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## **Twelve years of low nutrient input stimulates growth of trees and dwarf shrubs in the treeline ecotone**

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Running headline:

Nutrient addition stimulates growth at treeline

## Abstract

1. Almost all natural terrestrial ecosystems are nutrient limited in terms of growth, and we expect treeline vegetation to be no exception. However, direct constraints of low temperature on tissue formation may superimpose effects of low nutrient availability.

2. We examined growth responses of two tree (*Larix decidua* and *Pinus uncinata*) and two dwarf shrub species (*Vaccinium myrtillus* and *Vaccinium gaultherioides*) to 12 years of moderate fertilizer addition (NPK applied at a rate of 15 and 30 kg nitrogen ha<sup>-1</sup> a<sup>-1</sup>) along an elevation gradient within the treeline ecotone in the Swiss Alps (2083 to 2225 m a.s.l.). We measured annual top- and side-shoot increments as well as stem ring width in trees and shoot increments in dwarf shrubs.

3. Fertilizer addition increased soil nutrient availability, indicated by enhanced soil extractable N, higher concentrations of N, P and K in leaves, and higher foliar  $\delta^{15}\text{N}$ .
4. Fertilizer addition stimulated annual growth of all four species: by 11–20% for *L. decidua* and 15–36% for *P. uncinata* (depending on trait), and by 8–16% for the two dwarf shrub species. Growth stimulation by the higher fertilizer dose was not significantly stronger than by the lower dose (except for *V. gaultherioides*), suggesting an overall low nutrient demand for growth and saturation at a rather low nutrient input.
5. *Synthesis*. Even slightly enhanced nutrient availability can stimulate growth of trees and dwarf shrubs in an alpine treeline ecosystem. Ongoing atmospheric nutrient deposition, in conjunction with global warming, may accelerate plant growth at the treeline.

## KEYWORDS

fertilizer addition, *Larix decidua*, nitrogen isotope, *Pinus uncinata*, tree ring, shoot length, Swiss Alps, *Vaccinium gaultherioides*, *Vaccinium myrtillus*

## Introduction

Globally, the availability of nitrogen (N) and phosphorus (P) is considered to be a major factor limiting growth and productivity in terrestrial ecosystems (Vitousek & Howarth 1991). In recent decades, N availability for plants has increased in many areas owing to atmospheric deposition of nitrogenous compounds (Vitousek et al. 1997; Galloway et al. 2008). In addition, global warming is expected to increase turnover rates of soil organic matter (Kirschbaum 1995; Saxe et al. 2001; Kammer et al. 2009; Bai et al. 2013; Dawes et al. 2017b), possibly enhancing the availability of nutrients in general.

Arctic and alpine regions are expected to be especially low in plant-available nutrients because of temperature constraints on decomposition and mineralization processes in the soil (Körner 2003). Nitrogen availability generally decreases with an elevation-associated decline in temperature (Loomis et al. 2006 [arctic mountain ranges]; Thébault et al. 2014; Fajardo & Piper 2017 [Andes]; Solly et al. 2017a [Ural]). Substantial growth stimulation in response to fertilization has been observed for arctic and alpine ground-layer plants (e.g. Schächli & Körner 1996; Van Wijk 2004; Bassin et al. 2012). Even the vegetation of glacier forefields shows dramatic increases in biomass production when nutrients are added (Heer & Körner 2002). Thus, growth in cold ecosystems makes no exception to the general nutrient limitation of terrestrial ecosystems.

Growth responses to an improved nutrient supply, however, often differ among plant species and growth forms (e.g. Chapin et al. 1995; Thomas et al. 1999; Bassin et al. 2007; Klanderud 2008), mainly depending on a species' inherent ability to benefit from additional nutrients and the degree to which growth is limited by factors other than nutrient availability, such as temperature. Treelines follow a common isotherm at 5–7 °C (6.4 °C; Paulsen & Körner 2014) globally, suggesting that temperature limits tree growth at and above the treeline (Körner & Paulsen 2004). Because tree canopies are aerodynamically open to convective heat transfer (wind) and also shade the ground (causing reduced soil heat flux), they experience colder conditions than low-stature plants (including tree seedlings), which often profit from warmer microclimates (Scherrer & Körner 2010; Körner 2012). Growth chamber experiments have demonstrated that growth of juvenile trees is greatly reduced at temperatures below ca. 6 °C, even under an optimal nutrient supply (Iivonen et al. 1998; Alvarez-Uria & Körner 2007; Hoch 2013; Ferrari et al. 2016). This suggests that the growth responses to nutrient addition can be strongly constrained under critically low temperatures.

Globally, soil types and thus fertility vary considerably among treelines, indicating that nutrient availability does not consistently affect treeline position the way growing season temperature does (Körner 2012). While low nutrient availability may not be decisive for the establishment of seedlings, it may constrain the vigor of saplings and trees. However, a global assessment of foliar N concentrations in herbs and trees has demonstrated an increasing rather than a decreasing trend with increasing elevation (Körner 1989). Hence, tree life at high elevations is not associated with an obvious foliar nutrient deficiency. Accordingly, the needle N concentration of *Picea abies* and *Pinus cembra* does not change over the uppermost ca. 300 m of elevation in the Swiss Alps, although growth rate declines substantially (Birmann & Körner 2009). On the other hand, fertilizer addition experiments have reported growth responses of treeline trees to fertilization, similar to responses observed for other vegetation (see above). This indicates that trees in cold climates make no exception in taking advantage of added nutrients in terms of growth. Within three years of a single, high dose application of NPK (85–175 kg N ha<sup>-1</sup>), growth of *Betula pubescens* was found to respond more strongly at the treeline compared with two lower sites in northern Sweden (Sveinbjörnsson 1992). At the Finnish arctic treeline, 50-year-old *Pinus sylvestris* showed a tripling of annual growth in response to ca. 150 kg N ha<sup>-1</sup> a<sup>-1</sup> of NPK (Susiluoto et al. 2010). Sullivan et al. (2015) presented various lines of observational evidence (no nutrients added) suggesting that growth of arctic treeline trees in Alaska is predominantly limited by nutrients.

Here, we present results from a 12-year low-dose fertilization experiment at a treeline in the Swiss Alps. Over an elevation gradient of 140 m across an afforestation site in the current treeline ecotone, we annually added NPK fertilizer corresponding to 15 or 30 kg N ha<sup>-1</sup> a<sup>-1</sup> to plots containing 30-year-old (in the first year of fertilization) *Larix decidua* or 32-year old *Pinus uncinata* individuals with an understory of mainly ericaceous dwarf shrubs. To quantify growth, we measured annual shoot increments of trees and dwarf shrubs as well as stem ring width of trees. We also measured nutrient concentrations in the soil and assessed the foliar

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nutritional status of trees and dwarf shrubs. While the very high fertilizer doses used in previous studies (see above) may have prevented competition effects between trees and understory vegetation, we used doses not uncommon in the front ranges of the Alps to provide more realistic insight into growth responses to anthropogenic increases in nutrient availability. Atmospheric N deposition in the inner parts of the Alps is mostly below 20 kg N ha<sup>-1</sup> a<sup>-1</sup> (e.g. Hiltbrunner et al. 2005, Rogora et al. 2016) and empirically defined thresholds for measurable long-term effects on ecosystems (so called 'critical loads', Nilsson 1988) are in the range of 5–15 kg N ha<sup>-1</sup> a<sup>-1</sup> for comparable ecosystems, such as coniferous woodland and alpine shrub habitats (Bobbink & Hettelingh 2010). The estimated background deposition of ca. 4–8 kg N ha<sup>-1</sup> a<sup>-1</sup> in our test region (Rihm & Kurz 2001, Schmitt et al. 2005) may already have exerted some growth stimulation, and we expected that a doubling or tripling of such low rates would have clear effects.

We aimed to determine whether the growth stimulation observed in previous short-term fertilization trials conducted at treeline locations occurs in a longer-term study, and whether possible negative effects of nutrient addition offset the benefits of any growth stimulation. Given the general sensitivity of plant growth to nutrient addition in natural ecosystems, we expected (1) growth to be stimulated already by the lower NPK-fertilizer dose corresponding to 15 kg N ha<sup>-1</sup> a<sup>-1</sup> and (2) growth stimulation to differ between growth forms, with smaller effects of nutrient addition for trees than for dwarf shrubs, as upright trees are exposed to lower temperatures. Further, we expected (3) the effect of fertilization to vary across temperature gradients. On a spatial scale, we predicted that the response would decrease with increasing elevation within the treeline ecotone, owing to greater low temperature limitation of tissue formation. On a temporal scale, we predicted larger responses to fertilization in warmer years, when direct thermal constraints are relieved.

## Materials and methods

### STUDY SITE

The experiment was located within a 5 ha long-term afforestation site in the temperate continental climate zone of the inner Alps (Stillberg, Davos, Switzerland; 46°47'N, 9°52'E). In 1975, more than 92,000 tree seedlings of *Larix decidua* Mill., *Pinus mugo* ssp. *uncinata* [DC.] Domin (referred to hereafter as *P. uncinata*) and *Pinus cembra* L. were planted into the intact dwarf shrub heath (Kuoeh 1970), slightly above the treeline in the region (Barbeito et al. 2012). The afforestation site was partitioned into plantation quadrats 12.25 m<sup>2</sup> in area, each containing 25 evenly spaced individuals (Supporting Information, Fig. S1). From 1975 to 2005, vertical growth of all three tree species decreased substantially with increasing elevation (Barbeito et al. 2012). At the beginning of our study, tree height declined from ca. 3.2 m to 1.4 m in larch and from 1.6 m to 1.0 m in pine across the elevation range of 142 m (2083 to 2225 m a.s.l.) covered in the present experiment (Fig. S2). On-site climate data is available from a weather station situated at the lower end of the site (2090 m a.s.l.). On average, annual precipitation was 1159 mm and annual mean temperature was 2.0 °C over the last 40 years (1975 to 2015). January is the coldest and July the warmest month, with mean temperatures of -5.4 °C and 10.2 °C, respectively. The growing season lasts 3–4 months (June to September) at this location. A second weather station on the same slope at 2220 m. a.s.l. suggested a lapse rate of 0.55 K per 100 m of elevation for the summer months of 2011 and 2012. A 30-year comparison of summer temperatures measured on site and those measured at a weather station 8 km away at 2691 m a.s.l. suggested a lower lapse rate of ca. 0.48 K per 100 m of elevation. Thus, we expect that our experimental site covered a mean air temperature gradient of at least 0.7 K in a temperature range considered critical for tree growth. Date of snowmelt (Barbeito et al. 2012) and freezing events (Rixen et al. 2012) are important modulating factors for growth at the study site.

