



AperTO - Archivio Istituzionale Open Access dell'Università di Torino

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

This is the author's manuscript
Original Citation:
Availability:
This version is available http://hdl.handle.net/2318/1678981 since 2021-12-09T16:31:25Z
Published version:
DOI:10.1111/1365-2745.13073
Terms of use:
Open Access
Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

1	Twelve years of low nutrient input stimulates growth of trees and dwarf shrubs in
2	the treeline ecotone
3	Patrick Möhl <sup>1</sup> , Martin A. Mörsdorf <sup>2</sup> , Melissa A. Dawes <sup>1,3</sup> , Frank Hagedorn <sup>3</sup> , Peter
4	Bebi <sup>1</sup> , Davide Viglietti <sup>4</sup> , Michele Freppaz <sup>4</sup> , Sonja Wipf <sup>1</sup> , Christian Körner <sup>6</sup> , Frank M.
5	Thomas <sup>5</sup> , Christian Rixen <sup>1</sup>
6	
7	<sup>1</sup> WSL-Institute for Snow and Avalanche Research (SLF), Alpine Ecosystems, Davos,
8	Switzerland
9	<sup>2</sup> Department of Arctic and Marine Biology, The Arctic University of Norway,
10	Tromsø, Norway
11	<sup>3</sup> Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Zürcherstr.
12	111, CH-8903 Birmensdorf, Switzerland
13	<sup>4</sup> Department of Agricultural, Forest and Food Sciences, University of Turin,
14	Grugliasco, Italy
15	<sup>5</sup> Faculty of Regional and Environmental Sciences – Geobotany, University of Trier,
16	Trier, Germany
17	<sup>6</sup> Institute of Botany, University of Basel, Schönbeinstrasse 6, CH-4056 Basel,
18	Switzerland
19	
20	Corresponding author:
21	Patrick Möhl, WSL-Institute for Snow and Avalanche Research (SLF), Flüelastrasse
22	11, 7260 Davos Dorf

23 patrick.moehl@alumnibasel.ch

24

25 Running headline:

26 Nutrient addition stimulates growth at treeline

27

## 28 Abstract

29 1. Almost all natural terrestrial ecosystems are nutrient limited in terms of growth,

30 and we expect treeline vegetation to be no exception. However, direct constraints of

31 low temperature on tissue formation may superimpose effects of low nutrient

32 availability.

33 2. We examined growth responses of two tree (*Larix decidua* and *Pinus uncinata*) and

34 two dwarf shrub species (*Vaccinium myrtillus* and *Vaccinium gaultherioides*) to 12

35 years of moderate fertilizer addition (NPK applied at a rate of 15 and 30 kg nitrogen

 $ha^{-1}a^{-1}$  along an elevation gradient within the treeline ecotone in the Swiss Alps

37 (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.

39 3. Fertilizer addition increased soil nutrient availability, indicated by enhanced soil

40 extractable N, higher concentrations of N, P and K in leaves, and higher foliar  $\delta^{15}$ N.

4. Fertilizer addition stimulated annual growth of all four species: by 11–20% for *L*.

42 *decidua* and 15–36% for *P. uncinata* (depending on trait), and by 8–16% for the two

43 dwarf shrub species. Growth stimulation by the higher fertilizer dose was not

44 significantly stronger than by the lower dose (except for *V. gaultherioides*),

45 suggesting an overall low nutrient demand for growth and saturation at a rather low46 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.

51

52 KEYWORDS

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

55

#### 56 Introduction

57 Globally, the availability of nitrogen (N) and phosphorus (P) is considered to be a 58 major factor limiting growth and productivity in terrestrial ecosystems (Vitousek & 59 Howarth 1991). In recent decades, N availability for plants has increased in many 60 areas owing to atmospheric deposition of nitrogenous compounds (Vitousek et al. 61 1997; Galloway et al. 2008). In addition, global warming is expected to increase 62 turnover rates of soil organic matter (Kirschbaum 1995; Saxe et al. 2001; Kammer et 63 al. 2009; Bai et al. 2013; Dawes et al. 2017b), possibly enhancing the availability of 64 nutrients in general.

65

Arctic and alpine regions are expected to be especially low in plant-available nutrientsbecause of temperature constraints on decomposition and mineralization processes in

68 the soil (Körner 2003). Nitrogen availability generally decreases with an elevation-69 associated decline in temperature (Loomis et al. 2006 [arctic mountain ranges]; 70 Thébault et al. 2014; Fajardo & Piper 2017 [Andes]; Solly et al. 2017a [Ural]). 71 Substantial growth stimulation in response to fertilization has been observed for arctic 72 and alpine ground-layer plants (e.g. Schäppi & Körner 1996; Van Wijk 2004; Bassin 73 et al. 2012). Even the vegetation of glacier forefields shows dramatic increases in 74 biomass production when nutrients are added (Heer & Körner 2002). Thus, growth in 75 cold ecosystems makes no exception to the general nutrient limitation of terrestrial 76 ecosystems.

77

78 Growth responses to an improved nutrient supply, however, often differ among plant 79 species and growth forms (e.g. Chapin et al. 1995; Thomas et al. 1999; Bassin et al. 80 2007; Klanderud 2008), mainly depending on a species' inherent ability to benefit 81 from additional nutrients and the degree to which growth is limited by factors other 82 than nutrient availability, such as temperature. Treelines follow a common isotherm at 83 5–7 °C (6.4 °C; Paulsen & Körner 2014) globally, suggesting that temperature limits 84 tree growth at and above the treeline (Körner & Paulsen 2004). Because tree canopies 85 are aerodynamically open to convective heat transfer (wind) and also shade the 86 ground (causing reduced soil heat flux), they experience colder conditions than low-87 stature plants (including tree seedlings), which often profit from warmer 88 microclimates (Scherrer & Körner 2010; Körner 2012). Growth chamber experiments 89 have demonstrated that growth of juvenile trees is greatly reduced at temperatures 90 below ca. 6 °C, even under an optimal nutrient supply (Iivonen et al. 1998; Alvarez-91 Uria & Körner 2007; Hoch 2013; Ferrari et al. 2016). This suggests that the growth

92 responses to nutrient addition can be strongly constrained under critically low93 temperatures.

