

Resource availability drives plant–plant interactions of conifer seedlings across elevations under warming in Alaska

KYOKO OKANO,^{1,2,3,†} M. SYNDONIA BRET-HARTE,^{1,2} CHRISTA P. H. MULDER,^{1,2} AND GLENN P. JUDAY⁴

¹*Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, Alaska 99775 USA*

²*Department of Biology and Wildlife, University of Alaska Fairbanks, Fairbanks, Alaska 99775 USA*

³*Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011 USA*

⁴*School of Natural Resources and Extension, University of Alaska Fairbanks, Fairbanks, Alaska 99775 USA*

Citation: Okano, K., M. S. Bret-Harte, C. P. H. Mulder, and G. P. Juday. 2021. Resource availability drives plant–plant interactions of conifer seedlings across elevations under warming in Alaska. *Ecosphere* 12(5):e03508. 10.1002/ecs2.3508

Abstract. Future warming may alter plant stress at high-elevation treelines and forests, thereby changing plant–plant interactions. The relative importance of competition and facilitation may depend on the degree of resource or physical stress. According to the stress gradient hypothesis (SGH), physical stress on trees is more important at cold high elevations where facilitation predominates, and less important at low elevations where competition is the main interaction. Our goals were to investigate whether plant–plant interactions along elevational gradients corresponded to those predicted by the SGH, and to assess the effects of increasing temperatures on the growth of conifer seedlings in tundra–forest ecosystems in interior Alaska, USA. We established sites along two elevational gradients: one in tundra (four sites, 550–1170 m) and one in forest (three sites, 210–760 m). A field warming and neighbor removal experiment was conducted using transplanted seedlings of white spruce (*Picea glauca*). After three growing seasons, regardless of elevation, spruce seedling biomass was approximately 20% lower in seedlings with neighbor plants compared to those without neighbors. Therefore, there was no evidence for greater facilitation at high elevations across either tundra or forest elevational gradient. Seedlings in forest sites increased their shoot-to-root ratios and relative growth rates in height, suggesting competition for light, but this was not seen for seedlings growing in tundra sites. When warmed by greenhouses, seedling growth was stimulated in forests, especially at high elevations. In contrast, at tundra sites, warmed seedlings grew less than controls and the seedlings at high elevations showed water stress. Our results suggest that the resource stresses of low light and water availability are more important drivers of plant–plant interactions than the physical stress imposed by low temperature, possibly due to warming in the past 50 yr in this region. Further warming may increase growth of seedlings in forests, but is likely to decrease it in open tundra habitats, thereby slowing the upward movement of treeline in the near future.

Key words: alpine treeline; boreal forests; climate change; interior Alaska; plant–plant interaction; seedlings; stress gradient hypothesis; white spruce.

Received 3 October 2020; **accepted** 19 October 2020; **final version received** 8 March 2021. **Corresponding Editor:** Debra P. C. Peters.

Copyright: © 2021 The Authors. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

† **E-mail:** ko468@nau.edu

INTRODUCTION

The importance of different plant–plant interactions in structuring natural plant communities is a matter of continuing debate. In montane environments, physical stress typically increases

with increasing elevation, altering plant–plant interactions. This concept, known as the stress gradient hypothesis (SGH), suggests that facilitation between plants occurs because of increasing physical stress at high elevations, while

competition is important where physical stress is lower at low elevations (Bertness and Callaway 1994). Cold temperatures are thought to be an important source of physical stress (Körner et al. 2003). The SGH was refined by Maestre et al. (2009) to distinguish between physical and resource stress, such as that caused by low levels of soil nutrients, water availability, or light, because resource stress does not always vary as physical stress changes; thus, resources may be limiting even under conditions of high abiotic stress. Experimental studies have generally found support for the SGH (e.g., Choler et al. 2001, Callaway et al. 2002, Badano et al. 2007, Callaway 2007, Grau et al. 2013, Michalet et al. 2014a). At high elevations, treeline ecosystems occupy a transitional area between tundra and continuous forests that includes stunted and isolated trees (Batllori et al. 2009, Stueve et al. 2011). Sheltering effects of neighboring plants can reduce seedling mortality caused by physical stressors, such as strong wind, low temperature, abrasion by snow crystals, and exposure to solar radiation (Körner 2003, Batllori et al. 2009). At low elevations, in contrast, where plants experience less physical stress, limitation of resources can promote competition among plants (Callaway et al. 2002, Okano and Bret-Harte 2015, Ettinger and HilleRisLambers 2017).

However, both the type and magnitude of plant stress along elevational gradients may be altered by global warming (IPCC 2014). As climate warms, physical stress at high elevations caused by cold temperatures may be ameliorated. If frost damage is reduced at high elevations, competition for resources may become more important than facilitation. Competition might even become the dominant plant–plant interaction in alpine environments (Klanderud and Totland 2007). If so, plants may become limited by competition for light, water, or nutrients triggered by warming, thereby limiting or even reducing their growth. Warming likely leads to drier conditions, and competition for water may occur, as intraspecific competition of white spruce for water is reported in high basal area stands (Nicklen et al. 2019). Low nutrient availability in cold soil causes low rates of photosynthesis and growth (Sullivan et al. 2015), so increasing soil nitrogen (N) availability by warming may change plant competitive interactions

for nutrients. If stressors switch from physical to resource factors due to warming, plant–plant interactions may change in an unpredictable way (Maestre et al. 2009).

How climate change affects plant–plant interactions may also depend on the traits of the interacting species. Commonly, deciduous species acquire resources rapidly to achieve maximum growth, but have low stress tolerance, while evergreen species conserve resources and grow slowly, but tolerate stressful environments, illustrating contrasting evolutionary strategies of a trade-off between maximum growth and stress tolerance (Grime 1977, Chapin 1980, Díaz et al. 2004). Climate warming may favor resource-acquiring species: Growing season warming has been shown to stimulate mainly deciduous shrubs or dominant species in numerous experiments (e.g., Chapin et al. 1995, Bret-Harte et al. 2001, Graglia et al. 2001, Sistla et al. 2013, Zamin et al. 2014). Species in other growth forms, such as evergreen and non-vascular plants (which are categorized as resource-conserving species), either did not respond or showed negative responses to warming (e.g., Chapin et al. 1995, Kudo and Suzuki 2003, Rixen and Mulder 2009).

The effects of warming may be especially apparent in treeline ecosystems because plant growth at treeline is limited by temperature (Körner 2003). The response of treeline ecosystems to warming may be the first noticeable change on a mountain; therefore, it may be representative of tree seedling establishment in low-elevation forest ecosystems that we may see in a warmer future (Greenwood and Jump 2014). Studying the growth of treeline seedlings is important, because seedlings are the most vulnerable life stage of trees, and montane ecosystems in the future depend on establishment of seedlings (Woods et al. 2019).

Growth response of tree seedlings may differ by habitat type. Small tree seedlings may be shaded by fast-growing deciduous shrubs and graminoids. Such light limitation was found in Scandinavian and Pyrenean forests where seedlings of pines decreased in growth due to resource stress, particularly light competition (Grau et al. 2013). On the other hand, in tundra of montane Alaska, the treeline conifer white spruce (*Picea glauca* (Moench) Voss) has moved upslope into alpine tundra at high elevations and

invaded shrub tundra near forest at low elevations in response to recent warming (Hamm 2007, Stueve et al. 2011, Roland and Stehn 2014). Different habitat types exhibit different biotic and abiotic environments and affect what the limiting factor is; therefore, different plant performances may be seen.

Treeline–forest ecosystems in Alaska provide a unique opportunity to test the impacts of climate warming for two reasons. First, the magnitude of warming has been greater at high latitudes than in any other places in the world (McBean et al. 2005) and rapid warming is expected to continue in Alaska (Karl et al. 2009). Second, interior Alaska (the region between the Alaska Range and the Brooks Range) is underlain by discontinuous permafrost, resulting in tundra, which otherwise only occurs above altitudinal treelines, occurring at lower elevations in patches within forests (e.g., Roland and Stehn 2014). Thus, we can distinguish habitat effects from elevation when assessing plant–plant interactions, because both habitats occur over multiple elevations.

In order to assess changes in plant–plant interactions under climate change in tundra and forest habitats along elevational gradients, we established a species removal and passive warming experiment using transplanted seedlings of white spruce in interior Alaska, USA. This experiment followed up on our previous study (Okano and Bret-Harte 2015), which we established two tundra sites, one above treeline and one near treeline, and one forest site 50 m lower than the near treeline site. We saw a strong positive effect of warming at the above treeline site and a weak one at the near treeline site, but a negative warming effect at the forest site. Since these different effects of warming could be due to either habitat or elevation, we wished to separate these two effects. We also found that competition between spruce seedlings and neighbor plants was increased at all sites when the sites were warmed, and we suggested that belowground competition might have caused this effect. However, since we measured aboveground growth and photosynthetic rates, but not belowground growth, we could not test this idea. Therefore, in this experiment, we added two tundra and two forest sites, planted a new set of spruce seedlings, and grew the seedlings *in situ* for three growing seasons. We established two elevational

gradients, one with four tundra sites and one with three forest sites, both of which included species removal and warming treatments. At the end of the experiment, we harvested the seedlings and measured their biomass.

The objectives of this study, based on our previous experiment and literature review, were to find whether the patterns of plant–plant interactions predicted by the SGH would occur along our elevational gradients, to identify the factors limiting growth of seedlings in each habitat, and to understand how plant–plant interactions and seedling growth would be affected by climate warming. Our hypotheses were as follows: (1) Competition with neighbor plants reduces growth of spruce seedlings at all sites, but the magnitude of growth reduction will be smaller at higher elevations where physical stress is high due to colder temperatures, than at lower elevations where physical stress is low due to warmer temperatures, as predicted by the SGH. We expected to see this pattern in both habitat types. (2) Growth of seedlings in forest sites will show a different relationship with elevation than growth of seedlings in tundra sites. This will occur because seedlings in tundra sites mainly respond to physical stress imposed by temperature, while seedlings in shaded forest sites respond to physical stress due to temperature and resource stress imposed by low light availability. (3) When warmed with a greenhouse, the physical stress caused by cold summer temperatures at high elevations will be ameliorated, which will increase seedling growth, but temperatures in greenhouses at low elevations will exceed those that are optimal for photosynthesis, which will decrease seedling growth. Warming will also increase resource competition with neighboring plants. This experiment will provide insights into the conditions under which resource and physical stress affect plant–plant interactions, and how seedling performance of this important treeline/boreal forest species is likely to alter under changing climate.