The most common understory plants at the site are the ericaceous dwarf shrubs *Vaccinium myrtillus* L., *Vaccinium gaultherioides* Bigelow (group *V. uliginosum* aggr.) and *Empetrum nigrum* ssp. *hermaphroditum* (Hagerup) Böcher, reflecting the oligotrophic nature of these ecosystems. Soils are extremely acidic (pH 3.5 to 4.5), developed on siliceous bedrock (Schönenberger & Frey 1988) and have a sandy texture. Soil types at the afforestation site vary with microtopography, with rankers on the ridges and iron-humus podzols within gullies. All soils have 5 to 20 cm thick mor-type organic layers (Bednorz et al. 2000). Detailed descriptions of soil and microclimatic conditions at the site can be found in Blaser (1980), Bednorz et al. (2000), Senn & Schönenberger (2001) and Hagedorn et al. (2010).

## EXPERIMENTAL DESIGN

We chose 22 locations throughout the afforestation site (Fig. S1), hereafter referred to as blocks, that covered the whole range of micro-environmental conditions (see Senn & Schönenberger 2001). Half of the blocks included European larch (*L. decidua*) and the other half included mountain pine (*P. uncinata*). Within each block, we randomly selected three plantation quadrats as experimental plots. Each plot was assigned to a control (no fertilization) or to one of two fertilizer dose treatments. Treatments were assigned randomly but confined so that the location of fertilized plots within a block was not directly above control plots to avoid nutrient input from drainage. The elevation difference between treatment plots of the same block was  $5.4 \pm 0.7$  m (mean  $\pm$  SE). We annually applied the mid- to long-term fertilizer ARBOSTAR (N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O:Mg = 8:4:11:2; Geistlich Arbostar, HBG Düngevertrieb AG, Grossaffoltern, Switzerland) at an amount that corresponded to 15 kg N ha<sup>-1</sup> ('NPK15') or 30 kg N ha<sup>-1</sup> ('NPK30'). About 75% of the N in the fertilizer was in the form of NH<sub>4</sub><sup>+</sup> and the rest was derived from methylene urea. Fertilizer was first applied in September 2004 and then at the beginning (June) of each following growing season until 2016. Fertilizer granules were evenly distributed by hand over the entire area of a given plot.



## SOIL ANALYSES

Six soil cores (0–10 cm depth, 5 cm diameter) per plot were collected in September 2010. Soil samples were bulked plot-wise and sieved at 4 mm mesh size, and fine roots were removed. Subsequently, samples were stored at -20 °C until chemical analysis. For analyses of soil inorganic N, an aliquot of 20 g of soil was extracted with 100 ml of 0.5 M K<sub>2</sub>SO<sub>4</sub>, following the protocol of Brooks et al. (1996). The ammonium (NH<sub>4</sub><sup>+</sup>) concentration in the extracts was determined colorimetrically by automated flow injection analysis (RF-535 Fluorescence HPLC Monitor, Shimadzu). The nitrate (NO<sub>3</sub><sup>-</sup>) concentration was measured colorimetrically according to Navone (1964).

For the analysis of stable isotope ratios, soil aliquots were dried at 60 °C, milled and then analysed using an automated elemental analyser (Euro EA 3000, HEKAtech, Wegberg, Germany) interfaced with a continuous flow isotope ratio mass spectrometer (Delta-S, Thermo Finnigan, Bremen, Germany). The <sup>15</sup>N natural abundance in extracted NH<sub>4</sub><sup>+</sup> was determined by the diffusion technique (Schleppi et al. 2006), converting NH<sub>4</sub><sup>+</sup> to NH<sub>3</sub> and trapping it on acidified teflon filters. The filters were packed in tin (Sn) capsules and then measured immediately by mass spectrometry. The pH of the bulk soil was measured potentiometrically in H<sub>2</sub>O at a soil-to-solution volume ratio of 1:2.5.

## PLANT RESPONSES

In late August of 2010 and 2016, 20–25 new needles were sampled from three (2010) to five (2016) randomly chosen trees (larch or pine) per plot. The same number of leaves was collected from roughly 10 branchlets per plot for each of the two dominant dwarf shrubs *V. myrtillus* and *V. gaultherioides*. Needles were sampled from the upper canopy on the south side of the trees. Leaves from dwarf shrubs were sampled from the upper shrub canopy, thus

avoiding deep shade. Samples were dried at 65 °C for 24 hours, milled to a fine powder and weighed into tin capsules (1–2 mg per sample). Nitrogen per dry mass and  $\delta^{15}\text{N}$  were obtained by mass spectrometry in connection with an element analyser, as described for the soil samples. In 2010, we additionally determined phosphorus (P), potassium (K) and magnesium (Mg) concentrations of leaf samples. Phosphorus concentrations were analysed photometrically using an UV-VIS spectrometer (UV-160A, Shimadzu Europe GmbH, Germany; DIN EN ISO15681-1). Potassium and Mg were determined by atomic flame spectrometry (SpectrAA 220, Varian Deutschland GmbH, 64289 Darmstadt, Germany; DIN EN ISO 9964-2 for K and DIN EN ISO7980 for Mg).

In 2004, we selected three census trees in each plot that were visibly unaffected by frost damage or snow fungus and were not heavily shaded by surrounding trees. Tree and shrub growth was measured in 8 years from 2004 (pre-treatment year) to 2016. No measurements were made from 2011 to 2015. During this period, several of the originally selected trees died, were damaged or lost their labels, prompting us to select new trees for the final census in 2016 using the original criteria. There was no difference in tree ring width between the trees selected in 2004 and the trees selected in 2016 in four overlapping years (2007 to 2010, see Fig. S3). Thus, we are confident that selecting new trees did not have a qualitative effect on the results. In 2005 and 2015, the survival status (alive or dead) of all trees growing on the afforestation site was documented, allowing us to investigate the influence of the treatment on tree mortality.

For the annual growth census, one branch per tree at approx. half the tree height was marked to assess side-shoot growth. We obtained tree shoot increment data for 2004 to 2016 using obvious annual bud scars/branching nodes. The increment of top- and side-shoots in 2007 and

2009 and from 2011 to 2015 was back-measured in the subsequent years. Top-shoot increment of larch was not measured in 2009 and 2010.

In 2010 and 2016 we collected microcores (35 mm length, 2 mm diameter) from all marked trees using an increment puncher (Trephor, Università degli Studi di Padova, S. Voto di Cadore, Italy). Cores were extracted from two sides of the main stem (east and west in 2010 and north and west in 2016), above the curved section at the stem base, at a height of roughly 20–40 cm for pine and 60–100 cm for larch. After extraction, the cores were stored in 80% ethanol and later dried at room temperature for 10–12 hours before measuring ring width. We cut the cores lengthwise, applied chalk powder to the cut surface to improve the visibility of the growth rings, and measured ring width with a stereomicroscope at 40x magnification. Ring width of the two cores per tree were averaged prior to statistical analysis. For each tree surveyed in 2016, we additionally measured total tree height as well as tree diameter at two heights (roughly 20 and 80 cm above ground level for larch and 20 and 40 cm for pine), which we averaged for statistical analysis.

For the dwarf shrubs, the annual increments of the longest shoot of five randomly chosen branchlets per species were measured in each plot from 2004 to 2010. The annual increment formed in 2007 (*V. myrtillus* only) and 2009 was back-measured in the subsequent year (2008, 2010). Increments from 2014 to 2016 were measured in 2016, but we have no data for 2011 to 2013 because increments older than two years could not be clearly identified. In 2016, we measured the longest shoots of only three randomly chosen ramets (instead of five) per species. Shoot increments were always measured in autumn after annual growth was complete.

## STATISTICAL ANALYSES

We assessed the effects of fertilizer addition, other explanatory variables (treatment year; plot elevation; tree species in the plot [in shrub models]) and their potential two-way interactions with fertilization on measured soil properties and plant traits. Specifically, we fitted linear mixed-effects models in R version 3.4.1 (R Core Team 2017) using the `lme` function of the `nlme`-package by Pinheiro et al. (2016). The fertilizer dose (control, NPK15, NPK30) and treatment year (2005–2016) were generally analysed as categorical variables (factors), whereas elevation was analysed as a numerical variable (2083 to 2225 m a.s.l.). This approach resulted in separate, unbiased coefficients for each level of fertilizer dose and year, which were used to create Figures 3 and 4. The models for foliar nutrient concentration and nutrient ratios indicated linear relationships between these traits and the fertilizer dose, prompting us to rerun the model with fertilizer dose as a numeric variable (0, 15 or 30 kg N ha<sup>-1</sup> a<sup>-1</sup>). We analysed annual growth traits separately for each species, whereas the other response variables (foliar nutrient concentration and  $\delta^{15}\text{N}$ , diameter and height of trees) were analysed with one model for both species of the same growth form. Data from the pre-treatment year (2004) were tested separately for differences among treatment groups. Tree mortality was investigated with logistic regression (`glmmPQL` function) using survival data for the period between 2005 and 2015.