94

95 Globally, soil types and thus fertility vary considerably among treelines, indicating 96 that nutrient availability does not consistently affect treeline position the way growing 97 season temperature does (Körner 2012). While low nutrient availability may not be 98 decisive for the establishment of seedlings, it may constrain the vigor of saplings and 99 trees. However, a global assessment of foliar N concentrations in herbs and trees has 100 demonstrated an increasing rather than a decreasing trend with increasing elevation 101 (Körner 1989). Hence, tree life at high elevations is not associated with an obvious 102 foliar nutrient deficiency. Accordingly, the needle N concentration of Picea abies and 103 *Pinus cembra* does not change over the uppermost ca. 300 m of elevation in the Swiss 104 Alps, although growth rate declines substantially (Birmann & Körner 2009). On the 105 other hand, fertilizer addition experiments have reported growth responses of treeline 106 trees to fertilization, similar to responses observed for other vegetation (see above). 107 This indicates that trees in cold climates make no exception in taking advantage of 108 added nutrients in terms of growth. Within three years of a single, high dose 109 application of NPK (85–175 kg N ha<sup>-1</sup>), growth of *Betula pubescens* was found to 110 respond more strongly at the treeline compared with two lower sites in northern 111 Sweden (Sveinbjörnsson 1992). At the Finnish arctic treeline, 50-year-old Pinus 112 sylvestris showed a tripling of annual growth in response to ca. 150 kg N ha<sup>-1</sup> a<sup>-1</sup> of 113 NPK (Susiluoto et al. 2010). Sullivan et al. (2015) presented various lines of 114 observational evidence (no nutrients added) suggesting that growth of arctic treeline 115 trees in Alaska is predominantly limited by nutrients.

117 Here, we present results from a 12-year low-dose fertilization experiment at a treeline 118 in the Swiss Alps. Over an elevation gradient of 140 m across an afforestation site in 119 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 120 121 decidua or 32-year old Pinus uncinata individuals with an understory of mainly 122 ericaceous dwarf shrubs. To quantify growth, we measured annual shoot increments 123 of trees and dwarf shrubs as well as stem ring width of trees. We also measured 124 nutrient concentrations in the soil and assessed the foliar nutritional status of trees and 125 dwarf shrubs. While the very high fertilizer doses used in previous studies (see above) 126 may have prevented competition effects between trees and understory vegetation, we 127 used doses not uncommon in the front ranges of the Alps to provide more realistic 128 insight into growth responses to anthropogenic increases in nutrient availability. 129 Atmospheric N deposition in the inner parts of the Alps is mostly below 20 kg N ha<sup>-1</sup> 130 a<sup>-1</sup> (e.g. Hiltbrunner et al. 2005, Rogora et al. 2016) and empirically defined 131 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 132 133 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 & 134 135 Kurz 2001, Schmitt et al. 2005) may already have exerted some growth stimulation, 136 and we expected that a doubling or tripling of such low rates would have clear effects. 137

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,

140 and whether possible negative effects of nutrient addition offset the benefits of any 141 growth stimulation. Given the general sensitivity of plant growth to nutrient addition 142 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 143 144 differ between growth forms, with smaller effects of nutrient addition for trees than 145 for dwarf shrubs, as upright trees are exposed to lower temperatures. Further, we 146 expected (3) the effect of fertilization to vary across temperature gradients. On a 147 spatial scale, we predicted that the response would decrease with increasing elevation 148 within the treeline ecotone, owing to greater low temperature limitation of tissue 149 formation. On a temporal scale, we predicted larger responses to fertilization in 150 warmer years, when direct thermal constraints are relieved.

151

154

#### 152 Materials and methods

#### 153 STUDY SITE

155 continental climate zone of the inner Alps (Stillberg, Davos, Switzerland; 46°47'N, 156 9°52'E). In 1975, more than 92,000 tree seedlings of *Larix decidua* Mill., *Pinus mugo* 157 ssp. uncinata [DC.] Domin (referred to hereafter as P. uncinata) and Pinus cembra L. 158 were planted into the intact dwarf shrub heath (Kuoch 1970), slightly above the 159 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 160 161 (Supporting Information, Fig. S1). From 1975 to 2005, vertical growth of all three 162 tree species decreased substantially with increasing elevation (Barbeito et al. 2012). 163 At the beginning of our study, tree height declined from ca. 3.2 m to 1.4 m in larch

The experiment was located within a 5 ha long-term afforestation site in the temperate

164 and from 1.6 m to 1.0 m in pine across the elevation range of 142 m (2083 to 2225 m 165 a.s.l.) covered in the present experiment (Fig. S2). On-site climate data is available 166 from a weather station situated at the lower end of the site (2090 m a.s.l.). On average, 167 annual precipitation was 1159 mm and annual mean temperature was 2.0 °C over the 168 last 40 years (1975 to 2015). January is the coldest and July the warmest month, with 169 mean temperatures of -5.4 °C and 10.2 °C, respectively. The growing season lasts 3–4 170 months (June to September) at this location. A second weather station on the same 171 slope at 2220 m. a.s.l. suggested a lapse rate of 0.55 K per 100 m of elevation for the 172 summer months of 2011 and 2012. A 30-year comparison of summer temperatures 173 measured on site and those measured at a weather station 8 km away at 2691 m a.s.l. 174 suggested a lower lapse rate of ca. 0.48 K per 100 m of elevation. Thus, we expect 175 that our experimental site covered a mean air temperature gradient of at least 0.7 K in 176 a temperature range considered critical for tree growth. Date of snowmelt (Barbeito et 177 al. 2012) and freezing events (Rixen et al. 2012) are important modulating factors for 178 growth at the study site.

179

180 The most common understory plants at the site are the ericaceous dwarf shrubs 181 Vaccinium myrtillus L., Vaccinium gaultherioides Bigelow (group V. uliginosum 182 aggr.) and Empetrum nigrum ssp. hermaphroditum (Hagerup) Böcher, reflecting the 183 oligotrophic nature of these ecosystems. Soils are acidic (pH 3.5 to 4.5), developed on 184 siliceous bedrock (Schönenberger & Frey 1988) and have a sandy texture. Soil types 185 at the afforestation site vary with microtopography, with rankers on the ridges and 186 iron-humus podzols within gullies. All soils have 5 to 20 cm thick mor-type organic 187 layers (Bednorz et al. 2000). Detailed descriptions of soil and microclimatic

188 conditions at the site can be found in Blaser (1980), Bednorz et al (2000),

189 Schönenberg & Senn (2001) and Hagedorn et al. (2010).

190

## 191 EXPERIMENTAL DESIGN

192 We chose 22 locations throughout the afforestation site (Fig. S1), hereafter referred to 193 as blocks, that covered the whole range of micro-environmental conditions (see Senn 194 & Schönenberger 2001). Half of the blocks included European larch (L. decidua) and 195 the other half included mountain pine (P. uncinata). Within each block, we randomly 196 selected three plantation quadrats as experimental plots. Each plot was assigned to a 197 control (no fertilization) or to one of two fertilizer dose treatments. Treatments were 198 assigned randomly but confined so that the location of fertilized plots within a block 199 was not directly above control plots to avoid nutrient input from drainage. The 200 elevation difference between treatment plots of the same block was  $5.4 \pm 0.7$  m (mean 201  $\pm$  SE). We annually applied the mid- to long-term fertilizer ARBOSTAR 202  $(N:P_2O_5:K_2O:Mg = 8:4:11:2;$  Geistlich Arbostar, HBG Düngevertrieb AG, Grossaffoltern, Switzerland) at an amount that corresponded to 15 kg N ha<sup>-1</sup> 203 ('NPK15') or 30 kg N ha<sup>-1</sup> ('NPK30'). About 75% of the N in the fertilizer was in the 204 205 form of NH<sub>4</sub><sup>+</sup> and the rest was derived from methylene urea. Fertilizer was first 206 applied in September 2004 and then at the beginning (June) of each following 207 growing season until 2016. Fertilizer granules were evenly distributed by hand over 208 the entire area of a given plot.