METHODS

Description of the study sites

Field research was conducted at six sites located in private inholdings across a range of elevations in Denali National Park and Preserve

(DNPP), and one site at the University of Alaska Fairbanks (UAF), Fairbanks, AK, between 2012 and 2014 (Fig. 1, Table 1). Although DNPP and Fairbanks are about 190 km apart, both are north of the Alaska Range and have a typical interior Alaskan climate and vegetation. There were no forests at elevations as low as the Fairbanks site near the DNPP sites. Because the mean summer temperature at the Fairbanks site concurred with the relationship between elevation and

temperature at the DNPP sites, we assumed that this was what the forest would have looked like if there were a low enough elevation site in DNPP. Thus, we regarded the Fairbanks site as being part of the same elevational gradient as the forest sites in DNPP. The closest weather station to the Denali sites (approximately 3 km south) is at Wonder Lake, and the one closest to the UAF site (5 km away) is at the Fairbanks International Airport (FAI). In summer 2014, mean, maximum,

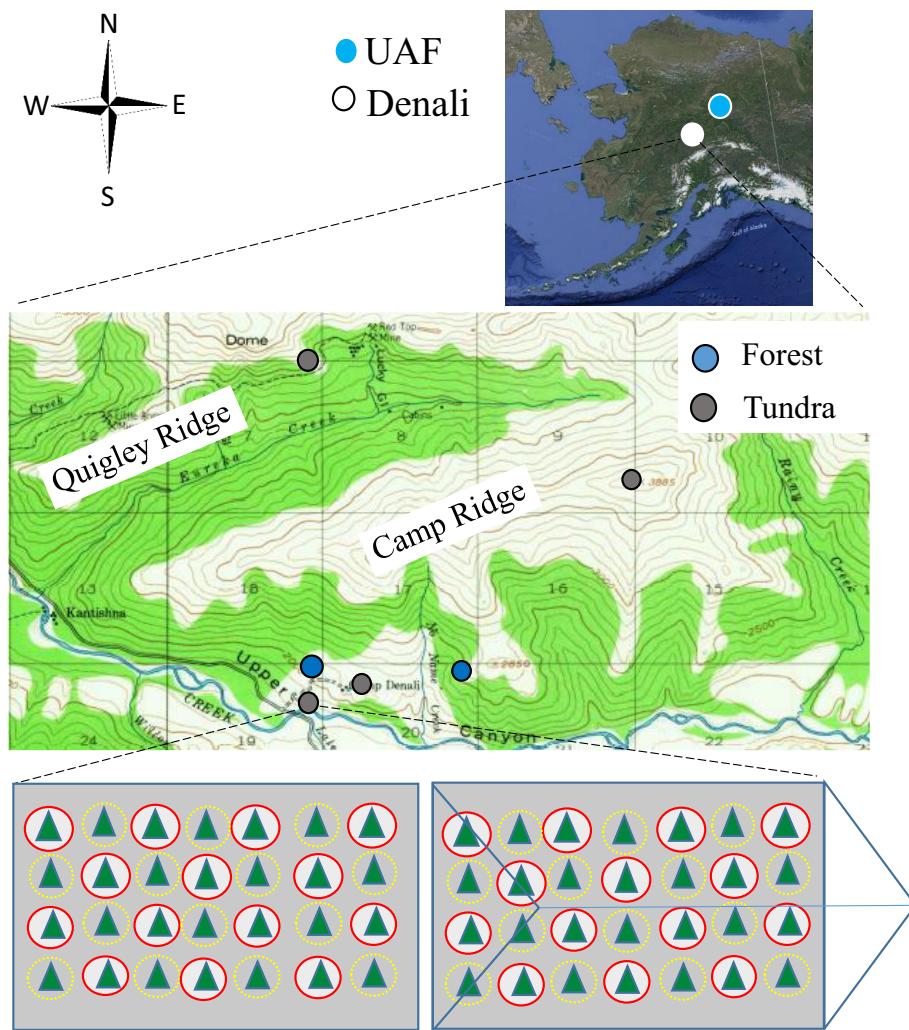


Fig. 1. Study sites and experimental design. One UAF site (210 m) and six Denali sites (550–1170 m) were established either in tundra or in forest habitat in interior Alaska in 2012. Each site had two temperature plots (140 × 120 cm): ambient temperature (left) and elevated temperature by greenhouse (right). Within each plot, each seedling was planted at a center of a 15-cm circle where neighboring plants were present (dotted circle) or removed (solid circle). In order to avoid the low ceilings at the long side edge, seedlings were planted at a buffer of 22.5 cm from the long edge of the greenhouse and a buffer of 2.5 cm from the short edge.

Table 1. Environmental data related to vegetation conditions at seven sites.

Characteristic	UAF:210	LowT:550	BelowF:620	NearT:670	HighF:760	HighT:940	AboveT:1170
Latitude (N)	64°51'31"	63°30'58"	63°31'10"	63°31'10"	63°31'20"	63°32'56"	63°32'18"
Longitude (W)	147°51'37"	150°54'10"	150°54'23"	150°53'54"	150°52'45"	150°54'34"	150°50'25"
Elevation (m)	210	550	620	670	760	940	1170
Habitat	Forest	Tundra	Forest	Tundra	Forest	Tundra	Tundra
Description	Fairbanks mature forest	Lowest tundra	Below treeline mature forest	Near treeline moist tundra	High-elevation young forest	High-elevation treeline tundra	Above treeline alpine tundra
Neighboring shrub height (cm) <i>n</i> = 10	27.9	41.7	44.1	39.9	51.1	35.9	12.6
Plot SE	(2.2)	(4.4)	(6.0)	(2.7)	(4.3)	(3.0)	(1.8)
Mean moss layer (cm)	8.2	15.1	18.5	16.9	13.3	11.8	2.2
Site SE	(0.9)	(1.0)	(1.5)	(1.3)	(0.8)	(2.1)	(0.3)
June 2012 removed biomass per seedling (g/m ²)	524.5	994.6	1368.5	1241.3	1509.0	1022.2	642.2
SE	(144.3)	(380.8)	(430.1)	(209.8)	(1005.8)	(276.0)	(166.4)
Light intensity (μ mol·m ⁻² ·s ⁻¹)	61.2	453.5	490.6	716.2	480.7	587.2	610.3
SE	(17.4)	(80.6)	(89.1)	(109.6)	(87.7)	(65.4)	(86.6)

Notes: Shrub height data were obtained in 2015. Site abbreviations are as follows: UAF:210, Fairbanks forest; LowT:550, lowest tundra; BelowF:620, below treeline forest; NearT:670, near treeline tundra; HighF:760, high-elevation forest; HighT:940, high-elevation tundra; and AboveT:1170, above treeline tundra. Shrub height data were obtained in 2015.

and minimum air temperatures were lower at Wonder Lake than at FAI, by 4.5°, 4.5°, and 2.2°C, respectively. Total summer precipitation at Wonder Lake was 25% more than at FAI (Appendix S1: Table S1; site-specific temperature data are in Table 2).

Of the six Denali sites, five were on Camp Ridge (Fig. 1). One tundra site (940 m) was on Quigley Ridge (Fig. 1), about 2 km north of Camp Ridge, because no manipulative experiments were permitted on Camp Ridge between 760 and 1170 m in land owned by DNPP. The 940-m site on Quigley Ridge was the best available alternative to Camp Ridge for that elevation. The sites consisted of four tundra and three forest sites, and are abbreviated in this paper from high to low elevations as follows: AboveT:1170 (above treeline alpine tundra, 1170 m), HighT:940 (high-elevation tundra, 940 m), HighF:760 (high-elevation forest, 760 m), NearT:670 (near treeline tundra, 670 m), BelowF:620 (below treeline forest, 620 m), LowT:550 (lowest tundra in Denali, 550 m), and UAF:210 (mature white spruce forest in Fairbanks, 210 m). Treelines in DNPP occur between 850 m on the north-facing slopes and 1100 m on the south-facing slopes (National Park Service;

<https://www.nps.gov/dena>). Site data are summarized in Fig. 2 (vegetation percent cover), Table 1 (vegetation condition), and Appendix S1: Table S2 (plant species list). Nomenclature follows Flora of North America (<http://www.efloras.org>).

The four tundra sites ranged in elevation from 550 to 1170 m. The deciduous shrub *Vaccinium uliginosum* occurred at all tundra sites, and some evergreen shrubs (e.g., *Vaccinium vitis-idaea*) occurred at three of them, but other species varied across sites. Shrub height (12–42 cm) and moss layer depth (0.5–22 cm) were also different across sites. AboveT:1170 was the highest, most exposed site with the lowest proportion of shrub cover. HighT:940 was at the upper limit of treeline with a few isolated mature trees nearby. NearT:670 was a moist tundra underlain by permafrost at which active layer depth did not differ from the forest 50 m lower in elevation in August 2010 (Okano and Bret-Harte 2015). LowT:550 was the lowest tundra site within the forest zone.

The two forest sites in DNPP are located at 620 m in a mature spruce forest and at 760 m in a secondary growth forest. Both forest sites had more shrub species in common than the tundra sites did (e.g., deciduous shrubs: *Betula*

Table 2. Air temperature (temp), relative humidity, and soil temperature by site, mean (and SE) of summer 2014.

Metric	UAF:210	LowT:550	BelowF:620	NearT:670	HighF:760	HighT:940	AboveT:1170
Elevation (m)	210	550	620	670	760	940	1170
Habitat	Forest	Tundra	Forest	Tundra	Forest	Tundra	Tundra
Mean air temp	13.71	11.76	10.8	11.2	10.29	10.51	9.65
SE	(0.16)	(0.29)	(0.24)	(0.27)	(0.26)	(0.28)	(0.23)
Raised by GH	1.74	1.97	1.62	2.33	1.68	1.66	1.98
Mean daily maximum	19.82	23.26	20.32	21.57	21.25	22.10	17.75
SE	(0.58)	(0.97)	(0.70)	(0.80)	(0.83)	(1.04)	(0.87)
Changed by GH	2.49	5.13	2.73	2.99	1.93	-1.29	3.36
Mean daily minimum	8.74	2.80	3.46	3.26	2.85	3.36	4.04
SE	(0.32)	(0.55)	(0.46)	(0.41)	(0.41)	(0.36)	(0.32)
Changed by GH	1.29	1.73	1.06	2.19	1.86	2.98	0.73
Relative humidity (%)	86.87	74.9	80.87	80.2	86.01	81.1	79.88
SE	(0.58)	(0.67)	(0.56)	(0.66)	(0.55)	(0.69)	(0.74)
Changed by GH	-6.47	0.23	0.81	-4.76	-5.56	-4.9	-3.82
July 2014 monthly mean temp	13.83	12.4	11.48	11.98	10.9	11.28	10.4
SE	(0.25)	(0.44)	(0.36)	(0.40)	(0.38)	(0.41)	(0.33)
Raised by GH	1.76	1.93	1.59	2.33	1.69	1.6	
GH SE	(0.26)	(0.47)	(0.38)	(0.41)	(0.37)	(0.34)	(0.38)
Mean soil temp	10.91	7.59	6.86	5.31	6.06	7.10	7.94
SE	(0.07)	(0.10)	(0.08)	(0.08)	(0.10)	(0.10)	(0.10)
Raised by GH	0.20	0.19	0.91	2.26	0.49	1.52	0.23
GH SE	(0.09)	(0.10)	(0.09)	(0.11)	(0.08)	(0.08)	(0.09)

Notes: Temperatures in Celsius (°C). Summer temperature was measured between 8 June and 11 August 2014. Site abbreviations as in legend to Table 1. GH = greenhouse treatment (elevated temperature).

glandulosa and *V. uliginosum*, and evergreen shrubs: *V. vitis-idaea* and *Empetrum nigrum*), but BelowF:620 was evergreen-dominated and HighF:760 was deciduous-dominated. Both forests had a relatively open tree canopy, but tall shrubs (e.g., *Salix* spp.) up to 250 cm tall shaded the understory shrubs (~45–50 cm tall). The UAF forest canopy was more closed, with shorter understory plants (<30 cm), and a few deciduous and evergreen shrubs (e.g., *Cornus canadensis* and *V. vitis-idaea*) were shared with the DNPP forest sites. The moss layer in the UAF forest was <11 cm deep, while the Denali forests had moss layers that were up to 35 cm deep.