We included random intercepts for experimental block in all models (a total of 11 blocks per tree species and 22 per shrub species, see Fig. S1). As each of the blocks contained three plots from which multiple samples were taken, we added plot identity as a nested random effect within block. For repeatedly measured traits on the same individual trees (top- and side-shoot increment, ring width), we also included tree identity as a nested random effect within block and plot, thereby allowing the model intercept to vary among trees. Further, we implemented an autocorrelation structure of order 1 (`corAR1` in `nlme`) in models with repeated measures to

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account for temporal autocorrelation, which improved the AIC (Akaike information criterion) of the models. We expected residual variance to differ between treatments, years and/or species. Therefore, we allowed the residual variance to differ between levels of these factors by using the `varIdent` function in `nlme` (Zuur et al. 2009). F-statistics and detailed model specifications are given in the Supporting Information (Tables S3–S5). Model assumptions of residual normality and homoscedasticity were verified visually (q-q plot, Tukey-Anscombe plot) for each model. To improve residual spread in cases of heteroscedasticity, the response variable was log or square root transformed (Supporting Information, Table S1). Model estimates for each year and treatment (averaged over all other factors in the model, as shown in Figures 2 and 3) were obtained with the `lsmeans` package in R (Lenth 2016). Using the same package, we calculated the average estimates across the twelve years (Table 1), performed t-tests on contrasts between treatments and back-transformed the estimates for presentation. Log-transformed values yield the geometric mean and square-root-transformed values an intermediate between arithmetic and geometric mean when back-transformed. Numerator degrees of freedom (df) are given for t-tests.

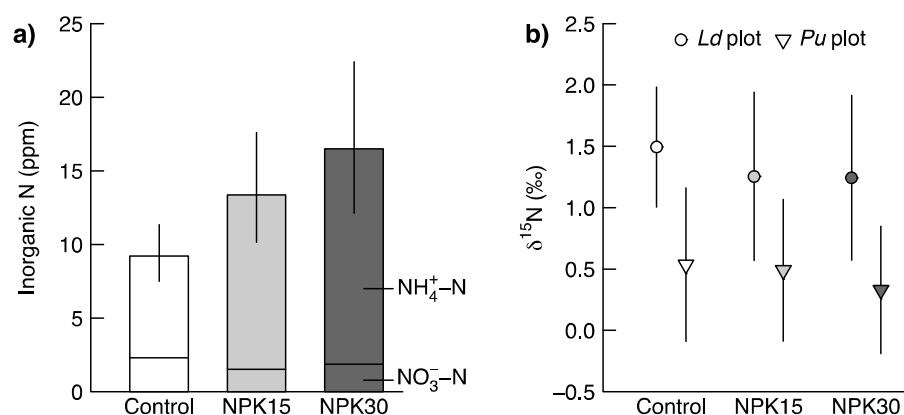
To test the influence of annual climatic conditions on the growth responses to fertilization (i.e. control vs. fertilized plots), we combined the data from the two fertilizer doses because there was no qualitative difference between the growth responses to NPK15 and NPK30. The factor 'year' was replaced by number of frost events per growing season and mean summer temperature (June, July and August together) in the air (2 m above ground). The statistical significance of the resulting trends was tested with t-tests and the corresponding *P*-values were corrected for multiple testing using the 'Bonferroni-Holm' method. We considered the start and end of the growing season as the dates when the daily mean soil temperature first rose above or fell below 3.2 °C for two consecutive days, respectively (Körner & 2004, Dawes et al. 2011). Dates with daily minimum air temperatures below -1 °C were considered as freezing events (Rixen et al. 2012). Freezing events that spanned contiguous dates were

counted only once. If not specified, results are presented as treatment means with the corresponding 95% confidence intervals.

## Results

### TREATMENT EFFECTS ON SOIL PROPERTIES

In 2010, end-of-season inorganic N concentrations in the soil (0–10 cm depth) increased by 45 [10, 90] % in NPK15 ( $df = 37$ ,  $t = 2.8$ ,  $P < 0.01$ ) and 79 [32, 142] % in NPK30 plots (Fig. 1;  $df = 21$ ,  $t = 3.9$ ,  $P < 0.001$ ). The higher inorganic N concentrations were solely caused by an increase in  $\text{NH}_4^+$ , which comprised ca. 75% of total inorganic N in control plots and 89% in the fertilized plots (Fig. 1). In contrast, the concentration of  $\text{NO}_3^-$ , which was not part of the fertilizer, remained unaffected in treated plots.  $\text{NH}_4^+$ , but not  $\text{NO}_3^-$ , decreased significantly with increasing elevation, by ca. 0.77 [0.07, 1.46] mg N  $\text{kg}^{-1}$  per 10 m of elevation ( $df = 37$ ,  $t = 2.4$ ,  $P = 0.021$ ). Elevation did not affect soil nutrient increases due to fertilization. There was a marginally significant trend of ca. 35% lower  $\text{NH}_4^+$  concentrations in plots with pine compared to in plots with larch ( $df = 20$ ,  $t = -1.8$ ,  $P = 0.089$ ), independent of fertilization treatment.



**Figure 1** End of season inorganic N (a) and  $\delta^{15}\text{N}$  (b) in the soil of control and fertilized plots, corresponding to 15 (NPK15) or 30 kg N ha<sup>-1</sup> a<sup>-1</sup> (NPK30), in 2010. Means and 95% confidence intervals are shown. a) Bars are subdivided for the fraction of inorganic N derived from ammonium (NH<sub>4</sub><sup>+</sup>-N) and nitrate (NO<sub>3</sub><sup>-</sup>-N). b) Soils of plots containing either *Larix decidua* (Ld) or *Pinus uncinata* (Pu) differed in  $\delta^{15}\text{N}$  and are displayed separately.

Bulk soil  $\delta^{15}\text{N}$  was not significantly affected by fertilization (Fig. 1; fertilizer  $\delta^{15}\text{N}$  was ca. 0.80 ‰). Similarly,  $\delta^{15}\text{N}$  in soil extractable NH<sub>4</sub><sup>+</sup> was not significantly affected by fertilization (control: 0.26 ‰, NPK15: 0.48 ‰, NPK30: 0.44 ‰). Soil  $\delta^{15}\text{N}$  signatures were significantly higher in plots with larch than in plots with pine (Fig. 1; df = 20,  $t = 2.9$ ,  $P = 0.010$ ). The treatment did not affect the soil pH of 3.79 [3.72, 3.86].

#### FOLIAR NUTRIENT CONCENTRATIONS AND ISOTOPE RATIOS

Across treatments, foliar N concentration of larch decreased with elevation in 2010, from 2.39 [2.22, 2.56] mg g<sup>-1</sup> at the lowest elevation to 2.05 [1.94, 2.18] mg g<sup>-1</sup> at the highest elevation (Table S2). This decrease led to a parallel decrease of the N:P and N:K ratios towards higher elevation for larch. However, this relationship between foliar N and elevation disappeared in leaves sampled in 2016 (Table S2). For pine, only the N:K ratio decreased from 3.57 [2.94, 4.34] at the lowest elevation to 2.08 [1.64, 2.63] at the highest elevation in 2010 (Table S2).

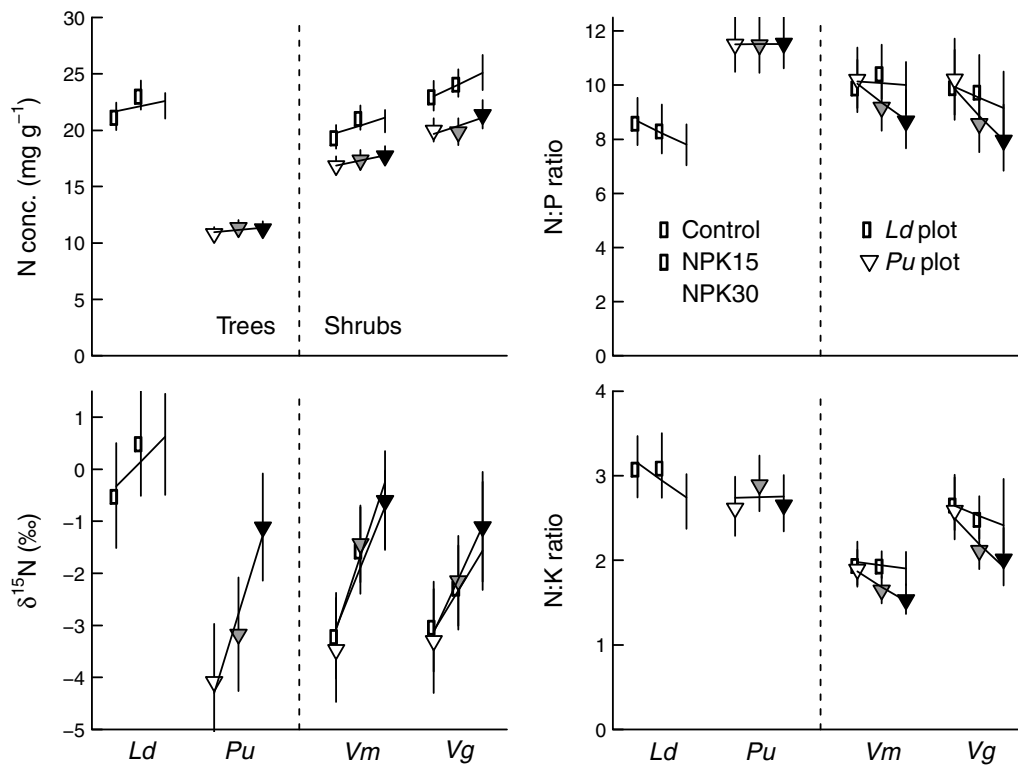
In 2010, no significant response to fertilization was found in foliar N concentration of trees, except for a stimulation in larch when grown in the NPK15 treatment (Fig. 2; df = 38,  $t = 2.4$ ,  $P = 0.024$ ). The second sampling in 2016 revealed a stronger and linear response for both species, amounting to a maximum of +10% in the NPK30 treatment (df = 34,  $t = 3.4$ ,  $P < 0.01$ ; Fig. S4). Phosphorus and K concentration (both measured in 2010 only) of larch leaves

increased considerably under fertilization, leading to lower foliar N:P (df = 40,  $t = -2.0$ ,  $P = 0.048$ ) and N:K ratios (df = 40,  $t = -2.4$ ,  $P = 0.024$ ), while no change in nutrient ratios was found for pine leaves (Fig. 2).