#### 210 SOIL ANALYSES

211 Six soil cores (0–10 cm depth, 5 cm diameter) per plot were collected in September

212 2010. Soil samples were bulked plot-wise and sieved at 4 mm mesh size, and fine

213 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 concentration in the extracts was determined colorimetrically by

automated flow injection analysis (RF-535 Fluorescence HPLC Monitor, Shimadzu).

218 The nitrate concentration (NO<sub>3</sub><sup>-</sup>) was measured colorimetrically according to Navone

219 (1964).

220

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,

223 Wegberg, Germany) interfaced with a continuous flow isotope ratio mass

224 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  $NH_4^+$  to  $NH_3$  and trapping it on acidified teflon filters. The

227 filters were packed in tin (Sn) capsules and then measured immediately by mass

 $228 \qquad \text{spectrometry. The pH of the bulk soil was measured potentiometrically in H_2O at a}$ 

soil-to-solution volume ratio of 1:2.5.

230

231 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

234 leaves was collected from roughly 10 branchlets per plot for each of the two dominant 235 dwarf shrubs V. myrtillus and V. gaultherioides. Needles were sampled from the 236 upper canopy on the south side of the trees. Leaves from dwarf shrubs were sampled 237 from the upper shrub canopy, thus avoiding deep shade. Samples were dried at 65 °C 238 for 24 hours, milled to a fine powder and weighed into tin capsules (1–2 mg per sample). Nitrogen per dry mass and  $\delta^{15}N$  were obtained by mass spectrometry in 239 240 connection with an element analyser, as described for the soil samples. In 2010, we 241 additionally determined phosphorus (P), potassium (K) and magnesium (Mg) 242 concentrations of leaf samples. Phosphorus concentrations were analysed 243 photometrically using an UV-VIS spectrometer (UV-160A, Shimadzu Europe GmbH, 244 Germany; DIN EN ISO15681-1). Potassium and Mg were determined by atomic 245 flame spectrometry (SpectrAA 220, Varian Deutschland GmbH, 64289 Darmstadt, 246 Germany; DIN EN ISO 9964-2 for K and DIN EN ISO7980 for Mg). 247

248 In 2004, we selected three census trees in each plot that were visibly unaffected by 249 frost damage or snow fungus and were not heavily shaded by surrounding trees. Tree 250 and shrub growth was measured in 8 years from 2004 (pre-treatment year) to 2016. 251 No measurements were made from 2011 to 2015. During this period, several of the 252 originally selected trees died, were damaged or lost their labels, prompting us to select 253 new trees for the final census in 2016 using the original criteria. There was no 254 difference in tree ring width between the trees selected in 2004 and the trees selected 255 in 2016 in four overlapping years (2007 to 2010, see Fig. S3). Thus, we are confident 256 that selecting new trees did not have a qualitative effect on the results. In 2005 and 257 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 treemortality.

260

261	For the annual growth census, one branch per tree at approx. half the tree height was
262	marked to assess side-shoot growth. We obtained tree shoot increment data for 2004
263	to 2016 using obvious annual bud scars/branching nodes. The increment of top- and
264	side-shoots in 2007 and 2009 and from 2011 to 2015 was back-measured in the
265	subsequent years. Top-shoot increment of larch was not measured in 2009 and 2010.

266

267 In 2010 and 2016 we collected microcores (35 mm length, 2 mm diameter) from all 268 marked trees using an increment puncher (Trephor, Università degli Studi di Padova, 269 S. Voto di Cadore, Italy). Cores were extracted from two sides of the main stem (east 270 and west in 2010 and north and west in 2016), above the curved section at the stem 271 base, at a height of roughly 20-40 cm for pine and 60-100 cm for larch. After 272 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 273 274 chalk powder to the cut surface to improve the visibility of the growth rings, and 275 measured ring width with a stereomicroscope at 40x magnification. Ring width of the 276 two cores per tree were averaged prior to statistical analysis. For each tree surveyed in 277 2016, we additionally measured total tree height as well as tree diameter at two 278 heights (roughly 20 and 80 cm above ground level for larch and 20 and 40 cm for 279 pine), which we averaged for statistical analysis.

281 For the dwarf shrubs, the annual increments of the longest shoot of five randomly 282 chosen branchlets per species were measured in each plot from 2004 to 2010. The 283 annual increment formed in 2007 (V. myrtillus only) and 2009 was back-measured in 284 the subsequent year (2008, 2010). Increments from 2013 to 2016 were measured in 285 2016, but we have no data for 2011 to 2013 because increments older than two years 286 could not be clearly identified. In 2016, we measured the longest shoots of only three 287 randomly chosen ramets (instead of five) per species. Shoot increments were always 288 measured in autumn after annual growth was complete.

289

#### 290 STATISTICAL ANALYSES

291 We assessed the effects of fertilizer addition, other explanatory variables (treatment 292 year; plot elevation; tree species in the plot [in shrub models]) and their potential two-293 way interactions with fertilization on measured soil properties and plant traits. 294 Specifically, we fitted linear mixed-effects models in R version 3.4.1 (R Core Team 295 2017) using the lme function of the nlme-package by Pinheiro et al. (2016). The 296 fertilizer dose (control, NPK15, NPK30) and treatment year (2005–2016) were 297 generally analysed as categorical variables (factors), whereas elevation was analysed 298 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 299 300 create Figures 3 and 4. The models for foliar nutrient concentration and nutrient ratios 301 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> 302 303 <sup>1</sup>). We analysed annual growth traits separately for each species, whereas the other response variables (foliar nutrient concentration and  $\delta^{15}$ N, diameter and height of 304

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.

309

310 We included random intercepts for experimental block in all models (a total of 11 311 blocks per tree species and 22 per shrub species, see Fig. S1). As each of the blocks 312 contained three plots from which multiple samples were taken, we added plot identity 313 as a nested random effect within block. For repeatedly measured traits on the same 314 individual trees (top- and side-shoot increment, ring width), we also included tree 315 identity as a nested random effect within block and plot, thereby allowing the model 316 intercept to vary among trees. Further, we implemented an autocorrelation structure of 317 order 1 (corAR1 in nlme) in models with repeated measures to account for temporal 318 autocorrelation, which improved the AIC (Akaike information criterion) of the 319 models. We expected residual variance to differ between treatments, years and/or 320 species. Therefore, we allowed the residual variance to differ between levels of these 321 factors by using the varIdent function in nlme (Zuur et al. 2009). F-statistics and 322 detailed model specifications are given in the Supporting Information (Tables S3–S5). 323 Model assumptions of residual normality and homoscedasticity were verified visually 324 (q-q plot, Tukey-Anscombe plot) for each model. To improve residual spread in cases 325 of heteroscedasticity, the response variable was log or square root transformed 326 (Supporting Information, Table S1). Model estimates for each year and treatment 327 (averaged over all other factors in the model, as shown in Figures 2 and 3) were 328 obtained with the lsmeans package in R (Lenth 2016). Using the same package, we 329 calculated the average estimates across the twelve years (Table 1), performed t-tests

330 on contrasts between treatments and back-transformed the estimates for presentation.