Seedling preparation

The treeline species white spruce is intermediate shade-tolerant and dominates on warm, south-facing uplands and on floodplains across a large portion of the boreal regions in North America (Burns and Honkala 1990). Spruce seedlings were germinated and grown in the UAF greenhouse. About two-third of the spruce seeds were from cones collected by the National Park Service in 2008 (from trees at 640 and 675 m in

DNPP; Roland et al. 2014) and stored at -18°C at UAF. The remaining seeds were collected at Camp Denali (from trees at 580 m) in September 2011. Seeds were cold-stratified at 4°C for four months starting in September 2011, germinated under 21°C and a 14-h photoperiod, planted in a peat-based medium (Pro-Mix BX; Premier Tech Horticulture, Quakertown, Pennsylvania, USA), and grown for 116–127 d with 10–30–20 fertilizer, applied at 50 ppm N first for a week, then increased up to 200 ppm N. Because of low germination rates, the procedure was repeated without cold stratification in January 2012 to provide more seedlings. Of all prepared seeds, 9% were planted and the survival rate after planting was 62% in May 2012, before seedlings were transplanted into the field.

Study design and treatments

In May–June 2012, 392 seedlings were transplanted across seven different elevations, either in tundra or in forest, under two temperature levels and two competition levels with 14 seedlings per treatment (Fig. 1). To minimize the effects of seed origin (locations of cones

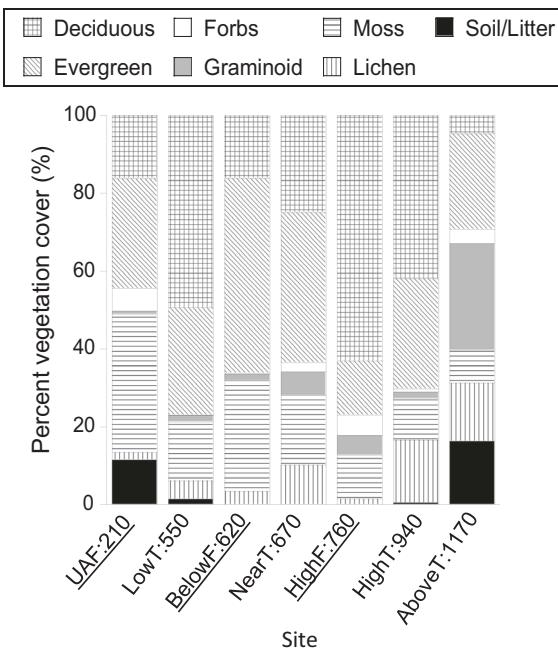


Fig. 2. Vegetation percent cover per site. Vegetation cover was measured by optical cramping (Young 2007) by using a 100-cm² grid separated into 100, 10 × 10 cm square areas to calculate percent cover ($n = 7$ or 8). The results were sorted by plant growth form. Lichen includes fungi. Forbs include *Lycopodium* spp. and *Equisetum* spp. Site abbreviations are as follows: UAF:210, Fairbanks forest (210 m); LowT:550, lowest tundra (550 m); BelowF:620, below treeline forest (620 m); NearT:670, near treeline tundra (670 m); HighF:760, high-elevation forest (760 m); HighT:940, high-elevation tundra (940 m); and AboveT:1170, above treeline tundra (1170 m). Underline shows forest sites.

harvested) and seedling size among the treatments, we grouped seedlings by origins and by height before assigning a treatment. Seedlings from each origin group were stratified into height categories, and within each origin and height category, seedlings were assigned randomly to each treatment. This ensured that seedling heights were roughly equal across groups at the beginning of the experiment. We excluded the seedlings that could not fit into any of the height categories.

At each field site, we established two different temperature treatments (Fig. 1). An elevated temperature plot (GH, Appendix S1: Fig. S1a, c)

was warmed by one A-frame wooden greenhouse (140 × 120 cm and 85 cm in height, hereafter “greenhouse”) covered with 6-mil (0.15 mm) plastic sheeting, with two 20- to 30-cm side vents, as used in our previous study (Okano and Bret-Harte 2015). One ambient plot (Out, Appendix S1: Fig. S1b, d) of the same footprint size was established per site. Greenhouses were set up each year at the beginning of June and disassembled at the end of August. Greenhouses of the same design as this study increased temperatures by 3°–6°C (Okano and Bret-Harte 2015), which was closer to the temperature projection for interior Alaska by the end of the century (2.2°–5.6°C, Chapin et al. 2014) than open-top chambers can achieve (up to 1.8°C; Marion et al. 1997, Danby and Hik 2007, Welshofer et al. 2018). The greenhouse excluded most precipitation and reduced both light intensity and soil moisture inside by more than 30% relative to outside, in our previous study (Okano and Bret-Harte 2015). However, differences in both light intensity and soil moisture between greenhouse and ambient plots were not significant in the previous study. In addition, the greenhouses did not reduce seedling growth inside compared with outside (Okano and Bret-Harte 2015), similar to the results of other studies using greenhouses (e.g., Chapin et al. 1995, Zamin et al. 2014).

Within each plot, we removed neighbor plants around half of the seedlings to assess the effects of interactions with neighbors. To create the absence of neighbors, we removed naturally occurring plants from a 15 cm diameter circle (Choler et al. 2001), cut the soil at the outside border of each circle to a depth of 20–25 cm with a knife, and then planted a seedling at the center. Shoots of neighbors and green mosses were removed, but non-photosynthetically active brown mosses and carpet mosses growing on soil could not be removed completely. We clipped regrowth to maintain the removal condition at the beginning and end of each growing season. Biomass of removed neighboring plants from each circle was dried, and a subset (10 circles per site) was weighed to estimate biomass removed per seedling per site. We planted seedlings with neighbors 20 cm away from the nearest removal treatment, at the center of a 15 cm diameter circle in which we did not remove naturally occurring plants except for a small hole

(diameter <3 cm) in which a seedling was planted (Fig. 1).

Our goal was to assess the growth of seedlings across treatments in the growing season (early June–late August). We expected that neighbors within the 15-cm circles would influence seedling growth during the summer, but that the size of the circles was too small to create differences due to the presence of neighbors in winter. The greenhouses were removed over the winter, so the winter environments differed only by elevation and habitat type. Because we could not access the sites in DNPP in winter, our experimental treatments started in early June. Both shoot growth (flushing) and secondary growth for white spruce in interior Alaska can start in May, and cease by the end of June (flushing) and July (secondary growth), and their cessation depends on photoperiod rather than on temperature (Burns and Honkala 1990, Hamilton et al. 2016). Although there could have been effects of the prior winter's conditions on summer growth, mortality over two winters in 2012–2013 and in 2013–2014 together was lower than mortality in summer 2013, one year prior to our biomass harvest reported here (Okano 2018). Therefore, we could capture most of the period of seedling growth in this experiment, and infer that growth reflected primarily conditions in summer.

Because there were only one greenhouse plot and one control plot per site, treatment effects could be confounded by natural variation among locations. Our experiment was limited by the need to conduct it within private inholdings in DNPP because of National Park Service regulations that did not allow manipulative experiments, so having more plots was not possible at most of the sites. We conducted two additional experiments where the main experiment was conducted to test whether, in the absence of treatments, seedlings growing within plots (in close proximity to each other) were more similar to each other than seedlings in different plots. Sixty seedlings (10 seedlings per plot, six plots) and 20 seedlings (five seedlings per plot, four plots) were planted without treatments (no greenhouse, no neighbor removal) in tundra (close to the NearT:670 site) and in forest (close to the BelowF:620 site), respectively, in 2014. We found no significant effects of different plots at the same site on seedling biomass, diameter, and

relative growth rate (RGR) in height ($P > 0.1$ for all, details in Appendix S1; see Appendix S1: Table S3, Fig. S2), suggesting that plot effects were unlikely to be driving differences in seedling growth within a site. Based on these additional experiments, we concluded that landscape variability in the absence of treatments affected seedling growth minimally.

Plant size measurements

In 2014, seedling height and basal stem diameter were measured in the field in the spring (28 May–3 June, except for the UAF:210 site on 23 May) as a baseline. These variables were measured again in the fall (26 August–3 September at DNPP and 6 September at UAF:210) before the seedlings were harvested. Relative growth rates (RGRs) in height were calculated as

$$RGR_{\text{height}} = \frac{\ln(\text{Height}_{\text{final}}) - \ln(\text{Height}_{\text{initial}})}{\text{Day}_{\text{final}} - \text{Day}_{\text{initial}}},$$

where $\text{Height}_{\text{final}}$ is the height measured in the fall, and $\text{Height}_{\text{initial}}$ is the spring height. $\text{Day}_{\text{final}}$ and $\text{Day}_{\text{initial}}$ are the day of the data collection in the fall and spring, respectively. Diameter values were compared using the average of two measurements at right angles measured in the fall. We measured stem diameters, but did not calculate relative growth rate in diameter because seedlings grew so little in diameter that our measurement precision was not sufficient to detect the change over one season.

Biomass, N pool size, and foliar $\delta^{13}\text{C}$, $\delta^{15}\text{N}$

Seedlings were harvested in fall 2014 on the same day as their sizes were measured. Each seedling was dug up with its surrounding soil and nearby vegetation, which were washed away by submerging the roots in water. Each seedling was separated into different tissue types: new needles, old needles, new stems, old stems, and roots. All tissues were dried at 60°C for more than 72 h.

New needles from a subset of seedlings ($n = 3$ per treatment) were ground and analyzed for C and N contents and stable isotope signatures. Analyses were performed using continuous-flow isotope ratio mass spectrometry (CFIRMS), which consists of a Costech ECS 4010 Elemental Analyzer (Costech Analytical Technologies, Valencia, California, USA) and Thermo Scientific

ConFlo IV interfaced with Thermo Scientific DeltaV Plus Mass Spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA), with a precision of <0.2‰, at the UAF Alaska Stable Isotope Facility. The pool size of foliar N was estimated from needle biomass and the average N concentration of the needles. Foliar $\delta^{13}\text{C}$ was analyzed to assess seedlings' drought stress levels (Farquhar et al. 1989, Ehleringer et al. 1993). Foliar $\delta^{15}\text{N}$ is an indicator of plants' ability to obtain nitrogen; establishing mycorrhizal associations and obtaining organic nitrogen through fungi make foliar $\delta^{15}\text{N}$ more depleted (Hobbie et al. 2009).