The foliar N concentration of dwarf shrubs was independent of elevation (Table S2) but increased with the fertilizer dose by up to 6–8 % in the NPK30 treatment in 2010 (df = 38,  $t = 4.1$ ,  $P < 0.001$ , Fig. 2). While *V. gaultherioides* had, on average, higher foliar N concentrations than *V. myrtillus* (df = 58,  $t = 12.0$ ,  $P < 0.001$ ), the response to fertilization did not differ between these species. Dwarf shrubs had 17 [11, 22] % higher foliar N concentrations when growing with larch instead of with pine (Fig. 2; df = 20,  $t = 5.9$ ,  $P < 0.001$ ). As observed for larch trees, the N:P mass ratios of the dwarf shrubs decreased significantly with increasing fertilization dose, but only in plots with pine (Fig. 2; df = 38,  $t = -2.5$ ,  $P = 0.016$ ) and not under larch. Similarly, foliar N:K ratios of dwarf shrubs decreased in fertilized plots with pine only (Fig. 2; df = 38,  $t = -3.9$ ,  $P < 0.001$ ). N:Mg ratios remained unaffected by the treatment for all species. These patterns for foliar N concentration were confirmed by the second analysis using leaves harvested in 2016 (Fig. S4).

Foliar  $\delta^{15}\text{N}$  in 2010 was unaffected by elevation, except for a slight increase at higher elevations for pine (Table S2), but increased significantly with fertilization for both tree species (Fig. 2, df = 40,  $t = 5.9$ ,  $P < 0.001$ ). This increase was much stronger for pine than for larch (df = 40,  $t = 3.0$ ,  $P < 0.01$ ), and larch leaves were significantly less depleted in  $^{15}\text{N}$  than pine leaves (df = 20,  $t = -4.9$ ,  $P < 0.001$ ). Foliar  $\delta^{15}\text{N}$  of dwarf shrubs was also unaffected by elevation and  $\delta^{15}\text{N}$  values increased with fertilization (Fig. 2, df = 38,  $t = 6.0$ ,  $P < 0.001$ ). The response was similar between species for dwarf shrubs and independent of the tree species growing in the plot. The second analysis in 2016 confirmed the  $\delta^{15}\text{N}$  signals observed in 2010 (Fig. S4).





**Figure 2** Effects of NPK fertilization, corresponding to 15 (NPK15) or 30 kg N ha<sup>-1</sup> a<sup>-1</sup> (NPK30), on N concentrations, mass ratios of N:P and N:K, and δ<sup>15</sup>N values in leaves of *Larix decidua* (Ld), *Pinus uncinata* (Pu), *Vaccinium myrtillus* (Vm) and *Vaccinium gaultherioides* (Vg) in 2010. Means and 95% confidence intervals are shown. Point estimates and regression lines were modelled separately by treating the fertilizer dose as either a categorical or a numeric variable.

## GROWTH OF TREE SPECIES

Before the first fertilizer application in 2004, there was no difference between plots assigned to the different treatments in any of the tree growth traits (top- and side-shoot increment, ring width;  $P > 0.2$  in each case). In 2016, the average ring width of larch trees growing in control plots was 1.7 [1.4, 2.1] mm at the lowest elevation and decreased to 1.3 [1.0, 1.5] mm at the highest elevation ( $df = 106$ ,  $t = -2.2$ ,  $P = 0.028$ ). Higher elevation was also associated with smaller tree diameter and tree height in larch (Table S3), with a decrease in diameter from

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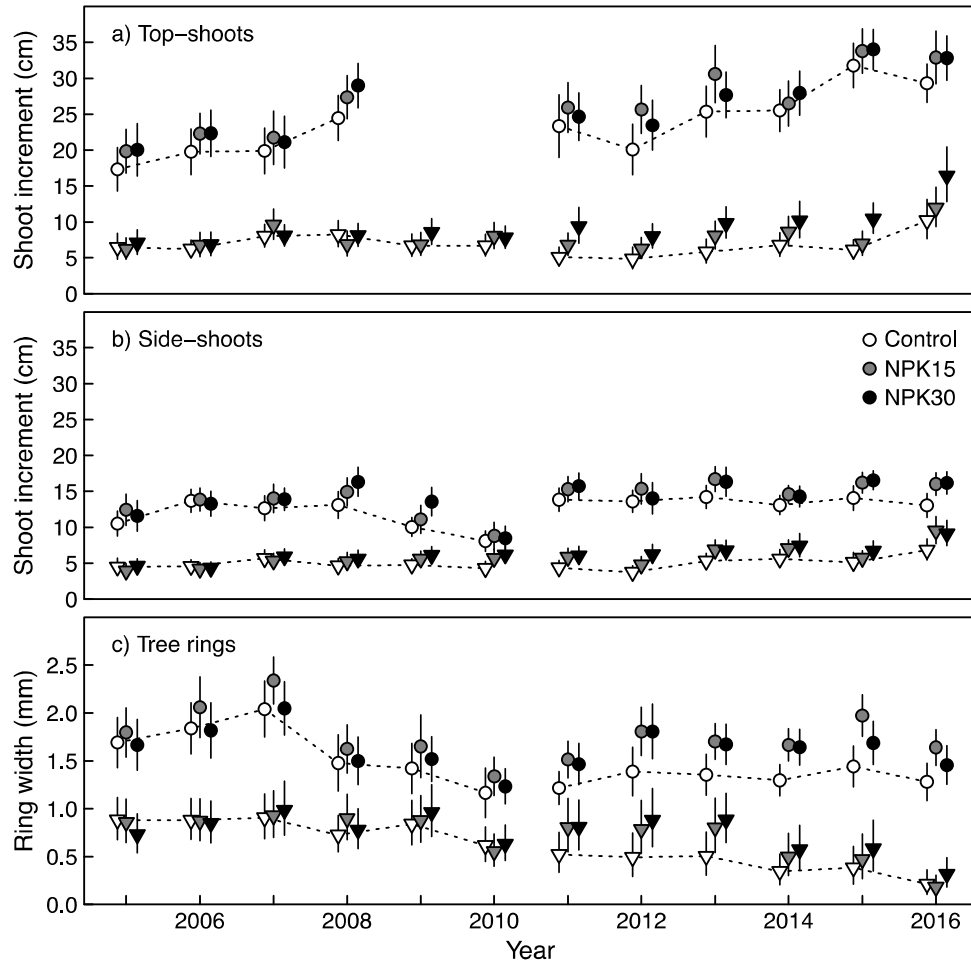
11.0 [8.7, 13.3] cm at the lowest elevation to 8.0 [6.2, 9.9] cm at the highest elevation ( $df = 97$ ,  $t = -1.8$ ,  $P = 0.071$ ). Larch height was 536 [452, 627] cm at the lowest and decreased to 374 [317, 437] cm at the highest elevation ( $df = 97$ ,  $t = -2.8$ ,  $P = 0.007$ ). Elevation did not affect individual annual top- and side-shoot increments of either tree species (Table S4), and it had no influence on tree height (166 [90, 266] at lowest vs. 139 [82, 211] at highest elevation) or diameter (4.9 [1.3, 8.4] vs. 6.0 [2.8, 9.1]) of pine.

In larch, nutrient addition stimulated all three annually measured growth traits (top- and side-shoot increment, ring width). The average effect across years varied from 13% to 20% in the NPK15 treatment and from 11% to 14% in the NPK30 treatment, depending on trait (Table 1). The differences between the two fertilizer doses were not statistically significant for any of the growth traits for larch ( $P > 0.14$  for each trait). Interannual differences in the response were statistically significant for side-shoot increment only (Table S4), where stimulation mainly occurred in the second half of the study period (2011–2016, Fig. 3). Fertilization led to a significantly larger tree diameter, but not height, for larch (Table 1).

For pine, the response to fertilization varied significantly among years for each trait (Table S4) and showed an initial phase with low fertilization effects followed by stimulation in the second half of the study period (Fig. 3). The average stimulation across years was significant for top- and side-shoot increment, ranging from 15 to 36% (Table 1), and marginally significant for ring width (+26% in the NPK30 treatment only). Top-shoot increment of pine responded significantly more to the NPK30 than to the NPK15 treatment (Table 1,  $df = 20$ ,  $t = 2.96$ ,  $P < 0.01$ ). Side-shoot increments were also somewhat greater in the NPK30 than in the NPK15 treatment, but the difference between the two fertilizer doses was not significant in this case. Almost all pine trees from the lowest three sites, spanning 40 m of the total 140

m of elevation investigated, died between 2005 and 2015. Fertilization had no effect on tree survival in either species (controls were similarly affected).

Elevation had no statistically significant influence on the tree growth response to fertilization, except for the side-shoot increment of pine in the NPK15 treatment (Table S4), where fertilizer stimulation decreased with increasing elevation ( $df = 91$ ,  $t = -2.4$ ,  $P = 0.02$ ). However, this finding was not supported by data from the NPK30 treatment.



**Figure 3** Annual growth of top-shoots, side-shoots and tree ring width in larch (*Larix decidua*, dots) and mountain pine (*Pinus uncinata*, triangles) growing in control and fertilized

plots, corresponding to 15 (NPK15) or 30 kg N ha<sup>-1</sup> a<sup>-1</sup> (NPK30). Means and 95% confidence intervals are shown. Dotted lines connecting control values illustrate that the same individuals were measured in these years and apply to all treatments (not only control plots). Fertilizer was first applied in September 2004.