331 Log-transformed values yield the geometric mean and square-root-transformed values

an intermediate between arithmetic and geometric mean when back-transformed.

333 Numerator degrees of freedom (df) are given for t-tests.

334

335 To test the influence of annual climatic conditions on the growth responses to 336 fertilization (i.e. control vs. fertilized plots), we combined the data from the two 337 fertilizer doses because there was no qualitative difference between the growth 338 responses to NPK15 and NPK30. The factor 'year' was replaced by number of frost 339 events per growing season and mean summer temperature (June, July and August 340 together) in the air (2 m above ground). The statistical significance of the resulting 341 trends was tested with t-tests and the corresponding *P*-values were corrected for 342 multiple testing using the 'Bonferroni-Holm' method. We considered the start and end 343 of the growing season as the dates when the daily mean soil temperature first rose 344 above or fell below 3.2 °C for two consecutive days, respectively (Körner and 345 Paulsen 2004, Dawes et al. 2011). Dates with daily minimum air temperatures 346 below -1 °C were considered as freezing events (Rixen et al. 2012). Freezing events 347 that spanned contiguous dates were counted only once. If not specified, results are 348 presented as treatment means with the corresponding 95% confidence intervals.

349

## 350 **Results**

## 351 TREATMENT EFFECTS ON SOIL PROPERTIES

352 In 2010, end-of-season inorganic N concentrations in the soil (0–10 cm depth)

353 increased by 45 [10, 90] % in NPK15 (df = 37, t = 2.8, P < 0.01) and 79 [32, 142] %

354	In NPK30 plots (Fig. 1; df = 21, $t = 3.9$ , $P < 0.001$ ). The higher inorganic N
355	concentrations were solely caused by an increase in $NH_4^+$ , which comprised ca. 75%
356	of total inorganic N in control plots and 89% in the fertilized plots (Fig. 1). In
357	contrast, the concentration of $NO_3^-$ , which was not part of the fertilizer, remained
358	unaffected in treated plots. $NH_4^+$ , but not $NO_3^-$ , decreased significantly with
359	increasing elevation, by ca. 0.77 [0.07, 1.46] mg N kg <sup>-1</sup> per 10 m of elevation (df =
360	37, $t = 2.4$ , $P = 0.021$ ). Elevation did not affect soil nutrient increases due to
361	fertilization. There was a marginally significant trend of ca. 35% lower $NH_4^+$
362	concentrations in plots with pine compared to in plots with larch (df = 20, $t$ = -1.8, $P$
363	= 0.089), independent of fertilization treatment.





365

366 Figure 1 End of season inorganic N (a) and  $\delta^{15}N$  (b) in the soil of control and fertilized plots,

367 corresponding to 15 (NPK15) or 30 kg N ha<sup>-1</sup> a<sup>-1</sup> (NPK30), in 2010. Means and 95% confidence

368 intervals are shown. a) Bars are subdivided for the fraction of inorganic N derived from ammonium

369 (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* 

370 (*Pu*) differed in  $\delta^{15}$ N and are displayed separately.

372	Bulk soil $\delta^{13}N$ was not significantly affected by fertilization (Fig. 1; fertilizer $\delta^{13}N$
373	was ca. 0.80 ‰). Similarly, $\delta^{15}$ N in soil extractable NH <sub>4</sub> <sup>+</sup> was not significantly
374	affected by fertilization (control: 0.26 ‰, NPK15: 0.48 ‰, NPK30: 0.44 ‰). Soil
375	$\delta^{15}$ N signatures were significantly higher in plots with larch than in plots with pine
376	(Fig. 1; df = 20, $t$ = 2.9, $P$ = 0.010). The treatment did not affect the soil pH of 3.79
377	[3.72, 3.86].

#### 379 FOLIAR NUTRIENT CONCENTRATIONS AND ISOTOPE RATIOS

380 Across treatments, foliar N concentration of larch decreased with elevation in 2010,

381 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

382 highest elevation (Table S2). This decrease led to a parallel decrease of the N:P and

383 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).

387

388 In 2010, no significant response to fertilization was found in foliar N concentration of 389 trees, except for a stimulation in larch when grown in the NPK15 treatment (Fig. 2; df 390 = 38, t = 2.4, P = 0.024). The second sampling in 2016 revealed a stronger and linear 391 response for both species, amounting to a maximum of +10% in the NPK30 treatment 392 (df = 34, t = 3.4, P < 0.01; Fig. S4). Phosphorus and K concentration (both measured 393 in 2010 only) of larch leaves increased considerably under fertilization, leading to 394 lower foliar N:P (df = 40, t = -2.0, P = 0.048) and N:K ratios (df = 40, t = -2.4, P = 395 0.024), while no change in nutrient ratios was found for pine leaves (Fig. 2).

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

Foliar  $\delta^{15}$ N in 2010 was unaffected by elevation, except for a slight increase at higher 411 412 elevations for pine (Table S2), but increased significantly with fertilization for both 413 tree species (Fig. 2, df = 40, t = 5.9, P < 0.001). This increase was much stronger for 414 pine than for larch (df = 40, t = 3.0, P < 0.01), and larch leaves were significantly less depleted in <sup>15</sup>N than pine leaves (df = 20, t = -4.9, P < 0.001). Foliar  $\delta^{15}$ N of dwarf 415 shrubs was also unaffected by elevation and  $\delta^{15}N$  values increased with fertilization 416 417 (Fig. 2, df = 38, t = 6.0, P < 0.001). The response was similar between species for 418 dwarf shrubs and independent of the tree species growing in the plot. The second analysis in 2016 confirmed the  $\delta^{15}$ N signals observed in 2010 (Fig. S4). 419



**422** 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 423 concentrations, mass ratios of N:P and N:K, and  $\delta^{15}$ N values in leaves of *Larix decidua* (*Ld*), *Pinus* 424 *uncinata* (*Pu*), *Vaccinium myrtillus* (*Vm*) and *Vaccinium gaultherioides* (*Vg*) in 2010. Means and 95% 425 confidence intervals are shown. Point estimates and regression lines were modelled separately by 426 treating the fertilizer dose as either a categorical or a numeric variable.