Environmental data collection and analyses

Environmental and basic descriptive data were recorded to characterize each site and effects of the greenhouse treatments. Site-specific light levels in photosynthetic photon flux density (PPFD) were measured ten times between 10:30 a.m. and 6 p.m. on a sunny day, using a photosynthetically active radiation (PAR) sensor attached to a photosynthesis system (LI-6400XT, LI-COR, Lincoln, Nebraska, USA). The measurements were taken at ground level.

Temperatures when greenhouses were in place at all sites (8 June–11 August 2014) were recorded using iButtons (Maxim Integrated, San Jose, California, USA) that logged temperatures every 2 h. At the center of each plot (greenhouse or control), air temperatures were measured 5–7 cm above the soil surface under a shade, and soil temperatures were measured 5 cm below the surface. From air temperatures, growing degree-days (GDD) were calculated using a baseline temperature of 5°C (Juday et al. 2003), which is the approximate temperature below which trees cease growth (Körner 2012).

Soil moisture levels in greenhouse and ambient plots were measured twice, using a moisture probe (HydroSense Soil Water Measurement System, Campbell Scientific, Logan, Utah, USA). Measurements were made just before and after greenhouses were set up in the spring and removed in the fall, respectively. Other soil data obtained to compare soil conditions by site included surface soil %N, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ of organic soil, and an estimate of plant-available inorganic N (NH_4^+ and NO_3^-) from ion exchange resins incubated 5 cm below the soil surface

in situ for 9 months from September 2013 and May 2014 for winter and for 3 months from June to August 2014 for summer (5 g per nylon stocking bag of J.T. Baker Mixed Bed Exchange Resin, Avantor Performance Materials, Center Valley, Pennsylvania, USA).

Statistics

In order to evaluate the effects of elevation, warming, neighbors, and habitat type on seedlings' growth performance, data for the 269 seedlings that survived until August 2014 were statistically analyzed using analysis of variance (ANOVA) in R (ver 4.0.2; R Development Core Team 2020). Explanatory variables included elevation (a continuous variable); habitat type: tundra vs. forest, greenhouse: inside vs. outside, and neighbors: with vs. without (three, two-level categorical variables); and all possible two-way and three-way interactions. Variables were entered according to their scaling order from largest to smallest; hence, type I ANOVA was used (Schmid et al. 2002, Nitschke et al. 2010). The four-way interactions were not included, because they were not readily interpretable. Elevation was a proxy for physical stress, and we assumed that changes in seedling growth along tundra and forest gradients were different. We chose to analyze the data from all sites together, rather than analyze data separately by habitat to compare the effects of habitat on response variables.

Response variables included those related to biomass, foliar N pool size, and size measurements (diameter and RGR in height). Foliar percent N, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ from the subset were also analyzed using the same models as other analyses. Initially, we included plot as a random effect to account for a possible plot effect. However, the random effect was usually <15% of the total residual of each analysis, and therefore, we dropped the random effect. Additionally, we also ran analyses by using the final biomass divided by the mean biomass at each elevation to reduce the influence of differences in plant size across treatments. The analyses essentially gave the same qualitative results as using biomass itself, so we do not report them here.

We performed ANCOVAs adding one of continuous variables (light level, neighbor plant height, or removed biomass in 2012) as a covariate to the original analyses in order to assess

whether these covariates explained any of the variation associated with the treatments. We compared all analyses once with and once without each one of the covariates. The same light levels were used for seedlings with and without neighbors at the same site. The light level inside a greenhouse was estimated to be 32% less than the outside at the same site (Okano and Bret-Harte 2015), which was calculated and applied to the inside plots. Mean neighbor height was used for all seedlings with neighbors at each site, and a height of zero was used for seedlings without neighbors. Likewise, the amount of biomass removed in 2012 per site was applied to the seedlings with neighbors, while zero neighbor biomass was applied to the seedlings without neighbors.

All response variables were tested for homogeneity of variance using Bartlett's test and for normality and were log-transformed if necessary. In order to avoid increasing type I errors by performing multiple ANOVAs, P values were adjusted by the Benjamini-Hochberg procedure with a false discovery rate of 0.15 (McDonald 2014). When interactions were significant, estimated marginal means were computed and compared with a P value of $\alpha = 0.05$ adjusted by the Dunnett adjustment (emmeans package, Lenth et al. 2020; multcompView package, Graves et al. 2019).

Table 3. ANOVA results for biomass and 2014 new biomass-to-total biomass ratio without covariates.

Source	Aboveground biomass		Belowground biomass		Total biomass		2014 biomass as a fraction of total	
	F	P	F	P	F	P	F	P
Elevation (E)	3.7	0.054	11.4	0.001**	5.4	0.021*	59.7	<0.001***
Habitat (H)	0.8	0.377	9.8	0.002**	2.6	0.107	12.2	0.001**
Greenhouse (G)	0.2	0.686	2.2	0.140	0.5	0.466	0.1	0.762
Neighbors (N)	5.9	0.016*	14.6	<0.001***	8.1	0.005**	0.9	0.347
E × H	2.4	0.125	8.7	0.003**	4.0	0.047	23.0	<0.001***
E × G	0.4	0.548	0.2	0.641	0.5	0.464	5.9	0.016*
H × G	6.5	0.011*	4.2	0.042*	6.1	0.014*	18.7	<0.001***
E × N	0.4	0.518	1.9	0.172	0.7	0.402	0.0	0.991
H × N	0.0	0.933	0.0	0.876	0.0	0.924	0.2	0.677
G × N	0.2	0.623	0.5	0.470	0.3	0.606	1.4	0.246
E × H × G	1.5	0.217	0.3	0.585	1.0	0.308	7.0	0.009**
E × H × N	0.0	0.895	0.0	0.835	0.0	0.840	15.8	<0.001***
E × G × N	2.4	0.126	1.7	0.190	2.4	0.126	1.9	0.164
H × G × N	0.4	0.531	0.1	0.817	0.4	0.543	0.2	0.664

Notes: Values in boldface were significant (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) after P values were adjusted by the Benjamini-Hochberg procedure with the false discovery rate of 0.15. For all analyses, each model factor had one numerator degree of freedom and 254 denominator degrees of freedom.

RESULTS

At the beginning of the experiment, the mean height of seedlings was 7.1 ± 0.2 cm (SE) and the mean number of needles measured by counting was 284 ± 13 (SE), which were not statistically different between treatments (one-way ANOVA, $F_{27,364} = 0.42$, $P > 0.9$ for height, $F_{27,364} = 0.09$, $P > 0.9$ for number of needles). This suggests that biomass of seedlings at the beginning was also not different across treatments. Thus, we assumed that differences in biomass at the end of the experiment were due to the experimental treatments (Tables 3, 4).

Environmental and site data

The summer of 2014 was cool at the beginning and wet throughout the growing season (Appendix S1: Table S1). Mean temperatures in June and July were 1.1° and 0.8°C , respectively, lower than the long-term normal value in Fairbanks. Total precipitation from June to August in Fairbanks was more than twice the long-term normal value. Summer air temperature of the UAF forest in Fairbanks was higher than the lowest forest site (BelowF:620) in Denali by 3°C (Table 2).

We confirmed that mean summer air temperature varied linearly, and was inversely related to elevation, for sites in each habitat type (tundra and forest; Fig. 3a,b), and thus was likely to

Table 4. ANOVA results for growth without covariates.

Source	Shoot-root ratio		Foliar N pool (g)		RGR in height		Diameter	
	F	P	F	P	F	P	F	P
Elevation (E)	9.5	0.002**	0.2	0.679	15.6	<0.001***	3.0	0.087
Habitat (H)	38.5	<0.001***	0.0	0.901	8.1	0.005**	2.3	0.132
Greenhouse (G)	10.8	0.001**	0.1	0.745	0.6	0.456	1.8	0.184
Neighbors (N)	17.4	<0.001***	7.3	0.007**	5.7	0.018*	3.9	0.048
E × H	7.1	0.008**	0.1	0.775	6.4	0.012*	1.5	0.218
E × G	0.0	0.871	0.3	0.580	3.9	0.050*	0.2	0.656
H × G	7.4	0.007**	8.7	0.003**	5.6	0.018*	2.8	0.097
E × N	8.2	0.005**	0.4	0.525	0.0	0.933	0.0	0.960
H × N	0.3	0.591	0.0	0.882	0.0	0.913	0.0	0.839
G × N	0.1	0.768	0.1	0.746	0.9	0.344	0.5	0.459
E × H × G	4.4	0.036*	3.7	0.056	7.2	0.008**	0.8	0.363
E × H × N	0.1	0.798	0.2	0.664	0.0	0.901	0.1	0.765
E × G × N	0.5	0.490	2.4	0.121	0.1	0.762	2.3	0.128
H × G × N	0.1	0.708	0.6	0.448	0.0	0.923	0.5	0.465

Notes: Values in boldface were significant (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) after P values were adjusted by the Benjamini-Hochberg procedure with the false discovery rate of 0.15. For all analyses, each model factor had one numerator degree of freedom and 254 denominator degrees of freedom.

create a gradient in physical stress for the plants growing there (Körner 2003). The slopes of the relationships between mean summer air temperature and elevation for tundra and forest were statistically different (t -test, $P = 0.014$). GDD also decreased as elevation increased, but the slopes of the relationships in the two habitats were not statistically different (t -test, $P = 0.56$; Fig. 3c). Temperatures and GDD in tundra sites tended to be higher than those of their closest forest sites. Thus, we defined the mean air temperature across elevations as a proxy for physical stress gradients in the two different habitats.

Greenhouses increased mean summer temperatures during the experimental period in 2014 by 1.7°–2.3°C at tundra sites and 1.6°–1.7°C at forest sites above ambient temperatures at the same site (Table 2). The slopes of the relationships between mean summer temperature and elevation for greenhouse plots were not different from those for ambient plots within the same habitat, so greenhouse-warmed tundra and greenhouse-warmed forest sites had significantly different slopes (t -test, $P = 0.04$; Fig. 3a,b), as was seen for ambient plots. Generally, greenhouses in tundra sites increased mean, maximum, and minimum temperatures more than in forest sites, except for minimum temperature at AboveT:1170. The effect of greenhouses on GDD was also similar regardless of habitat; GDDs were 10–40% higher

in greenhouses than in ambient temperature plots at all sites, particularly low elevations in tundra (Fig. 3c). Greenhouses decreased relative humidity (RH) by <7%, and two sites had almost the same (RH) inside and outside of greenhouses (Table 2). Greenhouses increased soil temperatures to a smaller extent than air temperatures (Table 2). Soil moisture was about 5–10% lower in the greenhouses than in ambient plots in the fall of 2014 (Fig. 3d). No trends by habitat or elevation were found in soil data, which are reported in Appendix S1: Table S4 and Fig. S3. In summary, greenhouses increased air temperature strongly, while other environmental factors were affected minimally.