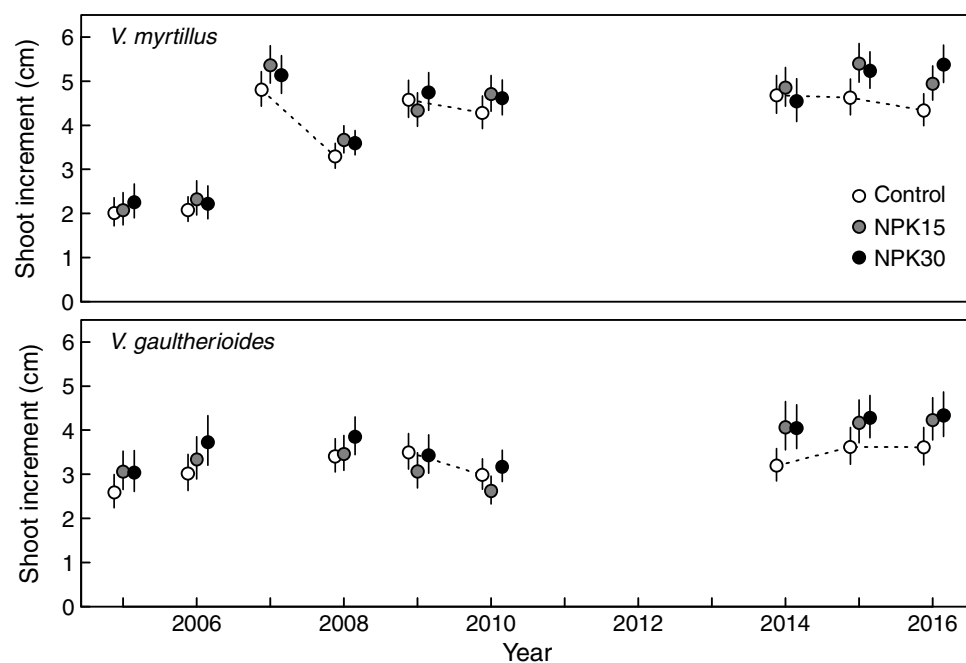
**Table 1** Model estimates for annual growth averaged across elevation and over all treatment years (2016 only for tree height and diameter). T-tests were used to determine the statistical significance of the differences between fertilized and control plots. Degrees of freedom were 20 for *L. decidua* (*Ld*) and *P. uncinata* (*Pu*) and 37 for *V. myrtillus* (*Vm*) and *V. gaultherioides* (*Vg*). Bold numbers highlight significant ( $P < 0.05$ ) and marginally significant ( $P < 0.1$ ) differences.

Species	Measure	Control		NPK15				NPK30			
		Mean	95% CI	Mean	95% CI	<i>t</i>	<i>P</i>	Mean	95% CI	<i>t</i>	<i>P</i>
<i>Ld</i>	Tree ring (mm)	1.47	1.30, 1.63	1.76	1.60, 1.92	3.3	<b>&lt;0.01</b>	1.63	1.46, 1.79	1.8	<b>0.09</b>
	Top-shoot (cm)	23.6	21.4, 26.0	26.7	24.3, 29.0	2.5	<b>0.02</b>	26.3	24.0, 28.6	2.3	<b>0.03</b>
	Side-shoot (cm)	12.5	11.4, 13.6	14.1	13.0, 15.2	2.6	<b>0.02</b>	14.2	13.1, 15.3	2.7	<b>0.01</b>
	Height 2016 (cm)	443	404, 484	468	428, 510	1.4	0.16	470	430, 512	1.5	0.13
	Diam 2016 (cm)	9.4	8.2, 10.5	11.1	9.9, 12.3	3.7	<b>&lt;0.01</b>	10.3	9.1, 11.5	2.0	<b>0.05</b>
<i>Pu</i>	Tree ring (mm)	0.58	0.44, 0.76	0.69	0.52, 0.88	1.3	0.20	0.73	0.56, 0.93	1.9	<b>0.07</b>
	Top-shoot (cm)	6.7	5.7, 7.7	7.7	6.6, 8.8	2.2	<b>0.04</b>	9.1	7.9, 10.3	5.1	<b>&lt;0.01</b>
	Side-shoot (cm)	4.9	4.2, 5.7	5.7	4.9, 6.6	2.8	<b>0.01</b>	6.2	5.4, 7.1	4.3	<b>&lt;0.01</b>
	Height 2016 (cm)	151	124, 180	147	121, 177	-0.3	0.79	173	143, 205	1.6	0.11
	Diam 2016 (cm)	5.5	4.2, 6.8	5.7	4.4, 6.9	0.4	0.73	6.3	4.9, 7.6	1.4	0.17
<i>Vm</i>	Shoot (cm)	3.7	3.5, 3.7	4.0	3.8, 4.2	2.7	<b>0.01</b>	4.0	3.8, 4.2	2.8	<b>&lt;0.01</b>
<i>Vg</i>	Shoot (cm)	3.2	2.9, 3.5	3.5	3.1, 3.8	1.4	0.18	3.7	3.4, 4.1	2.7	<b>0.01</b>

## GROWTH OF DWARF SHRUB SPECIES

As with trees, shoot increments of shrubs did not differ between treatments in 2004 before the fertilizer was applied ( $P > 0.5$ ). In control plots, the mean annual shoot increment was 3.7 cm for *V. myrtillus* and 3.2 cm for *V. gaultherioides*, independent of elevation (Table 1). The response to fertilizer addition varied among years (Fig. 4). On average, both fertilizer doses stimulated the annual shoot increment of *V. myrtillus* by ca. 8%, while *V. gaultherioides*

responded to the NPK30 treatment only (16% longer shoot increment, Table 1). The response to fertilization did not depend on elevation or the tree species growing in the plot (Table S5).



**Figure 4** Annual shoot increment of *Vaccinium myrtillus* and *Vaccinium gaultherioides* growing in control or fertilized plots, corresponding to 15 (NPK15) or 30 kg N ha<sup>-1</sup> a<sup>-1</sup> (NPK30). Means and 95% confidence intervals are shown. Dotted lines connecting control values indicate that the same individuals were measured in these years and apply to all treatments (not only control plots). Fertilizer was first applied in September 2004.

#### CLIMATIC EFFECTS ON THE FERTILIZER-RESPONSE

Summer (June, July and August) mean air temperatures were between 8.7 and 12.9 °C and did not significantly alter the response to fertilization, although the measured traits of trees and shrubs (except top- and side-shoot increment in pine) showed significantly increased growth in warmer years (Table S6). Our experiment covered 0–4 frost events per season.

Trees and shrubs showed reduced growth in years with more frequent frost events, except for top- and side-shoot increment in larch, which were not significantly affected. The growth stimulation by fertilizer addition in tree ring width of larch was significantly smaller in years with more frequent frost events ( $P = 0.012$ , Table S6).

## Discussion

Fertilizer addition to this high elevation treeline ecosystem for more than a decade stimulated growth in all four investigated tree and dwarf shrub species. Even the lower NPK-fertilizer dose with  $15 \text{ kg N ha}^{-1} \text{ a}^{-1}$  was enough to stimulate growth in the two tree species and in *V. myrtillus*. These findings strongly suggest that small increases in soil nutrient availability may suffice to stimulate growth in this treeline ecotone.

### LOW DOSE FERTILIZATION ENHANCES PLANT GROWTH

While previous experiments with trees applied  $85\text{--}175 \text{ kg N ha}^{-1} \text{ a}^{-1}$ , leading to a doubling or tripling of biomass production (Sveinbjörnsson 1992; Susiluoto et al. 2010), even our low dose treatment clearly enhanced growth. In cold regions, N availability is often low in absolute terms, but related to productivity (and the short growing season) the trends are not so obvious (Tranquillini 1979, Körner 2012) because the nutrient cycle is tuned with the carbon cycle. A recent  $^{15}\text{N}$  tracer study at this site showed that about 60% of N added as  $\text{NH}_4^+$  or glycine is rapidly immobilized in the soil (Dawes et al. 2017a), as is typical for high elevation ecosystems on acidic soils (Gerzabek et al. 2004). Nevertheless, higher concentrations of N, P and K in leaves, increased soil extractable N and higher  $\delta^{15}\text{N}$  values in foliage indicate that the mineral fertilizer indeed increased nutrient availability for plants in the present study. The increase in plant nutrient concentration by our comparably low fertilizer dose was similar to that in the Scandinavian treeline study with birch using a much higher dose of NPK-fertilizer

(Sveinbjörnsson 1992), but we do not know whether birch would have responded similarly to such low nutrient additions. We attribute the high responsiveness of the treeline ecosystem in our study to low rates of nutrient addition to a so far unexplored non-linearity of nutrient-growth relationships. Remarkably, little additional growth stimulation was observed with the higher (30 kg ha<sup>-1</sup> a<sup>-1</sup>) fertilizer dose compared with the lower dose. Our site has acidic soils covered with 5–20 cm thick organic layers and intrinsically low rates of nutrient cycling compared to forest soils in the temperate zone (e.g. Bednorz et al. 2000; Blaser et al. 2005; Hagedorn et al. 2010). Accordingly, the lack of a surplus stimulation by the higher fertilizer dose may indicate a low overall nutrient demand of growth, e.g. due to an inherently low growth rate or because of thermal constraints on meristematic activity (Hoch 2013). The 8–16% increase in dwarf shrub growth is low compared with results from an alpine grassland at 2500 m a.s.l., where a doubling in biomass was reported in response to 40 kg N ha<sup>-1</sup> a<sup>-1</sup> (Schäppi & Körner 1996).

#### *SPECIES-SPECIFIC GROWTH RESPONSES*

Trees and dwarf shrubs showed a similar stimulation by nutrient addition in contrast to our expectations that dwarf shrubs would be more responsive than trees because of a more favourable microclimate. This result may be related to spatial and temporal temperature variation. First, the microclimate in the understory vegetation does not warm up as much as it does in open terrain (Körner 2012). Thus, trees and dwarf shrubs experienced similar soil temperatures, at least in the rooting zone. Second, the ongoing climatic warming by 2 K (Rixen et al. 2012) during the last four decades has probably already relieved trees from thermal constraints of growth to some extent.

