal fungi, implies strong ^{15}N depletion in the plant tissues (Hobbie and Colpaert 2003). This suggests that most of the N supply to our plants was provided by dissolved N-containing organic compounds. This is in line with the results of several studies suggesting that foliar $\delta\ ^{15}N$ can be regarded as an index of N availability both at local and regional scale (see Craine *et al.* 2009 for review). If soil N availability decreases, mycorrhizal fungi transfer a lower proportion of N to the host plants (Hobbie *et al.* 2000). On the other hand, both NaOH-Pi and HCl-Pi can be solubilized by plants through their mycorrhizal associations (Cairney 2011). Mycorrhizal plants are also able to utilize Po by hydrolyzing organic compounds. Production of extracellular phosphatases has been demonstrated in a wide range of mycorrhizal fungi: ECM (Antibus *et al.* 1992), ErM (Gibson and Mitchell 2005) and AM (Koide and Kabir 2000). Po was in turn positively correlated with both NaOH-Pi and soil total P, as observed in other studies (Turner and Engelbrecht 2011). Therefore, phosphates and Po that can be solubilized or

Table 1: Mean (±1 SE) values of foliar chemistry variables across altitudinal gradients on different bedrocks

$\delta^{15}N_{leaf}$										
	V. myrtillus		*V. vitis-idaea*		*P.excelsa*		*H. alpina*		*C. villosa*	
	Carbonate	Silicate	Carbonate	Silicate	Carbonate	Silicate	Carbonate	Silicate	Carbonate	Silicate
1200 m	−6.58 ± 0.45 c	−1.75 ± 0.29 a	−6.23 ± 0.20 bc	−2.73 ± 0.37 a	−6.27 ± 0.43 a	−3.69 ± 0.24 a	−8.06 ± 0.79 c	−3.63 ± 0.40 c	−4.73 ± 0.50 bcd	−4.46 ± 0.17 ab
1400 m	−5.88 ± 0.21 bc	−5.08 ± 0.22 b	−7.51 ± 0.11 c	−5.58 ± 0.24 b	−9.31 ± 0.54 b	−5.82 ± 0.35 b	−7.15 ± 0.35 c	−4.74 ± 0.66 a	−5.65 ± 0.38 d	−5.68 ± 0.24 b
1600 m	−4.08 ± 0.34 ab	−4.07 ± 0.56 b	−5.18 ± 0.10 ab	−5.54 ± 0.19 b	−6.19 ± 0.44 a	−5.74 ± 0.56 b	−3.66 ± 0.36 a	−5.20 ± 0.38 ab	−2.53 ± 0.13 a	−3.65 ± 0.66 a
1800 m	−3.59 ± 0.56 ab	−4.82 ± 0.23 b	−4.02 ± 0.57 a	−6.35 ± 0.07 b	−4.67 ± 0.29 a	−5.80 ± 0.27 b	−4.65 ± 0.28 ab	−7.22 ± 0.48 bc	−3.18 ± 0.24 abc	−3.76 ± 0.20 ab
2000 m	−3.06 ± 0.46 a	−5.52 ± 0.87 b	−3.76 ± 0.25 a	−6.63 ± 1.06 b	−4.41 ± 0.42 a	−6.32 ± 0.39 b	−6.21 ± 0.47 bc	−7.12 ± 0.58 bc	−3.10 ± 0.51 ab	−5.00 ± 0.57 ab
2200 m	−5.32 ± 0.86 abc	−5.79 ± 0.42 b	−6.80 ± 0.94 bc	−6.95 ± 0.66 b	−5.63 ± 0.67 a	−7.39 ± 0.36 b	−7.62 ± 0.52 c	−7.62 ± 0.52 c	−4.95 ± 0.31 cd	−3.13 ± 0.50 a
$\delta^{15}N_{(leaf-soil)}$										
	V. myrtillus		*V. vitis-idaea*		*P.excelsa*		*H. alpine*		*C. villosa*	
	Carbonate	Silicate	Carbonate	Silicate	Carbonate	Silicate	Carbonate	Silicate	Carbonate	Silicate
1200 m	−2.97 ± 0.33 a	−1.85 ± 0.72 a	−2.62 ± 0.17 a	−2.82 ± 0.84 a	−2.66 ± 0.36 a	−3.79 ± 0.68 a	−4.46 ± 0.61 a	n.d.	−1.12 ± 1.54 a	−4.56 ± 1.35 a
1400 m	−2.25 ± 0.34 a	−1.91 ± 0.27 a	−3.88 ± 0.41 ab	−2.40 ± 0.43 a	−5.67 ± 0.66 bc	−2.65 ± 0.49 a	−3.52 ± 0.32 a	−1.56 ± 0.74 a	−2.02 ± 1.10 a	−2.50 ± 0.92 b
1600 m	−3.47 ± 0.98 a	−1.61 ± 1.05 a	−4.57 ± 0.59 ab	−3.08 ± 0.76 a	−5.58 ± 0.46 bc	−3.28 ± 1.08 a	−3.05 ± 0.82 a	−2.74 ± 0.77 a	−1.92 ± 1.73 a	−1.19 ± 1.46 b
1800 m	−2.56 ± 0.86 a	−2.35 ± 0.47 a	−3.07 ± 0.89 a	−3.89 ± 0.49 a	−3.72 ± 0.50 ab	−3.33 ± 0.54 a	−3.71 ± 0.69 a	−4.75 ± 0.47 a	−2.23 ± 1.33 a	−1.29 ± 1.13 b
2000 m	−4.97 ± 0.41 b	−5.61 ± 1.08 b	−5.67 ± 0.52 b	−6.71 ± 1.25 b	−6.32 ± 0.71 c	−6.41 ± 0.37 b	−8.12 ± 0.67 b	−7.20 ± 0.72 b	−5.01 ± 1.77 b	−5.08 ± 1.62 a
2200 m	−6.37 ± 1.45 b	−5.39 ± 0.85 b	−7.85 ± 1.05 c	−6.55 ± 0.86 b	−6.68 ± 1.30 c	−6.99 ± 0.48 b	−9.32 ± 0.68 b	−7.22 ± 0.85 b	−6.00 ± 2.13 b	−2.73 ± 1.43 b