#### 428 GROWTH OF TREE SPECIES

429 Before the first fertilizer application in 2004, there was no difference between plots

430 assigned to the different treatments in any of the tree growth traits (top- and side-

431 shoot increment, ring width; P > 0.2 in each case). In 2016, the average ring width of

432 larch trees growing in control plots was 1.7 [1.4, 2.1] mm at the lowest elevation and

433 decreased to 1.3 [1.0, 1.5] mm at the highest elevation (df = 106, t = -2.2, P = 0.028).

434 Higher elevation was also associated with smaller tree diameter and tree height in 435 larch (Table S3), with a decrease in diameter from 11.0 [8.7, 13.3] cm at the lowest 436 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 437 438 at the highest elevation (df = 97, t = -2.8, P = 0.007). Elevation did not affect 439 individual annual top- and side-shoot increments of either tree species (Table S4), and 440 it had no influence on tree height (166 [90, 266] at lowest vs. 139 [82, 211] at highest 441 elevation) or diameter (4.9 [1.3, 8.4] vs. 6.0 [2.8, 9.1]) of pine.

442

443 In larch, nutrient addition stimulated all three annually measured growth traits (top-444 and side-shoot increment, ring width). The average effect across years varied from 445 13% to 20% in the NPK15 treatment and from 11% to 14% in the NPK30 treatment, 446 depending on trait (Table 1). The differences between the two fertilizer doses were 447 not statistically significant for any of the growth traits for larch (P > 0.14 for each 448 trait). Interannual differences in the response were statistically significant for side-449 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 450 451 tree diameter, but not height, for larch (Table 1).

452

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

458	treatment only). Top-shoot increment of pine responded significantly more to the
459	NPK30 than to the NPK15 treatment (Table 1, df = 20, $t = 2.96$ , $P < 0.01$ ). Side-shoot
460	increments were also somewhat greater in the NPK30 than in the NPK15 treatment,
461	but the difference between the two fertilizer doses was not significant in this case.
462	Almost all pine trees from the lowest three sites, spanning 40 m of the total 140 m of
463	elevation investigated, died between 2005 and 2015. Fertilization had no effect on
464	tree survival in either species (controls were similarly affected).
465	
466	Elevation had no statistically significant influence on the tree growth response to

467 fertilization, except for the side-shoot increment of pine in the NPK15 treatment

468 (Table S4), where fertilizer stimulation decreased with increasing elevation (df = 91, t

469 = -2.4, P = 0.02). However, this finding was not supported by data from the NPK30

470 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)*

483 and *P. uncinata* (*Pu*) and 37 for *V. myrtillus* (*Vm*) and *V. gaultherioides* (*Vg*). Bold numbers highlight

Species	Measure	Control		NPK15				NPK30			
		Mean	95% CI	Mean	95% CI	t	Р	Mean	95% CI	t	Р
Ld	Tree ring (mm)	1.47	1.30, 1.63	1.76	1.60, 1.92	3.3	< 0.01	1.63	1.46, 1.79	1.8	0.09
	Top-shoot (cm)	23.6	21.4, 26.0	26.7	24.3, 29.0	2.5	0.02	26.3	24.0, 28.6	2.3	0.03
	Side-shoot (cm)	12.5	11.4, 13.6	14.1	13.0, 15.2	2.6	0.02	14.2	13.1, 15.3	2.7	0.01
	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	< 0.01	10.3	9.1, 11.5	2.0	0.05
Ри	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	0.07
	Top-shoot (cm)	6.7	5.7, 7.7	7.7	6.6, 8.8	2.2	0.04	9.1	7.9, 10.3	5.1	< 0.01
	Side-shoot (cm)	4.9	4.2, 5.7	5.7	4.9, 6.6	2.8	0.01	6.2	5.4, 7.1	4.3	< 0.01
	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
Vm	Shoot (cm)	3.7	3.5, 3.7	4.0	3.8, 4.2	2.7	0.01	4.0	3.8, 4.2	2.8	<0.01
Vg	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	0.01

484 significant (P < 0.05) and marginally significant (P < 0.1) differences.

486

485

#### 487 GROWTH OF DWARF SHRUB SPECIES

488 As with trees, shoot increments of shrubs did not differ between treatments in 2004

489 before the fertilizer was applied (P > 0.5). In control plots, the mean annual shoot

490 increment was 3.7 cm for *V. myrtillus* and 3.2 cm for *V. gaultherioides*, independent

491 of elevation (Table 1). The response to fertilizer addition varied among years (Fig. 4).

492 On average, both fertilizer doses stimulated the annual shoot increment of *V. myrtillus* 

493 by ca. 8%, while V. gaultherioides responded to the NPK30 treatment only (16%

494 longer shoot increment, Table 1). The response to fertilization did not depend on

495 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.

## 505 CLIMATIC EFFECTS ON THE FERTILIZER-RESPONSE

506 Summer (June, July and August) mean air temperatures were between 8.7 and 12.9  $^{\circ}$ 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
- 510 0–4 frost events per season. Trees and shrubs showed reduced growth in years with
- 511 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).

515

## 516 **Discussion**

517 Fertilizer addition to this high elevation treeline ecosystem for more than a decade

518 stimulated growth in all four investigated tree and dwarf shrub species. Even the

519 lower NPK-fertilizer dose with 15 kg N ha<sup>-1</sup> a<sup>-1</sup> was enough to stimulate growth in the

520 two tree species and in *V. myrtillus*. These findings strongly suggest that small

521 increases in soil nutrient availability may suffice to stimulate growth in this treeline

522 ecotone. In contrast to our hypothesis, the nutrient addition effect in larch did not

523 change with higher elevation in this ecotone, despite an almost halving of the height

and reduced annual radial growth in this species.

525

#### 526 LOW DOSE FERTILIZATION ENHANCES PLANT GROWTH

527 While previous experiments with trees applied 85-175 kg N ha<sup>-1</sup> a<sup>-1</sup>, leading to a

528 doubling or tripling of biomass production (Sveinbjörnsson 1992; Susiluoto et al.

529 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. Higher concentrations of N,

533 P and K in leaves, increased soil extractable N and higher  $\delta^{15}$ N values in foliage

indicate that the mineral fertilizer indeed increased nutrient availability for plants,

even though a recent <sup>15</sup>N tracer study at this site showed that about 60% of N added as

536 NH<sub>4</sub><sup>+</sup> or glycine is rapidly immobilized in the soil (Dawes et al. 2017a), as is typical 537 for high elevation ecosystems on acidic soils (Gerzabek et al. 2004). The increase in 538 plant nutrient concentration by our comparably low fertilizer dose was similar to that 539 in the Scandinavian treeline study with birch using a much higher dose of NPK-540 fertilizer (Sveinbjörnsson 1992), but we do not know whether birch would have 541 responded similarly to such low nutrient additions. We attribute the high 542 responsiveness of the treeline ecosystem in our study to low rates of nutrient addition 543 to a so far unexplored non-linearity of nutrient-growth relationships. Remarkably, little additional growth stimulation was observed with the higher  $(30 \text{ kg ha}^{-1} \text{ a}^{-1})$ 544 545 fertilizer dose compared with the lower dose. Our site has acidic soils covered with 5-546 20 cm thick organic layers and intrinsically low rates of nutrient cycling compared to 547 forest soils in the temperate zone (e.g. Bednorz et al. 2000; Blaser et al. 2005; 548 Hagedorn et al. 2010). Accordingly, the lack of a surplus stimulation by the higher 549 fertilizer dose may indicate a low overall nutrient demand of growth, e.g. due to an 550 inherently low growth rate or because of thermal constraints on meristematic activity 551 (Hoch 2013). The 8–16% increase in dwarf shrub growth is low compared with 552 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). 553

554

## 555 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 dwarfshrubs experienced similar soil temperatures, at least in the rooting zone. Second, the

562 ongoing climatic warming by 2 K (Rixen et al. 2012) during the last four decades has

563 probably already relieved trees from thermal constraints of growth to some extent.