Light intensity, removed neighbor biomass, and neighbor height were measured in order to include them in the analyses as covariates. Light was not correlated with either removed biomass ($r = 0.09$) or neighbor height ($r = -0.0002$). Light (in PPFD) in tundra sites was significantly higher than light in forest sites (t -test, $P < 0.01$; Table 1). Among the seven sites, light level at UAF:210 was significantly lower than at other sites (one-way ANOVA and Tukey-Kramer HSD, $F_{6,63} = 27.2$, $P < 0.001$), but six Denali sites were not statistically different from each other. However, except for the lowest site (LowT:550), tundra sites had higher light levels than the Denali forest sites (Table 1).

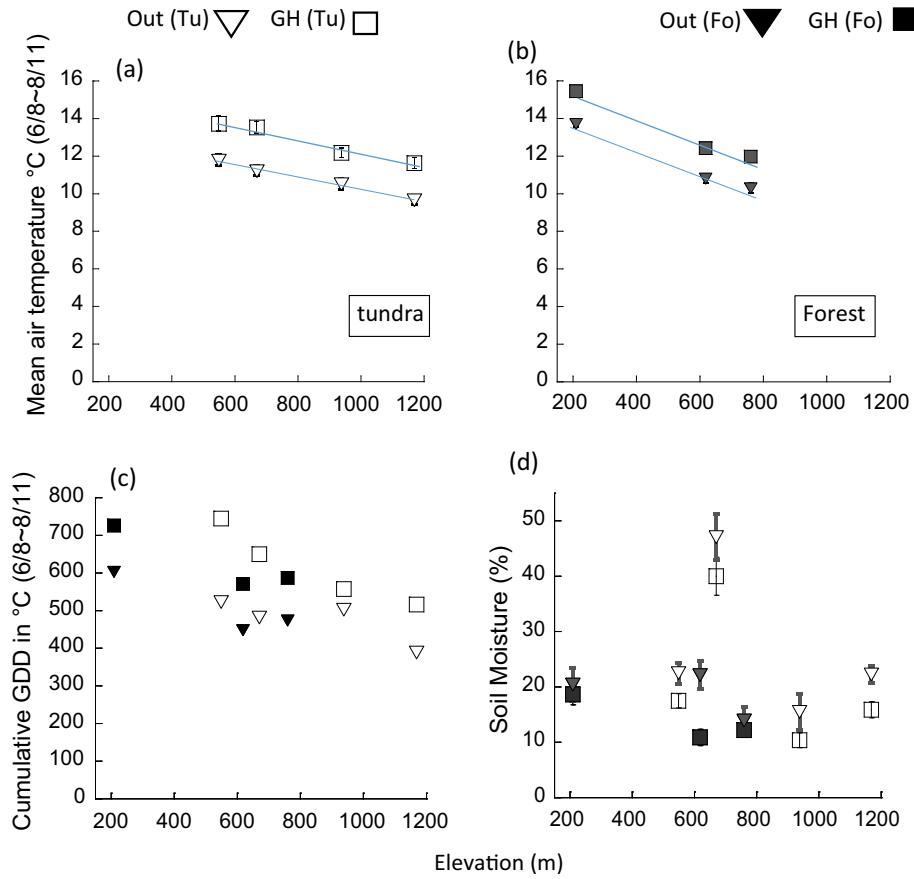


Fig. 3. Environmental data. (a) Mean air temperature during the summer (6/8–8/11/2014) by greenhouse treatment in tundra and (b) forest. Slopes of tundra and forest were different (Out: t -test $t_3 = -5.15$, $P = 0.014$), and greenhouse warming did not change the slopes for each habitat, so they were different under warming as well (GH: t -test $t_3 = -3.4$, $P = 0.04$). Warming increased mean temperatures, significantly in tundra ($F_{1,6} = 8.4$, $P = 0.03$), but not significantly in forest ($F_{1,4} = 1.2$, $P = 0.3$). (c) Growing degree-days (GDD) by greenhouse treatment. Slopes did not differ by greenhouse or by habitat. (d) Soil moisture by temperature treatment. Abbreviations are as follows: greenhouse treatment square, inside (GH); upside-down triangle, outside (Out); habitat filled, forest (Fo); and open, tundra (Tu). Error bars show one standard error.

Neighbor height and biomass at the highest and lowest elevation sites were less than those of other sites. Removed biomass in 2012 ranged from a minimum of 11.3 g/m^2 to a maximum of 4246.3 g/m^2 per seedling, and the mean biomass removed at the highest site (AboveT:1170) and the lowest site (UAF:210) was less than half of that at the Denali forest sites (Table 1, Fig. 4), which was significantly different (one-way ANOVA, $F_{6,63} = 6.1$, $P < 0.01$). Similarly, heights of surrounding understory neighbors were also shorter at AboveT:1170 and at UAF:210; compared with the neighbor heights of other sites in

the same habitat type, AboveT:1170 (tundra) had only one-third and UAF:201 (forest) had two-thirds of the heights of neighboring plants (Table 1). Neighbor height and removed biomass were highly correlated ($r = 0.97$).

Hypothesis 1: Did plant–plant interactions differ by elevation as predicted by the SGH?

We hypothesized that neighboring competition would reduce growth of spruce seedlings, but that the magnitude of reduction would be smaller at higher elevations where physical stress is high due to low temperatures than at lower

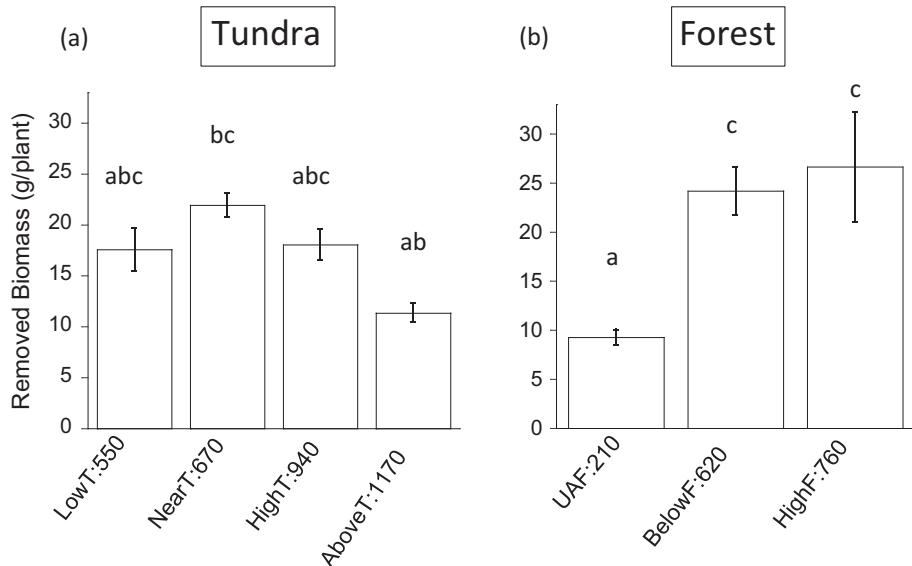


Fig. 4. Neighbor plant biomass removed in June 2012 in (a) tundra and (b) forest sites. A subset of removed biomass ($n = 10$ per site) was weighed and averaged. One-way ANOVA indicated significant differences among seven sites ($F_{(6,63)} = 6.1$, $P < 0.01$), which were analyzed by pairwise comparisons with family-wise $P = 0.05$ adjusted by the Dunnett methods. Sites that share a letter (a, b, or c) are not significantly different. Error bars indicate one standard error. Site abbreviations as in legend to Fig. 2.

elevations where stress is low due to warmer temperatures in both habitat types, as predicted by the SGH. Thus, we expected to see significant interactions between the effects of elevation (E) and neighbor plants (N), but instead found that the presence of neighbors by itself strongly affected seedling biomass. Having neighbors significantly reduced aboveground, belowground, and total seedling biomass, by approximately 20% each (Table 3, Fig. 5). The foliar N pool in seedlings without neighbors contained 20% more nitrogen than that of seedlings with neighbors (Table 4, Fig. 5g). Seedlings without neighbors had larger diameters than seedlings with neighbors by 10%, but this difference was not statistically significant (Fig. 6a,b). Seedlings with neighbors had significantly higher RGR in height than seedlings without neighbors, by 18% at all elevations (Fig. 6c,d).

There were significant interactions between elevation and neighbors (Figs. 5, 6, Tables 3, 4) only for shoot/root ratio and new/total biomass ratio. Seedlings with neighbors at low elevations had higher shoot/root ratios than seedlings without neighbors, but there was almost no difference

between neighbor treatments at higher elevations (Fig. 5h). The difference in shoot/root ratios with and without neighbors was larger in seedlings in forest sites than that in tundra sites, but the interaction between habitat and neighbors was not significant ($H \times N$, Table 4). Only the new/total biomass ratio in tundra sites showed the pattern that would have been predicted by the SGH: Seedlings with neighbors gained a higher proportion of new biomass than seedlings without neighbors as the elevation increased, but this was seen only in tundra sites, as indicated by a significant three-way interaction between elevation, habitat, and neighbors ($E \times H \times N$; Table 3, Fig. 5i–j). Overall, we found evidence for competition at all elevations, but we saw little evidence for statistically different magnitudes of neighbor effects along our elevational gradients in either habitat.

Hypothesis 2: Did seedling growth differ in tundra and in forest, due to resource stress?

We hypothesized that growth of seedlings in forest sites would show a different relationship with elevation than growth of seedlings in