Within each column, the means followed by different letters differ significantly ($P < 0.05$) based on Fisher's LSD post-hoc tests. Abbreviations: n.d., not defined; SE, standard error.

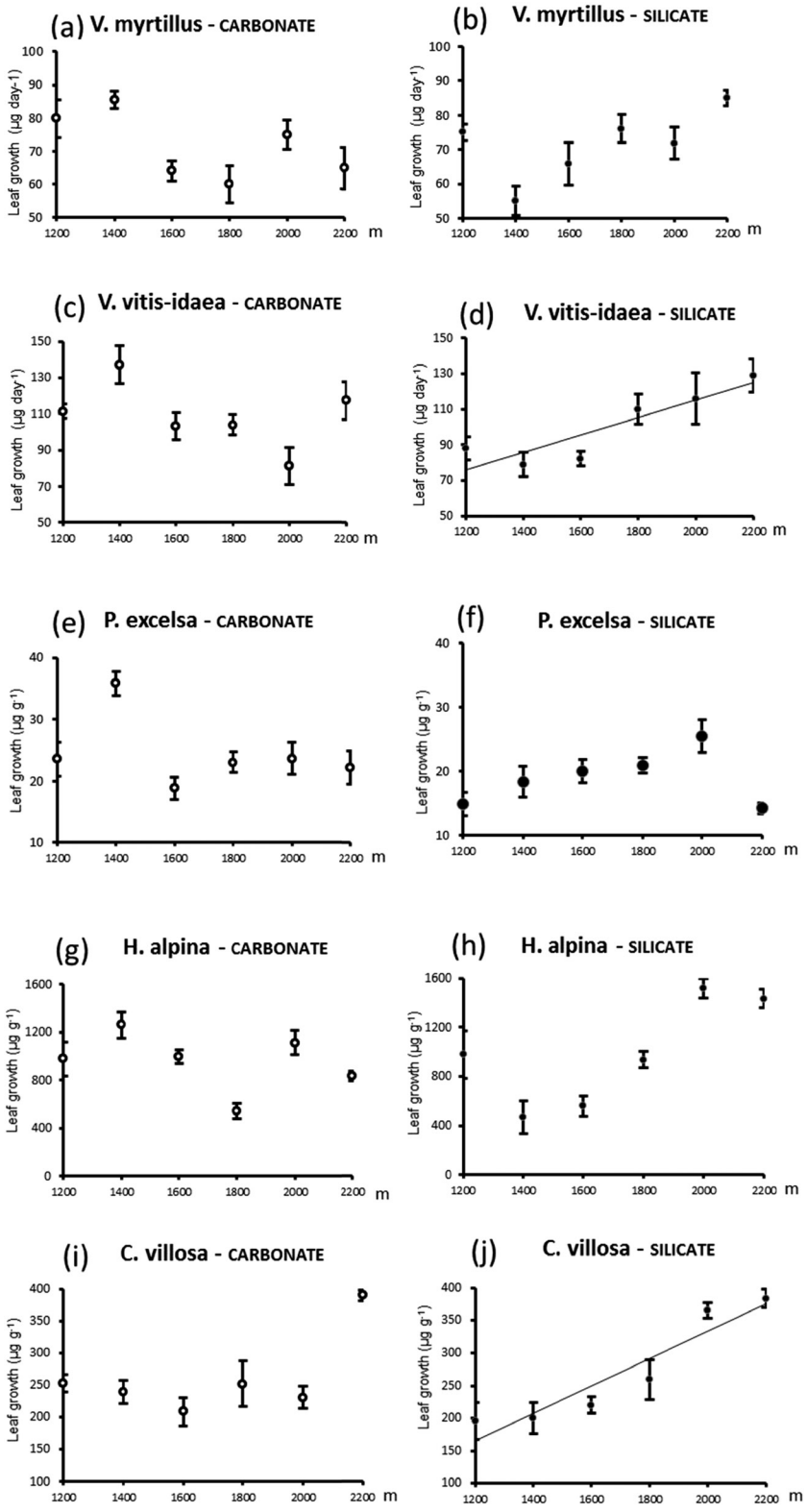

Figure 4: mean (±1SE) leaf growth rates for five species across altitudinal gradients on carbonate bedrock (empty symbols, dashed arrows) and silicate bedrock (full symbols, full arrows). Significant ($P < 0.05$) linear regressions of the means against altitude are for *V. vitis-idaea* on silicate bedrock (leaf growth rate = 17.37 + 0.049 × Altitude; $r^2 = 0.80$) and *C. villosa* on silicate bedrock (leaf growth rate = −87.30 + 0.21 × Altitude; $r^2 = 0.89$). Abbreviation: SE = standard error.

Table 2: Pearson's correlation coefficients (*P*-levels in parenthesis) of leaf growth rates with foliar chemistry and foliar δ ^{15}N for five species across altitudinal gradients on carbonate bedrock and silicate bedrock (N = 60)

	V. myrtillus	*V. vitis-idaea*	*P.excelsa*	*H. alpina*	*C. villosa*
Foliar N concentration	−0.28 (0.03)*	−0.37 (0.003)**	−0.28 (0.03)*	−0.27 (0.03)*	0.12 (0.35)
Foliar P concentration	−0.41 (0.001)**	−0.56 (<0.001)**	−0.36 (0.004)**	−0.46 (<0.001)**	−0.36 (0.005)**
Foliar N: P ratio	0.35 (0.006)**	0.24 (0.07)	0.26 (0.05)*	0.38 (0.003)**	0.41 (0.001)**
Foliar δ ^{15}N	−0.09 (0.48)	−0.39 (0.002)**	−0.38 (0.003)**	−0.35 (0.006)**	−0.02 (0.88)

*P values significant at α = 0.05.
**P values significant at α = 0.01.

hydrolyzed, respectively, by mycorrhizal partners appeared to be the main sources of P to our plants.

The main difference in soil chemistry across the altitudinal gradient on the two bedrock types consisted in the increasing concentrations of different soil P fractions, paralleled by a concomitant decline in soil C concentrations, across the gradient on carbonate bedrock. This means that the organic matter content in the soil decreased with increasing altitude on carbonate bedrock but not on silicate bedrock. Our data cannot provide a mechanistic explanation for the altitudinal patterns of soil P fractions on carbonate bedrock. However, some indirect support for suggesting possible causes can be drawn by soil δ ^{15}N signature and soil Ca, Al and Fe concentrations. The strongly negative correlations between soil δ ^{15}N and soil total C concentration can suggest that bulk soil δ ^{15}N reflects the degree of stabilization of soil organic matter (Craine *et al.