564

565 Although there was a similar fertilization effect on both growth forms, there was a 566 difference in the response between the two dwarf shrubs. While shoot increment 567 length of V. gaultherioides was stimulated by the higher fertilizer dose only, 568 responses of V. myrtillus saturated at the lower fertilizer dose (no difference between 569 the two doses). In a separate experiment within the same afforestation used in our 570 study, Dawes et al. (2011) found that growth of V. myrtillus responded more than that 571 of V. gaultherioides to six years of experimental soil warming by 4 K, a treatment that 572 led to enhanced nitrogen availability (Dawes et al. 2017b). Vaccinium myrtillus has a 573 wider elevational distribution range than V. gaultherioides (Lauber et al. 2012) and 574 might have a competitive advantage under changing growth conditions. However, it 575 was also found to be more prone to freezing damage when growing in warmer soils 576 (Martin et al. 2010), which could ultimately have a larger impact than growth 577 stimulation by warming or increased nutrient availability. It had been shown, although with higher addition rates of N (>100 kg ha<sup>-1</sup>), that fertilization delays bud formation 578 579 in autumn, and thus winter dormancy and freezing tolerance, in V. myrtillus (Körner 580 1984).

581

582 Positive effects of fertilization on the rate of tissue formation are commonly observed,
583 but may be counteracted by increased pathogen impact (Herms & Mattson 1992,

584 Rühmann et al. 2002, Blodgett et al. 2005) or reduced freezing tolerance (Hellergren 585 1981). In addition, faster growth from additional N may result in lower tissue 586 concentrations of lignin (Pitre et al. 2007, Li et al. 2012), which in turn could increase 587 the susceptibility to mechanical damage from snow breakage, wind, rock fall or 588 avalanches and ultimately lead to biomass loss. There is also evidence that fertilizer 589 addition can reduce the success of afforestation with small saplings, largely due to 590 fungal diseases—work that actually had been conducted at our study site (Keller 591 1970). We found that frequent frost events reduce, but not reverse, the growth 592 stimulation in larch. Nevertheless, stimulated growth and unchanged mortality in 593 response to twelve years of moderate nutrient addition in the current experiment 594 suggests that the adult trees examined here were not negatively affected by the 595 fertilizer treatment. Negative consequences of improved nutrition, and thus vigor, 596 could be associated with stochastic phenomena such as heavy snow pack (mechanical 597 damage), certain snow melt regimes (fungal pathogens) or epidemic insect outbreaks 598 (e.g. larch budmoth), and may take more time to materialize.

599

## 600 GROWING SEASON TEMPERATURE DID NOT MODULATE THE

# 601 FERTILIZER RESPONSE

602 Despite the positive association between summer temperature and annual growth, the

603 interannual variation of the fertilizer response was unrelated to interannual

604 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 <sup>15</sup>N studies in forest soils reveal added N to be 608 609 rapidly bound to stable soil organic matter pools (Hagedorn et al., 2005; Schleppi et 610 al. 2017). The lack of interaction between summer temperature and fertilizer is in line 611 with the absence of such an interactive effect across elevation within the ecotone. The 612 overall lack of fertilizer-temperature interaction is in contrast to the mountain birch 613 data that revealed the greatest responsiveness to nutrient addition at the highest 614 (coldest) of three sites spanning roughly 200 m in elevation (Sveinbjörnsson et al. 615 1992). These differences may reflect site-specific effects, differences between evergreen and deciduous species, or the different experimental durations and rates of 616 617 fertilizer addition.

618

619 Within the treeline ecotone covered in this study, growth of pine and larch decreased 620 considerably with increasing elevation during the 30 years before our experiment 621 started (Barbeito et al. 2012). This is typical for high elevation treelines, where the 622 distance between closed montane forest and the krummholz belt is often not more 623 than 50–100 m in elevation (Körner 2012). The elevation-associated decline in larch 624 ring width observed in our study indicates that temperature is still a major growth-625 limiting factor for this species within the afforestation site. Notably, apical and lateral 626 increments in shoots showed no significant elevation-associated patterns, but these 627 traits may not reflect the overall crown increment, which depends on the number of 628 shoots per crown surface area, branching patterns, and annual shoot wasting. Thus, 629 apical and lateral increments of single shoots may overestimate growth of larch at 630 higher elevation, where trees were clearly much smaller. The lack of an elevation-631 associated decline of growth in pine during the study period may relate to the 632 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).

637

638 A recent decrease of dwarf shrub cover at the lowest locations, presumably coupled 639 to an increase in visibly more vigorous (and therefore nutrient-demanding) understory 640 species, could be another explanation (see Fig. S5). The growing season temperature 641 increased by ca. 2 K in the region since the afforestation site was established in 1975 642 (Rixen et al. 2012). This warming (corresponding to 300 to 400 m of elevation) may 643 have masked the smaller spatial and year-to-year variation in temperature. In a study 644 close to our site, similar seedling survival at low and high elevations suggested that 645 the current treeline position lags behind climatic changes (Zurbriggen et al. 2013). 646 This indicates that temperature limitation of growth has been reduced over the past 647 few decades, as was also observed for other treelines in the Swiss Alps (Jochner et al. 648 2018).

649

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 657 critically low temperatures. Our data suggest that even the uppermost trees grew far658 from a thermal limit of growth.