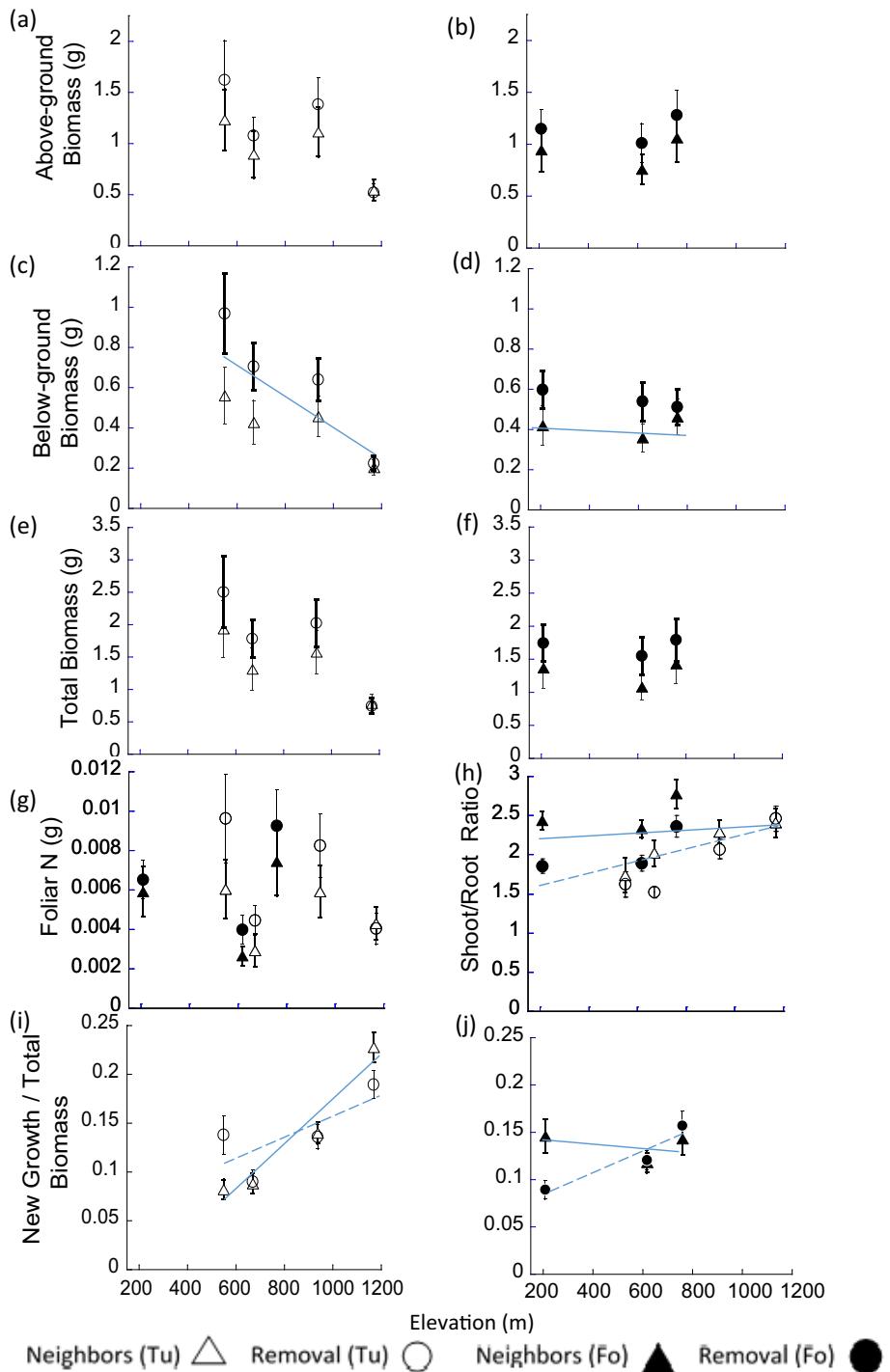


Fig. 5. Interactions between neighbor effects and elevation to assess the stress gradient hypothesis (SGH). (a) Aboveground biomass (needles + stems) in tundra and (b) in forest. (c) Belowground biomass (roots) in tundra, $R^2 = 0.80$ (no difference by neighbor effect) and (d) in forest, $R^2 = 0.90$ (no difference by neighbor effect). Lines applied show significant interactions between elevation and habitat. (e) Total biomass (aboveground + belowground) in tundra and (f) in forest. (g) Foliar nitrogen (N) pool. (h) Shoot-to-root ratio. Solid line Neighbors,

(Fig. 5. *Continued*)

$R^2 = 0.03$; dash line Removal, $R^2 = 0.40$. (i) New biomass as a fraction of total biomass in tundra (solid line Neighbors, $R^2 = 0.94$; dash line Removal, $R^2 = 0.54$) and (j) forest (solid line Neighbors, $R^2 = 0.36$; dash line Removal, $R^2 = 0.95$). (a, b) and (e, f) Slopes of each habitat did not differ by ANOVAs, which became different by adding a light covariate (significant interaction between elevation and habitat). (i, j) Slopes of removal between habitats (dash lines) were statistically not different. Neighbor treatment: triangle, neighbors present (Neighbors); and circle, neighbors removed (Removal). Habitat: filled, forest (Fo); and open, tundra (Tu). Error bars show one standard error. Lines applied show significant interactions with elevation. Neighbor effects were significant, but no significant interaction between neighbors and elevation, except for (h) two-way interaction and (i, j) three-way interaction between habitat, neighbors, and elevation.

tundra sites, because of resource stress imposed by low light availability in forest sites. Our results revealed that mostly seedlings showed different growth patterns along the elevational gradients in each habitat. Despite having a slightly steeper temperature gradient with

elevation in forest sites than in tundra sites (Fig. 3a,b), we found steeply decreasing biomass, foliar nitrogen, and diameter as elevation increased in tundra sites, but not in forest sites (Figs. 5a–g, 6a,b). The differences between tundra and forest sites were large for belowground

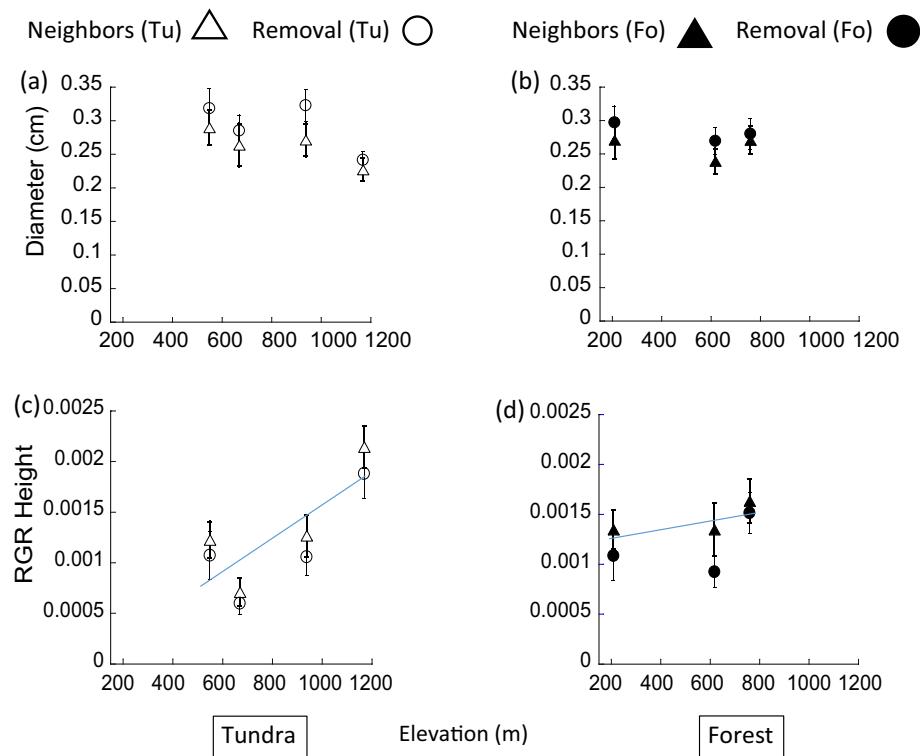


Fig. 6. Non-destructive growth in size to assess the SGH. (a) Basal stem diameter in tundra and (b) forest. (c) Relative growth rate (RGR) in height along an elevational gradient in tundra and (d) forest. Lines show different habitats (no difference by neighbor effect): Tundra $R^2 = 0.62$, Forest $R^2 = 0.32$. Diameter and height growth were measured at the beginning and the end of the growing season (before harvest for biomass) in 2014. Treatment abbreviations as in legend to Fig. 5. Error bars show one standard error. Statistically significant effects are shown by different fitted lines.

Table 5. Results that differed from Tables 3, 4 (Original), after inclusion of a covariate of (A) light, (B) neighbor height, and (C) removed biomass in 2012 (ANCOVA).

Covariate and response variable	Covariate <i>P</i> value	Affected model effect	Significance	
			Original	ANCOVA
(A) Light				
Aboveground biomass	0.7	E × H	NS	SG
Total biomass	0.48	E × H	NS	SG
New biomass/total	<0.001***	(E × H) (E × G)	SG SG	NS NS
Shoot/root ratio	0.01**	(E × H)	SG	NS
Foliar N pool (g)	0.03*	E × H	NS	SG
RGR in height	<0.001**	(E × H) (E × G) E × H × G	SG SG SG	NS NS NS
(B) Neighbor height				
New biomass/total	<0.001***	(E × H)	SG	NS
RGR in height	0.018*	E × H × N	SG	NS
(C) Removed biomass				
New biomass/total	<0.001***	(E × H) H × N	SG NS	NS SG
RGR in height	0.006*	E × H × N (E × H) E × H × N	SG SG NS	NS NS SG

Notes: Bolds show the results to interpret. Parenthesis indicates other factors are involved to interpret the result. Left column has the same model effects as Tables 3, 4. Affected model effect abbreviations are as follows: E, elevation; H, habitat; G, greenhouse; N, neighbor effects; and × = interaction. Significance abbreviations are as follows: SG, significant; and NS, not significant. Asterisks show significant covariates (**P* < 0.05, ***P* < 0.01, ****P* < 0.001) after *P* values were adjusted by the Benjamini-Hochberg procedure with the false discovery rate of 0.15. For all analyses, each model factor had one numerator degree of freedom and 253 denominator degrees of freedom.

biomass, shown by a significant elevation by habitat interaction (E × H, Table 3). When light as a covariate was added to the analyses, this interaction (E × H) also became significant for aboveground biomass, total biomass, and foliar nitrogen (Table 5, Appendix S1: Table S5). The ANCOVA results suggest that the interaction between habitat and elevation was masked by variations in light between tundra and forest. In addition, we found steeply increasing relative growth rates (RGRs) in height as elevation increased in tundra, but not in forest (E × H, Fig. 6c,d). These growth results suggest that other factors than temperature gradient, likely light availability, played a role in the forest sites.

Removing neighbors made the slopes of the relationships between new/total biomass ratio of seedlings and elevation the same for both habitats (seedlings growing under removal treatments in tundra vs. forest: *t*-test, *P* = 0.99;

Fig. 5i-j). In tundra, having neighbors increased seedling biomass at high elevations and decreased seedling biomass at low elevations, but in forest, the opposite trend was true (as shown by a significant three-way interaction: E × H × N; Table 3). However, including either neighbor height or neighbor biomass covariate in the model made this three-way interaction insignificant (Table 5, Appendix S1: Tables S6–S7). The two-way habitat by neighbor interaction (H × N) in ANCOVAs remained significant when neighbor biomass was added as a covariate and marginally significant when neighbor height was added as a covariate (*P* = 0.08; Appendix S1: Table S6). These results suggest that differences in new/total biomass ratio across elevational gradients in both habitats were at least partially due to neighbor height and biomass affecting the light environment of the seedlings, but other factors affected the H × N interaction.

Table 6. ANOVA table for foliar C and N using a subset of data ($n = 84$).