* 2015). The higher, i.e. less negative, soil δ ^{15}N values at high altitude on carbonate bedrock were related to lower C concentrations and the greater abundance of mineral particles in the upper soil layer. On the other hand, soil δ ^{15}N can also reflect SWC as soil δ ^{15}N usually are higher in arid regions (Liu and Wang 2009). This may suggest that SWC decreased with increasing altitude on carbonate bedrock. Although we did not observe any difference in SWC during humid periods, we cannot rule out the hypothesis that soil moisture in high-altitude carbonate soils is lower during dry periods because of faster infiltration. Indeed, infiltration capacity is strongly affected by soil organic C concentration (Fischer *et al.* 2015). Independent of the reason why the mineral fraction was enriched at high-altitude sites on carbonate bedrock, the close association between some P fractions and mineral particles was clearly reflected in the positive correlation between soil Al + Fe and Ca concentrations on one hand, and NaOH-Pi, HCl-Pi and Po on the other hand. Consistent with the correlations observed in our study, NaOH-Pi and Po are usually sorbed on the surface of Fe and Al minerals while HCl-Pi is incorporated in calcium phosphates (Crews *et al.* 1995; Giesler *et al.* 2005). All of these three P species can be absorbed by plants thanks to their mycorrhizal partners, as discussed above.

The strong difference between altitudinal patterns of leaf growth and nutrient concentrations on the two parent materials emphasized the importance of bedrock geology as a source of variation in foliar chemistry, which gives support to hypothesis 3. Concentrations of inorganic and, especially, organic N forms were similar across the gradient on both parent materials which met the species-specific plant N demand. In contrast, the varying concentrations of P compounds across the gradient on the two bedrock types resulted in an increasing P-concentration trend across the altitudinal gradient on carbonate bedrock and a decreasing P-concentration trend across the altitudinal gradient on silicate bedrock. When the plants absorbed P in excess of their metabolic demand this appeared to bring about stoichiometric imbalance between N and P content in the tissues (Peñuelas *et al.* 2013).

In conclusion, the varying patterns of leaf growth rates and nutrient concentrations across the altitudinal gradient in relation to parent material indicate that bedrock geology interacts with altitude in controlling the foliar nutrient status mainly owing to availability of soil P and its effect on foliar nutrient stoichiometry.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Plant Ecology online*.

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