Although there was a similar fertilization effect on both growth forms, there was a difference in the response between the two dwarf shrubs. While shoot increment length of *V. gaultherioides* was stimulated by the higher fertilizer dose only, responses of *V. myrtillus* saturated at the lower fertilizer dose (no difference between the two doses). In a separate experiment within the same afforestation used in our study, Dawes et al. (2011) found that growth of *V. myrtillus* responded more than that of *V. gaultherioides* to six years of experimental soil warming by 4 K, a treatment that led to enhanced nitrogen availability (Dawes et al. 2017b). *Vaccinium myrtillus* has a wider elevational distribution range than *V. gaultherioides* (Lauber et al. 2012) and might have a competitive advantage under changing growth conditions. However, it was also found to be more prone to freezing damage when growing in warmer soils (Martin et al. 2010), which could ultimately have a larger impact than growth stimulation by warming or increased nutrient availability. It had been shown, although with higher addition rates of N ( $>100 \text{ kg ha}^{-1}$ ), that fertilization delays bud formation in autumn, and thus winter dormancy and freezing tolerance, in *V. myrtillus* (Körner 1984).

Positive effects of fertilization on the rate of tissue formation are commonly observed, but may be counteracted by increased pathogen impact (Herms & Mattson 1992, Rühmann et al. 2002, Blodgett et al. 2005) or reduced freezing tolerance (Hellergrén 1981). In addition, faster growth from additional N may result in lower tissue concentrations of lignin (Pitre et al. 2007, Li et al. 2012), which in turn could increase the susceptibility to mechanical damage from snow breakage, wind, rock fall or avalanches and ultimately lead to biomass loss. There is also evidence that fertilizer addition can reduce the success of afforestation with small saplings, largely due to fungal diseases — work that actually had been conducted at our study site (Keller 1970). We found that frequent frost events reduce, but not reverse, the growth stimulation in larch. Nevertheless, stimulated growth and unchanged mortality in response to twelve years of moderate nutrient addition in the current experiment suggests that the adult trees examined here were not negatively affected by the fertilizer treatment. Negative



consequences of improved nutrition, and thus vigor, could be associated with stochastic phenomena such as heavy snow pack (mechanical damage), certain snow melt regimes (fungal pathogens) or epidemic insect outbreaks (e.g. larch budmoth), and may take more time to materialize.

## GROWING SEASON TEMPERATURE DID NOT MODULATE THE FERTILIZER RESPONSE

Despite the positive association between summer temperature and annual growth, the interannual variation of the fertilizer response was unrelated to interannual temperature variation among growing seasons in trees and shrubs. To some extent, these results may be confounded by an accumulation of added nutrients in the plant-soil-microbe system over the study duration. However, we expect this effect to be small, as trees and shrubs generally did not respond to a surplus of nutrients (the higher fertilizer dose) and longer term  $^{15}\text{N}$  studies in forest soils reveal added N to be rapidly bound to stable soil organic matter pools (Hagedorn et al., 2005; Schleppi et al. 2017). The lack of interaction between summer temperature and fertilizer is in line with the absence of such an interactive effect across elevation within the ecotone. The overall lack of fertilizer-temperature interaction is in contrast to the mountain birch data that revealed the greatest responsiveness to nutrient addition at the highest (coldest) of three sites spanning roughly 200 m in elevation (Sveinbjörnsson et al. 1992). These differences may reflect site-specific effects, differences between evergreen and deciduous species, or the different experimental durations and rates of fertilizer addition.

Within the treeline ecotone covered in this study, growth of pine and larch decreased considerably with increasing elevation during the 30 years before our experiment started (Barbeito et al. 2012). This is typical for high elevation treelines, where the distance between closed montane forest and the krummholz belt is often not more than 50–100 m in elevation

(Körner 2012). The elevation-associated decline in larch ring width observed in our study indicates that temperature is still a major growth-limiting factor for this species within the afforestation site. Notably, apical and lateral increments in shoots showed no significant elevation-associated patterns, but these traits may not reflect the overall crown increment, which depends on the number of shoots per crown surface area, branching patterns, and annual shoot wasting. Thus, apical and lateral increments of single shoots may overestimate growth of larch at higher elevation, where trees were clearly much smaller. The lack of an elevation-associated decline of growth in pine during the study period may relate to the generally low vigor and often crippled shape of these trees at the site. While Barbeito et al. (2012) found decreasing growth of pine with increasing elevation within the afforestation during the three decades before our experiment, the effect diminished over time and was comparably weak in the most recent decade before our experiment started (1995 to 2005).

A recent decrease of dwarf shrub cover at the lowest locations, presumably coupled to an increase in visibly more vigorous (and therefore nutrient-demanding) understory species, could be another explanation (see Fig. S5). The growing season temperature increased by ca. 2 K in the region since the afforestation site was established in 1975 (Rixen et al. 2012). This warming (corresponding to 300 to 400 m of elevation) may have masked the smaller spatial and year-to-year variation in temperature. In a study close to our site, similar seedling survival at low and high elevations suggested that the current treeline position lags behind climatic changes (Zurbriggen et al. 2013). This indicates that temperature limitation of growth has been reduced over the past few decades, as was also observed for other treelines in the Swiss Alps (Jochner et al. 2018).

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For seedlings of the same tree species as investigated in this study, it was shown that fertilizer responses were diminished at critically low mean temperatures of around 6 °C in a growth chamber, but that warmer air or soil temperature (12 °C) enabled positive growth responses to fertilization (Hoch 2013). Similarly, Iivonen et al. (1999) found that 1-year-old *Pinus sylvestris* seedlings grown at different root zone temperatures showed growth stimulation by fertilization only above 13 °C. Hoch (2013) argued that growth stimulation by nutrient addition may occur only above such critically low temperatures. Our data suggest that even the uppermost trees grew far from a thermal limit of growth.

#### FERTILIZATION ALTERS NUTRIENT BALANCES

While primary production of most terrestrial ecosystems on a global scale is considered to be N limited (Vitousek & Howarth, 1991), we found lower foliar N:P ratios with fertilization in three out of four species, suggesting that growth is limited more by P than N (Güsewell 2004). The only previous study in which trees at treeline received N and P additions separately was that with mountain birch, where no indication of P limitation was observed while N addition clearly stimulated growth (Sveinbjörnsson 1992). Perhaps continuous atmospheric N deposition during the last century has decreased the capacity for further growth stimulation by N addition at our site. Alternatively, lower N:P ratios may be related to a greater retention of N than of P in the soil due to microbial immobilization, a lack of P sorption in the thick organic layers, or a higher supply of P compared to N, a likely scenario when considering that our fertilizer had an N:P ratio of five while the foliar N:P ratios of the investigated plants were about twice as high. Altered mechanisms of nutrient uptake are supported by higher  $\delta^{15}\text{N}$  values in all plant species already at low levels of fertilization (corresponding to 15 kg N ha<sup>-1</sup> a<sup>-1</sup>). This finding indicates either that the plants took up more N from the supplied NH<sub>4</sub><sup>+</sup> (Garten 1993, Craine et al. 2009, Miller & Bowman 2002) or that there was a decrease in the proportion of organic N acquired by plants through mycorrhizal

association (Emmert et al. 2001, Hobbie & Colpaert 2003). Mycorrhizal activity has frequently been observed to decline with N fertilization (Wallander & Nylund 1992; Nilsson & Wallander 2003; Högberg et al. 2010). Further, in an experiment near our study site where soil warming led to greater N availability, Solly et al. (2017b) observed a shift in the fungal community composition towards more nitrophilic species.

### *[Conclusions]*

Our findings demonstrate that even a relatively low dose of fertilizer can enhance growth of woody species near the treeline, confirming the sensitivity of low temperature ecosystems to changes in nutrient availability in the soil. Hence, despite a limitation of plant growth by low temperatures at high elevation, even a small improvement of nutrient supply, for instance by increased atmospheric deposition or enhanced nutrient mineralization in warmer soils, can cause changes in high elevation treeline ecosystems. Our experimental site and all 'natural' treelines in the region experienced a 2 K warming over the past four decades, which has reduced thermal limitation of growth (Jochner et al. 2018) and may have masked interactions between fertilizer addition and more subtle year-to-year or spatial temperature differences. Thus, while treeline trees and associated dwarf shrubs benefit from nutrient addition in terms of growth, our study does not allow to answer whether nutrient availability influences the position of treeline. A central remaining question is whether greater tree vigor and/or higher tissue nutrient concentrations exert negative long-term fitness effects in such marginal habitats. Implications of faster growth for the long-term fitness of treeline species await additional research focusing on reproduction, life-span (mortality) and stress tolerance. Nevertheless, faster growth rates with increased nutrient availability may speed up the anticipated upward shift of treeline ecotones as a consequence of climate warming.

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## Authors' contributions

CR designed the study in consultation with PB, CK and SW. PM, MM, DV, and FH collected data, and PM, MM and MD completed the data analysis. PM led the writing with revisions and contributions from all authors. All authors approved the final version.

## Data accessibility

All relevant data used in this study is available from the Figshare repository, <http://dx.doi.org/10.6084/m9.figshare.7025858> (Möhl et al., 2018)

## References

- Alvarez-Uria, P. & Körner, C. (2007) Low temperature limits of root growth in deciduous and evergreen temperate tree species. *Functional Ecology*, **21**, 211–218.
- Bai, E., Li, S., Xu, W., Li, W., Dai, W. & Jiang, P. (2013) A meta-analysis of experimental warming effects on terrestrial nitrogen pools and dynamics. *New Phytologist*, **199**, 431–440.
- Barbeito, I., Dawes, M.A., Rixen, C., Senn, J. & Bebi, P. (2012) Factors driving mortality and growth at treeline: A 30-year experiment of 92 000 conifers. *Ecology*, **93**, 389–401.
- Bassin, S., Volk, M., Suter, M., Buchmann, N. & Fuhrer, J. (2007) Nitrogen deposition but not ozone affects productivity and community composition of subalpine grassland after 3 yr of treatment. *New Phytologist*, **175**, 523–534.