Source	Percent N		$\delta^{15}\text{N}$		$\delta^{13}\text{C}$	
	F	P	F	P	F	P
Elevation (E)	5.4	0.023*	0.4	0.524	7.8	0.007**
Habitat (H)	5.2	0.025*	1.2	0.281	1.9	0.176
Greenhouse (G)	0.1	0.709	6.7	0.012*	13.4	<0.001***
Neighbors (N)	0.2	0.663	1.2	0.274	0.2	0.643
E × H	9.9	0.002**	6.5	0.013*	11.2	0.001**
E × G	0.4	0.543	1.0	0.315	0.2	0.635
H × G	0.2	0.634	1.1	0.288	0.3	0.586
E × N	0.1	0.763	0.1	0.711	0.5	0.468
H × N	0.8	0.383	5.3	0.024*	0.5	0.498
G × N	0.2	0.688	0.2	0.643	0.1	0.739
E × H × G	0.05	0.831	0.2	0.640	9.3	0.003**
E × H × N	0.02	0.892	2.1	0.156	0.3	0.595
E × G × N	0.1	0.782	0.02	0.893	0.003	0.955
H × G × N	0.1	0.728	2.7	0.104	0.1	0.760

Notes: Values in boldface were significant (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) after P values were adjusted by the Benjamini-Hochberg procedure with the false discovery rate of 0.1. For all analyses, each model factor had one numerator degree of freedom and 69 denominator degrees of freedom.

A subset of seedling foliar elements was analyzed in order to investigate the effects of below-ground resources (N and water availability) on seedling growth. Although there was a significant interaction between elevation and habitat for all three analyses, there was no significant effect of neighbors, unlike most of our main results. Only the $\delta^{15}\text{N}$ signature was significantly higher (less depleted) when seedlings grew with neighbors in tundra sites than seedlings without neighbors or in forests (Appendix S1: Fig. S4a–b). Differences in foliar N content, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (Table 6, Fig. 7, Appendix S1: Fig. S4), did not explain the patterns of seedling growth that we saw in the main analyses (Tables 3, 4).

In summary, seedling growth differed more with elevation in tundra than in forests, as we hypothesized. Seedlings in tundra decreased most in their growth variables as the elevation increased, while seedling growth in forests did not vary as much with elevation, partially because light levels were mostly lower across forest sites. These results suggest that seedlings in tundra sites responded mainly to the gradient in physical stress associated with temperature, while resource stress caused by the light environment was more important for seedling responses in forest sites for several variables, resulting in a different pattern of seedling growth in tundra and in forest sites.

Hypothesis 3: Did warming alter seedling growth and patterns of plant–plant interactions?

We hypothesized that when warmed, physical stress caused by cold temperature at high elevations would be ameliorated, which would increase seedling growth, while temperatures at low elevations would exceed those that were optimal for photosynthesis, which would decrease seedling growth. Thus, we expected to find a significant interaction between elevation and greenhouse warming (E × G) in both tundra and forest sites. For shoot/root ratio (Fig. 8a,b), new/total biomass ratio (Fig. 8c,d), and RGR in height (Fig. 8e,f), we found a significant three-way interaction between elevation, habitat, and greenhouse (E × H × G, Tables 3, 4). However, there were no significant E × G interactions for most other variables; instead, we found significant interactions between habitat and greenhouse (H × G, Tables 3, 4).

At ambient temperature (greenhouse Out), seedlings growing in tundra increased their fraction of new biomass, shoot/root ratio, and RGR in height as elevation increased, but decreased them as elevation increased in forests (Out: $t_{254} = 4.7$, $P < 0.0001$ for new/total biomass ratio; $t_{254} = 3.6$, $P = 0.004$ for shoot/root ratio; $t_{257} = 3.4$, $P = 0.007$ for RGR in height). When warmed (greenhouse GH), for all of the three variables, seedlings in tundra did not change

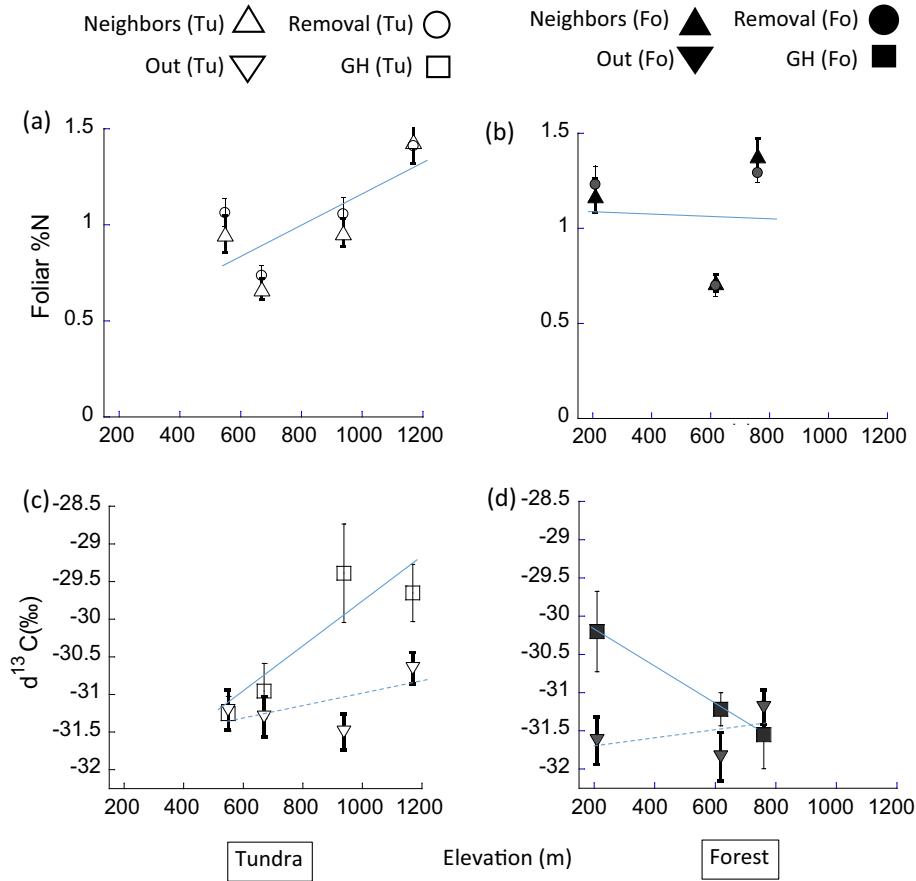


Fig. 7. Subset of foliar N and $\delta^{13}\text{C}$. (a) Foliar percent N for seedlings, interaction between elevation and neighbor effect in tundra ($R^2 = 0.59$, no neighbor effect) and (b) forest ($R^2 = 0.004$, no neighbor effect). (c) Foliar $\delta^{13}\text{C}$, interaction between greenhouse and elevation in tundra (solid line GH, $R^2 = 0.80$; dash line Out, $R^2 = 0.32$) and (d) forest (solid line GH, $R^2 = 0.99$; dash line Out, $R^2 = 0.18$). Slopes of Out between habitats were statistically not different ($t_{(254)} = -0.2$, $P = 0.84$). Treatment abbreviations as in legend to Figs. 3, 5. Error bars show one standard error. Statistically significant effects shown by fitted lines.

their trends from ambient temperature, but seedlings in forest increased these growth variables as elevation increased, which eliminated the statistically significant differences between habitats (i.e., the slopes of the relationship between each of these variables and elevation became the same between habitats; Fig. 8). Thus, warming did not significantly affect the relationships between elevation and either new/total biomass ratio, shoot/root ratio, or RGR in height in tundra sites, but warming caused these three growth variables to change their negative relationships with elevation to positive relationships in forest sites (Fig. 8).

We found more evidence that warming affected seedling growth and biomass differently in tundra and forest sites (significant $H \times G$ interactions; Table 3, 4, Fig. 9a,b), but the two-way interactions between elevation and greenhouse were not significant for any seedling growth variables ($E \times G$; Table 3, 4). In tundra sites, seedlings growing in greenhouses had significantly lower aboveground, belowground, and total biomass, and a lower foliar N pool than those outside. In forest sites, seedlings growing in greenhouses had higher biomass variables than those outside, but the difference was significant only for the foliar N

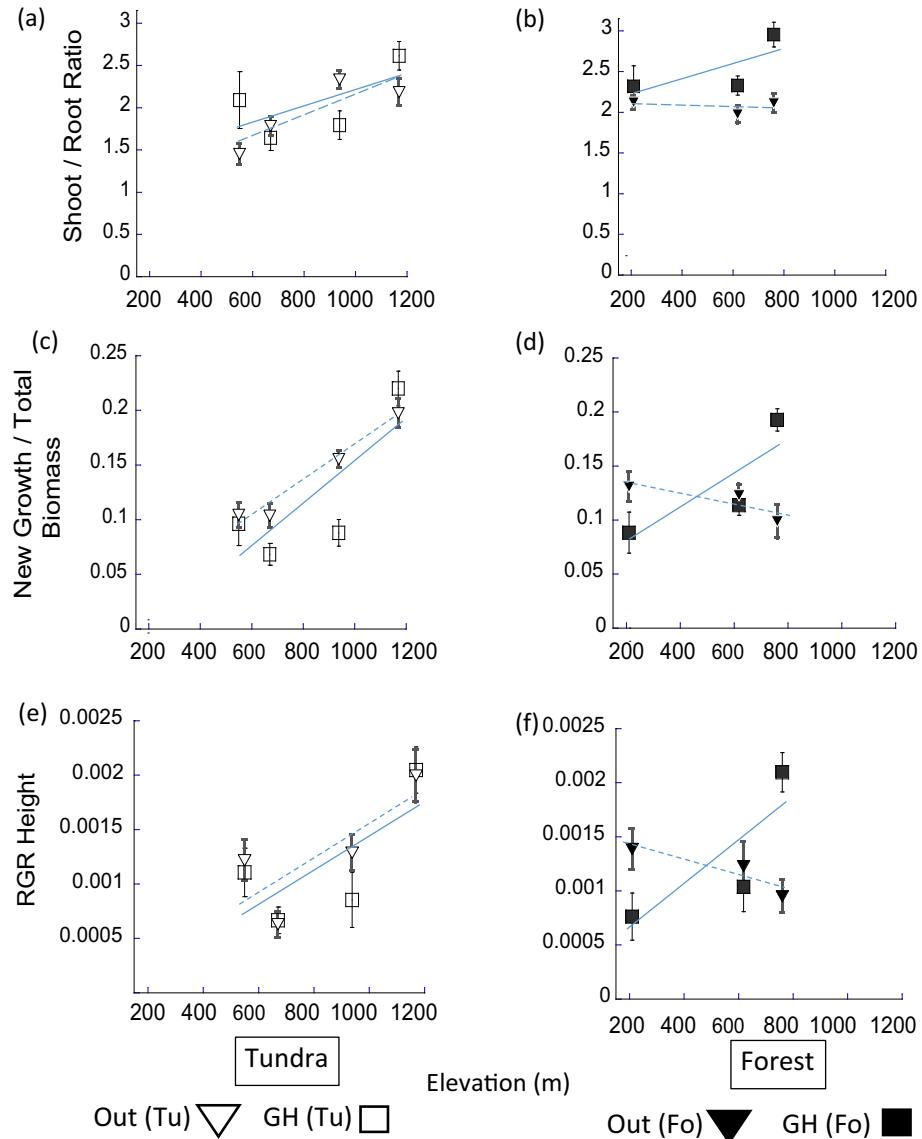


Fig. 8. Greenhouse effects across elevation by habitat. (a) Shoot/root ratio in tundra (solid line GH, $R^2 = 0.37$; dash line Out, $R^2 = 0.75$) and (b) forest (solid line GH, $R^2 = 0.50$; dash line Out, $R^2 = 0.11$). (c) New biomass as a fraction of total biomass in tundra (solid line GH, $R^2 = 0.62$; dash line Out, $R^2 = 0.96$; no difference by greenhouse effect, $t_{(254)} = 1.72$, $P = 0.09$) and (d) forest (solid line GH, $R^2 = 0.72$; dash line Out, $R^2 = 0.72$). (c, d) Slopes of GH between habitats were statistically not different ($t_{(254)} = 0.62$, $P = 0.54$). (e) RGR in height in tundra (solid line GH, $R^2 = 0.50$; dash line Out, $R^2 = 0.61$, slopes not different, $t_{(254)} = -0.56$, $P = 0.58$) and (f) forest (solid line GH, $R^2 = 0.68$; dash line Out, $R^2 = 0.82$). (e, f) Slopes of GH between habitats were not different ($t_{(254)} = -0.68$, $P = 0.50$). Other treatment abbreviations as in legend to Fig. 3. Error bars show one standard error. Statistically significant differences are shown by different fitted lines.

pool (Fig. 9b). The results were unexpected because we predicted that the effects of warming would not be different between habitat types.