659

## 660 FERTILIZATION ALTERS NUTRIENT BALANCES

661 While primary production of most terrestrial ecosystems on a global scale is 662 considered to be N limited (Vitousek & Howarth, 1991), we found lower foliar N:P 663 ratios with fertilization in three out of four species, suggesting that growth is limited 664 more by P than N (Güsewell 2004). The only previous study in which trees at treeline 665 received N and P additions separately was that with mountain birch, where no 666 indication of P limitation was observed while N addition clearly stimulated growth 667 (Sveinbjörnsson 1992). Perhaps continuous atmospheric N deposition during the last 668 century has decreased the capacity for further growth stimulation by N addition at our 669 site. Alternatively, lower N:P ratios may be related to a greater retention of N than of 670 P in the soil due to microbial immobilization, a lack of P sorption in the thick organic 671 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 672 673 plants were about twice as high. Altered mechanisms of nutrient uptake are supported by higher  $\delta^{15}$ N values in all plant species already at low levels of fertilization 674 (corresponding to 15 kg N ha<sup>-1</sup> a<sup>-1</sup>). This finding indicates either that the plants took 675 up more N from the supplied NH4<sup>+</sup> (Garten 1993, Craine et al. 2009, Miller & 676 677 Bowman 2002) or that there was a decrease in the proportion of organic N acquired 678 by plants through mycorrhizal association (Emmerton et al. 2001, Hobbie & Colpaert 679 2002). Mycorrhizal activity has frequently been observed to decline with N 680 fertilization (Wallander & Nylund 1992; Nilsson & Wallander 2003; Högberg et al.

- 681 2010). Further, in an experiment near our study site where soil warming led to greater
- 682 N availability, Solly et al. (2017b) observed a shift in the fungal community
- 683 composition towards more nitrophilic species.
- 684

685 [Conclusions]

686 Our findings demonstrate that even a relatively low dose of fertilizer can enhance 687 growth of woody species near the treeline, confirming the sensitivity of low 688 temperature ecosystems to changes in nutrient availability in the soil. Hence, despite a 689 limitation of plant growth by low temperatures at high elevation, even a small 690 improvement of nutrient supply, for instance by increased atmospheric deposition or 691 enhanced nutrient mineralization in warmer soils, can cause changes in high elevation 692 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 693 694 limitation of growth (Jochner et al. 2018) and may have masked interactions between 695 fertilizer addition and more subtle year-to-year or spatial temperature differences. 696 Thus, while treeline trees and associated dwarf shrubs benefit from nutrient addition 697 in terms of growth, our study does not allow to answer whether nutrient availability 698 influences the position of treelines. A central remaining question is whether greater 699 tree vigor and/or higher tissue nutrient concentrations exert negative long-term fitness 700 effects in such marginal habitats. Implications of faster growth for the long-term 701 fitness of treeline species await additional research focusing on reproduction, life-702 span (mortality) and stress tolerance. Nevertheless, faster growth rates with increased 703 nutrient availability may speed up the anticipated upward shift of treeline ecotones as 704 a consequence of climate warming.

706	Acknowledgements
707	The authors thank S. Stolz, A. Nussbaumer, K. Birmann, S. Mannes, M. Pattaroni, N.
708	Hardegger and T. Schmid for assistance with field work. We also thank two
709	anonymous reviewers for their critical comments on the manuscript.
710	
711	Authors' contributions
712	CR designed the study in consultation with PB, CK and SW. PM, MM, DV, and FH
713	collected data, and PM, MM and MD completed the data analysis. PM led the writing
714	with revisions and contributions from all authors. All authors approved the final
715	version.
716	
717	Data accessibility
718	The data used in this study will be available at figshare.com.
719	References
720	Alvarez-Uria, P. & Körner, C. (2007) Low temperature limits of root growth in deciduous and
721	evergreen temperate tree species. Functional Ecology, 21, 211–218.
722	Bai, E., Li, S., Xu, W., Li, W., Dai, W. & Jiang, P. (2013) A meta-analysis of experimental warming
723	effects on terrestrial nitrogen pools and dynamics. New Phytologist, <b>199</b> , 431–440.
724	Barbeito, I., Dawes, M.A., Rixen, C., Senn, J. & Bebi, P. (2012) Factors driving mortality and growth
725	at treeline: A 30-year experiment of 92 000 conifers. <i>Ecology</i> , <b>93</b> , 389–401.

- 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.
- 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.
- Bednorz, F., Reichstein, M., Broll, G., Holtmeier, F.-K. & Urfer, W. (2000) Humus forms in the forestAlpine 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.
- 739 Blaser P., Zimmermann S., Luster J., Walther L., Lüscher P. (2005) Waldböden der Schweiz. Band 2
  740 Regionen Alpen und Alpensüdseite. Birmensdorf, Eidgenössische Forschungsanstalt WSL. Hep
  741 Verlag, Bern
- 742 Bliss, L.C. (1971) Arctic and Alpine Plant Life Cycles. *Annual Review of Ecology and Systematics*, 2,
  743 405–438.
- 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.
- 746 Bobbink, R. & Hettelingh, J.P. (2010) Review and revision of empirical critical loads-response
  747 relationships. *Proceedings of an expert workshop, Noordwijkerhout, 23-25 June 2010.*
- 748 Brooks, P.D., Williams, M.W. & Schmidt, S.K. (1996) Microbial activity under alpine snowpacks,
  749 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.
- 752 Craine, J.M., Elmore, A.J., Aidar, M.P.M., Bustamante, M., Dawson, T.E., Hobbie, E. A., Kahmen, A.,
- 753 Mack, M.C., McLauchlan, K.K., Michelsen, A., Nardoto, G.B., Pardo, L.H., Peñuelas, J., Reich,
- 754 P.B., Schuur, E.A.G., Stock, W.D., Templer, P.H., Virginia, R.A., Welker, J.M. & Wright, I.J.
- 755 (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.
- 758 Dawes, M.A., Hagedorn, F., Zumbrunn, T., Handa, I.T., Hättenschwiler, S., Wipf, S. & Rixen, C.
- 759 (2011) Growth and community responses of alpine dwarf shrubs to in situ CO<sub>2</sub> enrichment and
  760 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.
- Eurola, S. & Hakala, A. (1977) The bird cliff vegetation of Svalbard. *Aquilo Ser Bot*, 1–18.
- 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.
- 773 *Oecologia*, **181**, 25–37.

774	Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A.,
775	Seitzinger, S.P. & Sutton, M.A. (2008) Transformation of the Nitrogen Cycle: Recent Trends,
776	Questions, and Potential Solutions. Science, 320, 889-892.
777	Garten, C.T. (1993) Variation in Foliar <sup>15</sup> N Abundance and the Availability of Soil Nitrogen on Walker
778	Branch Watershed. Ecology, 74, 2098–2113.
779	Gerzabek, M.H., Haberhauer, G., Stemmer, M., Klepsch, S. & Haunold, E. (2004) Long-term
780	behaviour of <sup>15</sup> N in an alpine grassland ecosystem. <i>Biogeochemistry</i> , <b>70</b> , 59–69.
781	Güsewell, S. (2004) N : P ratios in terrestrial plants: variation and functional significance. New
782	Phytologist, <b>164</b> , 243–266.
783	Hagedorn, F., Maurer, S., Bucher, J.B. & Siegwolf, R.T.W. (2005) Immobilization, stabilization and
784	remobilization of nitrogen in forest soils at elevated CO2: A <sup>15</sup> N and <sup>13</sup> C tracer study. Global
785	<i>Change Biology</i> , <b>11</b> , 1816–1827.
786	Hagedorn, F., Martin, M., Rixen, C., Rusch, S., Bebi, P., Zürcher, A., Siegwolf, R.T.W., Wipf, S.,
787	Escape, C., Roy, J. & Hättenschwiler, S. (2010) Short-term responses of ecosystem carbon fluxes
788	to experimental soil warming at the Swiss alpine treeline. <i>Biogeochemistry</i> , 97, 7–19.
789	Handa, I.T., Hagedorn, F. & Hättenschwiler, S. (2008) No stimulation in root production in response to
790	4 years of in situ CO <sub>2</sub> enrichment at the Swiss treeline. <i>Functional Ecology</i> , <b>22</b> , 348–358.
791	Heer, C. & Körner, C. (2002) High elevation pioneer plants are sensitive to mineral nutrient addition.