- Bassin, S., Schalajda, J., Vogel, A. & Suter, M. (2012) Different types of sub-alpine grassland respond similarly to elevated nitrogen deposition in terms of productivity and sedge abundance. *Journal of Vegetation Science*, **23**, 1024–1034.
- Bednorz, F., Reichstein, M., Broll, G., Holtmeier, F.-K. & Urfer, W. (2000) Humus forms in the forest-Alpine tundra ecotone at Stillberg (Dischmatal, Switzerland): spatial heterogeneity and classification. *Arctic, Antarctic, and Alpine Research*, **32**, 21–29.
- Birmann, K. & Körner, C. (2009) Nitrogen status of conifer needles at the alpine treeline. *Plant Ecology & Diversity*, **2**, 233–241.
- Blaser, P. (1980) Der Boden als Standortsfaktor bei Aufforstungen in der subalpinen Stufe (Stillberg, Davos). *Eidg. Anst. Forstliche Vers.*, **56**, 527–611.
- Blaser P., Zimmermann S., Luster J., Walther L., Lüscher P. (2005) *Waldböden der Schweiz. Band 2 Regionen Alpen und Alpensüdseite*. Birmensdorf, Eidgenössische Forschungsanstalt WSL. Hep Verlag, Bern
- Blodgett, J.T., Herms, D.A. & Bonello, P. (2005) Effects of fertilization on red pine defense chemistry and resistance to *Sphaeropsis sapinea*. *Forest Ecology and Management*, **208**, 373–382.
- Bobbink, R. & Hettelingh, J.P. (2010) Review and revision of empirical critical loads-response relationships. *Proceedings of an expert workshop, Noordwijkerhout, 23-25 June 2010*.
- Brooks, P.D., Williams, M.W. & Schmidt, S.K. (1996) Microbial activity under alpine snowpacks, Niwot Ridge, Colorado. *Biogeochemistry*, **32**, 93–113.
- Chapin, F.S., Shaver, G.R., Giblin, A.E., Nadelhoffer, K.J. & Laundre, J.A. (1995) Responses of arctic tundra to experimental and observed changes in climate. *Ecology*, **76**, 694–711.
- Craine, J.M., Elmore, A.J., Aida, M.P.M., Bustamante, M., Dawson, T.E., Hobbie, E. A., Kahmen, A., Mack, M.C., McLauchlan, K.K., Michelsen, A., Nardoto, G.B., Pardo, L.H., Peñuelas, J., Reich, P.B., Schuur, E.A.G., Stock, W.D., Templer, P.H., Virginia, R.A., Welker, J.M. & Wright, I.J. (2009) Global patterns of foliar nitrogen isotopes and their relationships with climate,

- mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. *New Phytologist*, **183**, 980–992.
- Dawes, M.A., Hagedorn, F., Zumbunn, T., Handa, I.T., Hättenschwiler, S., Wipf, S. & Rixen, C. (2011) Growth and community responses of alpine dwarf shrubs to in situ CO<sub>2</sub> enrichment and soil warming. *New Phytologist*, **191**, 806–818.
- Dawes, M.A., Schleppi, P. & Hagedorn, F. (2017a) The fate of nitrogen inputs in a warmer alpine treeline ecosystem: a <sup>15</sup>N labelling study. *Journal of Ecology*, **105**, 1723–1737.
- Dawes, M.A., Schleppi, P., Hättenschwiler, S., Rixen, C. & Hagedorn, F. (2017b) Soil warming opens the nitrogen cycle at the alpine treeline. *Global Change Biology*, **23**, 421–434.
- Emmerton, K.S., Callaghan, T. V., Jones, H.E., Leake, J.R., Michelsen, A. & Read, D.J. (2001) Assimilation and isotopic fractionation of nitrogen by mycorrhizal and nonmycorrhizal subarctic plants. *New Phytologist*, **151**, 513–524.
- Fajardo, A. & Piper, F.I. (2017) An assessment of carbon and nutrient limitations in the formation of the southern Andes tree line. *Journal of Ecology*, **105**, 517–527.
- Ferrari, A., Hagedorn, F. & Niklaus, P.A. (2016) Experimental soil warming and cooling alters the partitioning of recent assimilates: evidence from a <sup>14</sup>C-labelling study at the alpine treeline. *Oecologia*, **181**, 25–37.
- Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P. & Sutton, M.A. (2008) Transformation of the Nitrogen Cycle: Recent Trends, Questions, and Potential Solutions. *Science*, **320**, 889–892.
- Garten, C.T. (1993) Variation in Foliar <sup>15</sup>N Abundance and the Availability of Soil Nitrogen on Walker Branch Watershed. *Ecology*, **74**, 2098–2113.
- Gerzabek, M.H., Haberhauer, G., Stemmer, M., Klepsch, S. & Haunold, E. (2004) Long-term behaviour of <sup>15</sup>N in an alpine grassland ecosystem. *Biogeochemistry*, **70**, 59–69.

- Güsewell, S. (2004) N : P ratios in terrestrial plants: variation and functional significance. *New Phytologist*, **164**, 243–266.
- Hagedorn, F., Maurer, S., Bucher, J.B. & Siegwolf, R.T.W. (2005) Immobilization, stabilization and remobilization of nitrogen in forest soils at elevated CO<sub>2</sub>: A <sup>15</sup>N and <sup>13</sup>C tracer study. *Global Change Biology*, **11**, 1816–1827.
- Hagedorn, F., Martin, M., Rixen, C., Rusch, S., Bebi, P., Zürcher, A., Siegwolf, R.T.W., Wipf, S., Escape, C., Roy, J. & Hättenschwiler, S. (2010) Short-term responses of ecosystem carbon fluxes to experimental soil warming at the Swiss alpine treeline. *Biogeochemistry*, **97**, 7–19.
- Heer, C. & Körner, C. (2002) High elevation pioneer plants are sensitive to mineral nutrient addition. *Basic and Applied Ecology*, **3**, 39–47.
- Hellergren, J. (1981) Frost hardiness development in *Pinus silvestris* seedlings in response to fertilization. *Physiologia Plantarum*, **52**, 297–301.
- Hermes, D.A. & Mattson, W.J. (1992) The Dilemma of Plants: To Grow or Defend. *The Quarterly Review of Biology*, **67**, 283–335.
- Hiltbrunner, E., Schwikowski, M. & Körner, C. (2005) Inorganic nitrogen storage in alpine snow pack in the Central Alps (Switzerland). *Atmospheric Environment*, **39**, 2249–2259.
- Hobbie, E.A. & Colpaert, J. V. (2003) Nitrogen availability and colonization by mycorrhizal fungi correlate with nitrogen isotope patterns in plants. *New Phytologist*, **157**, 115–126.
- Hoch, G. (2013) Reciprocal root-shoot cooling and soil fertilization effects on the seasonal growth of two treeline conifer species. *Plant Ecology & Diversity*, **6**, 21–30.
- Högberg, M.N., Briones, M.J.I., Keel, S.G., Metcalfe, D.B., Campbell, C., Midwood, A.J., Thornton, B., Hurry, V., Linder, S., Näsholm, T. & Högberg, P. (2010) Quantification of effects of season and nitrogen supply on tree below-ground carbon transfer to ectomycorrhizal fungi and other soil organisms in a boreal pine forest. *New Phytologist*, **187**, 485–493.



- Iivonen, S., Rikala, R., Ryypö, A. & Vapaavuori, E. (1999) Responses of Scots pine (*Pinus sylvestris*) seedlings grown in different nutrient regimes to changing root zone temperature in spring. *Tree Physiology*, **19**, 951–958.
- Jochner, M., Bugmann, H., Nötzli, M. & Bigler, C. (2018) Tree growth responses to changing temperatures across space and time: a fine-scale analysis at the treeline in the Swiss Alps. *Trees - Structure and Function*, **32**, 645–660.
- Kammer, A., Hagedorn, F., Shevchenko, I., Leifeld, J., Guggenberger, G., Goryacheva, T., Rigling, A. & Moiseev, P. (2009) Treeline shifts in the Ural mountains affect soil organic matter dynamics. *Global Change Biology*, **15**, 1570–1583.
- Keller, T. (1970) Wuchsleistung, Gaswechsel, Überlebensprozente und Schneeschimmelpilzbefall gedüngter Ballenpflanzen an der oberen Waldgrenze. *Mitt. schweiz. Anst. forstl. Vers.-wes.*, **46**, 1–31.
- Kirschbaum, M.U.F. (1995) The temperature dependence of soil organic matter decomposition, and the effect of global warming on soil organic C storage. *Soil Biology and Biochemistry*, **27**, 753–760.
- Klanderud, K. (2008) Species-specific responses of an alpine plant community under simulated environmental change. *Journal of Vegetation Science*, **19**, 363–372.
- Körner, C. (1984) Auswirkungen von Mineraldünger auf alpine Zwergsträucher. *Verhandlungen der Gesellschaft für Ökologie*, **12**, 123–136.
- Körner, C. (1989) The nutritional status of plants from high altitudes. *Oecologia*, **81**, 379–391.
- Körner, C. (2003) *Alpine Plant Life*. Springer, Heidelberg.
- Körner, C. (2012) *Alpine Treelines*. Springer, Basel.
- Körner, C. & Paulsen, J. (2004) A world-wide study of high altitude treeline temperatures. *Journal of Biogeography*, **31**, 713–732.