- 792 Basic and Applied Ecology, 3, 39–47.
- 793 Hellergren, J. (1981) Frost hardiness development in *Pinus silvestris* seedlings in response to
- fertilization. *Physiologia Plantarum*, **52**, 297–301.
- Herms, 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.
- 803 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.
- 807 Iivonen, S., Rikala, R., Ryyppo, A. & Vapaavuori, E. (1999) Responses of Scots pine (Pinus sylvestris)
  808 seedlings grown in different nutrient regimes to changing root zone temperature in spring. *Tree*809 *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.
- 826 Körner, C. (2003) Alpine Plant Life. Springer, Heidelberg.
- 827 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.
- 830 Kuoch, R. (1970) Die Vegetation auf Stillberg (Dischmatal, Kt. Graubünden). *Mitteilungen der*
- 831 *Eidgenössischen Anstalt für das forstliche Versuchswesen*, **46**, 329–342.
- 832 Lauber, K., Wagner, G. & Gygax, A. (2012) Flora Helvetica. Haupt Verlag.
- Lenth, R. V. (2016) Least-Squares Means: The R Package Ismeans. *Journal of Statistical Software*, 69, 1–33.
- 835 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
- 837 physiology, and wood properties of slow- and fast-growing *Populus* species. *Journal of*
- 838 *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.

844	Mayor, J.R., Sanders, N.J., Classen, A.T., Bardgett, R.D., Clément, J.C., Fajardo, A., Lavorel, S.,
845	Sundqvist, M.K., Bahn, M., Chisholm, C., Cieraad, E., Gedalof, Z., Grigulis, K., Kudo, G.,
846	Oberski, D.L. & Wardle, D.A. (2017) Elevation alters ecosystem properties across temperate
847	treelines globally. Nature, 542, 91–95.
848	Miller, A.E. & Bowman, W.D. (2002) Variation in nitrogen-15 natural abundance and nitrogen uptake
849	traits among co-occurring alpine species: do species partition by nitrogen form? Oecologia, 130
850	609–616.
851	Navone, R. (1964) Proposed Method for Nitrate in Potable Waters. American Water Works
852	Association, <b>56</b> , 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.
- 865 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.

- 868 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.
- 875 Saxe, H., Cannell, M.G.R., Johnsen, Ø., Ryan, M.G. & Vourlitis, G. (2002) Tree and forest functioning
  876 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.
- 879 Scherrer, D. & Körner, C. (2009) Infra-red thermometry of alpine landscapes challenges climatic
  880 warming projections. *Global Change Biology*, 16, 2602–2613.
- 881 Schleppi, P., Bucher-Wallin, I., Saurer, M., Jäggi, M. & Landolt, W. (2006) Citric acid traps to replace
- 882 sulphuric acid in the ammonia diffusion of dilute water samples for <sup>15</sup>N analysis. *Rapid*
- 883 *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.
- 887 Schönenberger, W. & Frey, W. (1988) Untersuchungen zur Ökologie und Technik der
- Hochlagenaufforstung. Forschungsergebnisse aus dem Lawinenanrissgebiet Stillberg. *Schweiz. Z. Forstwes.*, 139, 735–820.
- 890 Senn, J. & Schönenberger, W. (2001) Zwanzig Jahre Versuchsaufforstung Stillberg: Überleben und
  891 Wachstum einer subalpinen Aufforstung in Abhängigkeit vom Standort. Schweiz. Z. Forstwes.,
- **152**, 226–246.

893	Solly, E.F., Djukic, I., Moiseev, P.A., Andreyashkina, N.I., Devi, N.M., Göransson, H., Mazepa, V.S.,
894	Shiyatov, S.G., Trubina, M.R., Schweingruber, F.H., Wilmking, M. & Hagedorn, F. (2017a)
895	Treeline advances and associated shifts in the ground vegetation alter fine root dynamics and
896	mycelia production in the South and Polar Urals. <i>Oecologia</i> , <b>183</b> , 571–586.
897	Solly, E.F., Lindahl, B.D., Dawes, M.A., Peter, M., Souza, R.C., Rixen, C. & Hagedorn, F. (2017b)
898	Experimental soil warming shifts the fungal community composition at the alpine treeline. New
899	Phytologist, <b>215</b> , 766–778.
900	Sullivan, P.F., Ellison, S.B.Z., McNown, R.W., Brownlee, A.H. & Sveinbjörnsson, B. (2015) Evidence
901	of soil nutrient availability as the proximate constraint on growth of treeline trees in northwest
902	Alaska. <i>Ecology</i> , <b>96</b> , 716–727.
903	Susiluoto, S., Hilasvuori, E. & Berninger, F. (2010) Testing the growth limitation hypothesis for
904	subarctic Scots pine. Journal of Ecology, 98, 1186–1195.
905	Sveinbjornsson, B., Nordell, O. & Kauhanen, H. (1992) Nutrient Relations of Mountain Birch Growth
906	at and Below the Elevational Tree-Line in Swedish Lapland. Functional Ecology, 6, 213.
907	Thébault, A., Clément, JC., Ibanez, S., Roy, J., Geremia, R.A., Pérez, C.A., Buttler, A., Estienne, Y.
908	& Lavorel, S. (2014) Nitrogen limitation and microbial diversity at the treeline. Oikos, 123, 729–
909	740.
910	Thomas, S.C., Halpern, C.B., Falk, D. a, Liguori, D. a & Austin, K.A. (1999) Plant diversity in
911	managed forests: Understory responses to thinning and fertilization. Ecological Applications, 9,
912	864–879.
913	Tranquillini, W. (1979) Physiological Ecology of the Alpine Timberline. Tree Existence at High
914	Altitudes with Special Reference to the European Alps. Springer-Verlag Berlin Heidelberg.
915	Van Wijk, M.T., Clemmensen, K.E., Shaver, G.R., Williams, M., Callaghan, T. V., Chapin, F.S.,
916	Cornelissen, J.H.C., Gough, L., Hobbie, S.E., Jonasson, S., Lee, J.A., Michelsen, A., Press, M.C.,
917	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.
- 920 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.
- 923 Vitousek, P. & Howarth, R. (1991) Nitrogen limitation on land and in the sea: How can it occur?
  924 *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.
- 28 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.