- Kuoch, R. (1970) Die Vegetation auf Stillberg (Dischmatal, Kt. Graubünden). *Mitteilungen der Eidgenössischen Anstalt für das forstliche Versuchswesen*, **46**, 329–342.
- Lauber, K., Wagner, G. & Gygax, A. (2012) *Flora Helvetica*. Haupt Verlag.
- Lenth, R. V. (2016) Least-Squares Means: The R Package lsmeans. *Journal of Statistical Software*, **69**, 1–33.
- Li, H., Li, M., Luo, J., Cao, X., Qu, L., Gai, Y., Jiang, X., Liu, T., Bai, H., Janz, D., Polle, A., Peng, C. & Luo, Z.-B. (2012) N-fertilization has different effects on the growth, carbon and nitrogen physiology, and wood properties of slow- and fast-growing *Populus* species. *Journal of Experimental Botany*, **63**, 6173–6185.
- Loomis, P.F., Ruess, R.W., Sveinbjörnsson, B. & Kielland, K. (2006) Nitrogen cycling at treeline: Latitudinal and elevational patterns across a boreal landscape. *Ecoscience*, **13**, 544–556.
- Martin, M., Gavazov, K., Körner, C., Hättenschwiler, S. & Rixen, C. (2010) Reduced early growing season freezing resistance in alpine treeline plants under elevated atmospheric CO<sub>2</sub>. *Global Change Biology*, **16**, 1057–1070.
- Mayor, J.R., Sanders, N.J., Classen, A.T., Bardgett, R.D., Clément, J.C., Fajardo, A., Lavorel, S., Sundqvist, M.K., Bahn, M., Chisholm, C., Cieraad, E., Gedalof, Z., Grigulis, K., Kudo, G., Oberski, D.L. & Wardle, D.A. (2017) Elevation alters ecosystem properties across temperate treelines globally. *Nature*, **542**, 91–95.
- Miller, A.E. & Bowman, W.D. (2002) Variation in nitrogen-15 natural abundance and nitrogen uptake traits among co-occurring alpine species: do species partition by nitrogen form? *Oecologia*, **130**, 609–616.
- Möhl, Patrick; Mörsdorf, Martin A.; Dawes, Melissa; Hagedorn, Frank; Bebi, Peter; Viglietti, Davide; et al. (2018): Twelve years of low nutrient input stimulates growth of trees and dwarf shrubs in the treeline ecotone. figshare. <https://doi.org/10.6084/m9.figshare.7025858>

- Navone, R. (1964) Proposed Method for Nitrate in Potable Waters. *American Water Works Association*, **56**, 781–783.
- Nilsson, J. (1988) Critical Loads for Sulphur and Nitrogen. *Air Pollution and Ecosystems*, pp. 85–91. Springer Netherlands, Dordrecht.
- Nilsson, L.O. & Wallander, H. (2003) Production of external mycelium by ectomycorrhizal fungi in a norway spruce forest was reduced in response to nitrogen fertilization. *New Phytologist*, **158**, 409–416.
- Paulsen, J. & Körner, C. (2014) A climate-based model to predict potential treeline position around the globe. *Alpine Botany*, **124**, 1–12.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. & R Core Team (2016) *nlme: Linear and Nonlinear Mixed Effects Models*. R package version 3.1-131
- Pitre, F.E., Pollet, B., Lafarguette, F., Cooke, J.E.K., Mackay, J.J. & Lapierre, C. (2007) Effects of Increased Nitrogen Supply on the Lignification of Poplar Wood Effects of Increased Nitrogen Supply on the. *Journal of Agricultural and Food Chemistry*, **55**, 10306–10314.
- R Core Team (2017) R: A language and environment for statistical computing.
- Rihm, B. & Kurz, D. (2001) Deposition and critical loads of nitrogen in Switzerland. *Water, Air, and Soil Pollution*, **130**, 1223–1228.
- Rixen, C., Dawes, M.A., Wipf, S. & Hagedorn, F. (2012) Evidence of enhanced freezing damage in treeline plants during six years of CO<sub>2</sub> enrichment and soil warming. *Oikos*, **121**, 1532–1543.
- Rogora, M., Colombo, L., Marchetto, A., Mosello, R. & Steingruber, S. (2016) Temporal and spatial patterns in the chemistry of wet deposition in Southern Alps. *Atmospheric Environment*, **146**, 44–54.
- Rühmann, S., Leser, C., Bannert, M. & Treutter, D. (2002) Relationship Between Growth, Secondary Metabolism, and Resistance of Apple. *Plant Biology*, **4**, 137–143.

- Saxe, H., Cannell, M.G.R., Johnsen, Ø., Ryan, M.G. & Vourlitis, G. (2002) Tree and forest functioning in response to global warming. *New Phytologist*, **149**, 369–399.
- Schäppi, B. & Körner, C. (1996) Growth responses of an alpine grassland to elevated CO<sub>2</sub>. *Oecologia*, **105**, 43–52.
- Scherrer, D. & Körner, C. (2009) Infra-red thermometry of alpine landscapes challenges climatic warming projections. *Global Change Biology*, **16**, 2602–2613.
- Schleppi, P., Bucher-Wallin, I., Saurer, M., Jäggi, M. & Landolt, W. (2006) Citric acid traps to replace sulphuric acid in the ammonia diffusion of dilute water samples for <sup>15</sup>N analysis. *Rapid Communications in Mass Spectrometry*, **20**, 629–634.
- Schleppi, P., Curtaz, F. & Krause, K. (2017) Nitrate leaching from a sub-alpine coniferous forest subjected to experimentally increased N deposition for 20 years, and effects of tree girdling and felling. *Biogeochemistry*, **134**, 319–335.
- Schmitt, M., Thöni, L., Waldner, P. & Thimonier, A. (2005) Total deposition of nitrogen on Swiss long-term forest ecosystem research (LWF) plots: Comparison of the throughfall and the inferential method. *Atmospheric Environment*, **39**, 1079–1091.
- Schönenberger, W. & Frey, W. (1988) Untersuchungen zur Ökologie und Technik der Hochlagenaufforstung. Forschungsergebnisse aus dem Lawinenanrissgebiet Stillberg. *Schweiz. Z. Forstwes.*, **139**, 735–820.
- Senn, J. & Schönenberger, W. (2001) Zwanzig Jahre Versuchsaufforstung Stillberg: Überleben und Wachstum einer subalpinen Aufforstung in Abhängigkeit vom Standort. *Schweiz. Z. Forstwes.*, **152**, 226–246.
- Solly, E.F., Djukic, I., Moiseev, P.A., Andreyashkina, N.I., Devi, N.M., Göransson, H., Mazepa, V.S., Shiyatov, S.G., Trubina, M.R., Schweingruber, F.H., Wilmking, M. & Hagedorn, F. (2017a) Treeline advances and associated shifts in the ground vegetation alter fine root dynamics and mycelia production in the South and Polar Urals. *Oecologia*, **183**, 571–586.

- Solly, E.F., Lindahl, B.D., Dawes, M.A., Peter, M., Souza, R.C., Rixen, C. & Hagedorn, F. (2017b) Experimental soil warming shifts the fungal community composition at the alpine treeline. *New Phytologist*, **215**, 766–778.
- Sullivan, P.F., Ellison, S.B.Z., McNown, R.W., Brownlee, A.H. & Sveinbjörnsson, B. (2015) Evidence of soil nutrient availability as the proximate constraint on growth of treeline trees in northwest Alaska. *Ecology*, **96**, 716–727.
- Susiluoto, S., Hiltasvuori, E. & Berninger, F. (2010) Testing the growth limitation hypothesis for subarctic Scots pine. *Journal of Ecology*, **98**, 1186–1195.
- Sveinbjörnsson, B., Nordell, O. & Kauhanen, H. (1992) Nutrient Relations of Mountain Birch Growth at and Below the Elevational Tree-Line in Swedish Lapland. *Functional Ecology*, **6**, 213.
- Thébaud, A., Clément, J.-C., Ibanez, S., Roy, J., Geremia, R.A., Pérez, C.A., Buttler, A., Estienne, Y. & Lavorel, S. (2014) Nitrogen limitation and microbial diversity at the treeline. *Oikos*, **123**, 729–740.
- Thomas, S.C., Halpern, C.B., Falk, D. a, Liguori, D. a & Austin, K.A. (1999) Plant diversity in managed forests: Understory responses to thinning and fertilization. *Ecological Applications*, **9**, 864–879.
- Tranquillini, W. (1979) Physiological Ecology of the Alpine Timberline. Tree Existence at High Altitudes with Special Reference to the European Alps. Springer-Verlag Berlin Heidelberg.
- Van Wijk, M.T., Clemmensen, K.E., Shaver, G.R., Williams, M., Callaghan, T. V., Chapin, F.S., Cornelissen, J.H.C., Gough, L., Hobbie, S.E., Jonasson, S., Lee, J.A., Michelsen, A., Press, M.C., Richardson, S.J. & Rueth, H. (2004) Long-term ecosystem level experiments at Toolik Lake, Alaska, and at Abisko, Northern Sweden: Generalizations and differences in ecosystem and plant type responses to global change. *Global Change Biology*, **10**, 105–123.

Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H. & Tilman, D.G. (1997) Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications*, **7**, 737–750.

Vitousek, P. & Howarth, R. (1991) Nitrogen limitation on land and in the sea: How can it occur? *Biogeochemistry*, **13**, 87–115.

Wallander, H. & Nylund, J.-E. (1992) Effects of excess nitrogen and phosphorus starvation on the extramatrical mycelium of ectomycorrhizas of *Pinus sylvestris* L. *New Phytologist*, **120**, 495–503.

Zurbriggen, N., Hättenschwiler, S., Frei, E.S., Hagedorn, F. & Bebi, P. (2013) Performance of germinating tree seedlings below and above treeline in the Swiss Alps. *Plant Ecology*, **214**, 385–396.

Zuur, A.F., Leno, E.N., Walker, N.J., Saveliev, A.A. & Smith, G.M. (2011) Mixed effects models and extensions in ecology with R. Springer, New York.