We added light as a covariate to our models to assess the effects of light attenuation by greenhouse plastic sheeting on seedling growth, but this did not affect the significance of our results

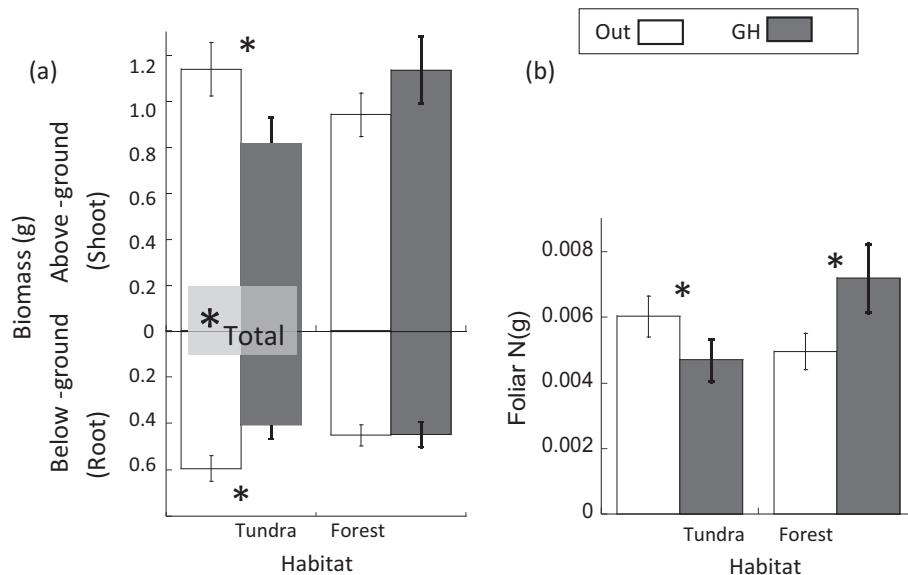


Fig. 9. Greenhouse effects by habitat. (a) Greenhouse-habitat interactions of aboveground biomass, belowground biomass, and total biomass. (b) Greenhouse-habitat interaction of foliar N pools. White bar is greenhouse outside (Out), and dark bar is greenhouse inside (GH). Other treatment abbreviations as in legend to Fig. 3. Error bars show one standard error. Statistically significant differences are indicated by an asterisk (*).

involving the greenhouse (G) except in one case, which was RGR in height, according to ANCOVAs (Table 5, Appendix S1: Table S5). The three-way interaction ($E \times H \times G$) was significant originally, but became marginally significant ($P = 0.06$, Table 5, Appendix S1: Table S5) when light was added as a covariate. However, the two-way interaction between habitat and greenhouse ($H \times G$) remained significant in the ANCOVA, suggesting that light conditions did not explain the effect of greenhouse. We concluded that light reductions inside greenhouses by 32% relative to outside did not affect the growth of our seedlings.

A subset of foliar $\delta^{13}\text{C}$ was analyzed as an indicator of water stress, and we found a significant three-way interaction between elevation, habitat, and greenhouse ($E \times H \times G$; Table 6, Fig. 7c,d). This occurred because at ambient temperature (greenhouse Out), the slope of the relationships between foliar $\delta^{13}\text{C}$ and elevation was the same for both tundra and forest (Out: $t_{69} = -0.21$, $P = 0.84$). However, when warmed with greenhouses, the foliar $\delta^{13}\text{C}$ of seedlings in tundra sites at high elevations became less depleted than the foliar $\delta^{13}\text{C}$ at ambient

temperatures, while the foliar $\delta^{13}\text{C}$ in forests at lower elevations became less depleted than that at ambient temperatures, so the slopes of the relationships between foliar $\delta^{13}\text{C}$ and elevation were altered. The results corresponded to the reduction in growth by warming at HighT:940 (the second highest elevation in tundra), reducing shoot/root ratio (Fig. 8a), new/total biomass ratio (Fig. 8c), and RGR in height (Fig. 8e), which may also reflect the reduction of biomass and foliar N in seedlings in tundra inside compared with outside greenhouses (Fig. 9a,b). For forest sites, both RGR in height (Fig. 8f) and new/total biomass (Fig. 8d) were reduced at UAF (the lowest forest) and increased at HighF:760 (the high-elevation forest), which corresponded to changes in foliar $\delta^{13}\text{C}$ that indicated water stress levels. These results suggest that warmer temperatures led to seedlings experiencing greater drought stress at high elevations in tundra and at low elevations at forest habitats.

We additionally hypothesized that resource competition against fast-growing neighboring plants would become increased by warming. However, we did not find evidence that neighbor plant growth was increased by warming within

the time frame of this experiment, because there were no significant interactions between greenhouse treatments and neighbors for any variables ($G \times N$, Table 3, 4). Adding covariates did not lead to significant interactions between neighbors and greenhouse for any variables, either (Table 5, Appendix S1: Tables S5–S7). Thus, we did not find evidence to suggest that warming altered the patterns of plant–plant interactions, at any elevations or habitat types.

DISCUSSION

Our goal in this study was to assess whether the patterns of plant–plant interactions predicted by the SGH would occur for white spruce seedlings planted along two elevational gradients, one in tundra and one in forested sites, and whether these interactions and seedling growth would be altered by experimental warming in interior Alaska. Although we acknowledge that not all species may respond in the same way, seedlings of white spruce are a key life stage of a widespread species that has been moving up in elevation in recent years, and thus, their response is of interest.

We found that competition was the strongest plant–plant interaction affecting the growth of spruce seedlings and occurred at all elevations in both the tundra and forest gradients. Further, the magnitude of competition did not vary with elevation enough to create a statistically significant elevation by neighbor interaction, in either tundra or forest sites. Thus, we did not see the patterns of plant–plant interactions predicted by the SGH (Table 3, 4). However, seedling growth differed along the two elevational gradients. Growth of seedlings in tundra sites decreased with elevation, as would be expected if they were predominantly responding to physical stress associated with decreasing temperature along the elevational gradient. In contrast, seedlings in forest sites, where light availability was less, increased their allocation to aboveground shoots and grew taller, indicating competition for light. These results suggest that, within the forest elevational gradient, resource stress (due to lack of light) may have been as important as physical stress in determining the growth responses of seedlings, as suggested by Maestre et al. (2009). Greenhouses reduced growth of seedlings in

tundra sites and at low elevations in forests, but enhanced growth at high elevations in forested sites. However, greenhouses did not alter the patterns of plant–plant interactions along the elevational gradients, in either tundra or forested sites.

Strong competition effects but no increase in the effects along the elevations

We hypothesized that competition with neighbors would decrease seedling growth at all sites, but its magnitude would be less at high elevations where the physical stress is higher. As we expected, competition decreased seedling biomass, height growth, and foliar N content, but there were no significant interactions between neighbor effects and elevation. Our results contrast with those of previous studies suggesting that at high elevations, physical stress promotes facilitation (e.g., Choler et al. 2001, Callaway et al. 2002, Michalet et al. 2014a). However, our results agree with recent studies that show decreasing or neutral relationships between facilitation and elevation (e.g., Cavieres et al. 2006, Dvorský et al. 2013, Soliveres and Maestre 2014).

One reason that we did not observe facilitation may be due to the life histories of white spruce seedlings and their neighbors. White spruce can be classified as a resource-conserving species (at least in mature trees), while many of its neighbors are faster-growing, stress-intolerant species, including deciduous shrubs such as *Betula* spp. (Fig. 2), which are mostly competitive species (Grime 1977, Bret-Harte et al. 2008, Kattge et al. 2011, Díaz et al. 2016). Michalet et al. (2006) related facilitation to life-history strategies (Grime 1977) and suggested that at intermediate environmental severity, facilitation is important for stress-intolerant species, while competition is important for stress-tolerant species. If no facilitation at any sites indicates the stress severity for seedlings was low to intermediate in our study sites, it is understandable that the interaction between seedlings of this stress-tolerant white spruce and their fast-growing neighbors could have resulted in competition.

At the highest site (AboveT:1170), there was almost no difference in biomass between seedlings with and without neighbors (Fig. 5a, c, e). It is possible that at only this site, physical stresses reduced competition, but that the stress was not

severe enough to trigger facilitation (He et al. 2013). Alternatively, facilitation may not have occurred because there was not enough neighboring plant biomass at this site to protect the seedlings (Michalet et al. 2014b), as the neighbor biomass removed from AboveT:1170 in 2012 was only two-thirds to half of the biomass from other tundra sites (Fig. 4, Table 1).

Resource stress altered patterns of plant response

We hypothesized that plant responses to physical stress gradients in temperature would differ between forest and tundra sites, because of resource stress due to low light in the forest sites. We saw evidence for light competition in the allocation patterns of seedlings growing in forested sites (Figs. 5h, 6c–d). Plants allocate photosynthate to leaves first when light limits growth, and to roots first when nutrients or water limit growth (e.g., Shipley and Peters 1990, Lambers et al. 2008, Annighöfer 2018). Allocation to shoots and height over roots in response to shading has been seen in other studies of conifer seedlings such as *Pinus sylvestris*, *P. nigra*, and *Larix decidua* (Stuiver et al. 2014, Chmura et al. 2017). Because small seedlings do not have much stored carbon, seedlings in forests allocated photosynthates preferentially toward shoots over roots to obtain more sunlight.

Whether competition or facilitation occurs may depend on the type of stress (Michalet et al. 2006, Maestre et al. 2009). A meta-analysis by He et al. (2013) reported that increasing physical stresses changed plant–plant interactions from competitive to facilitative, but low resources such as light, water, or nutrients did not. When resource stress co-occurred with physical stress, plants were more abundant and their growth variables increased without neighboring plants than with neighbors (Dvorský et al. 2013, Liancourt et al. 2017). With increasing resource stress, the plant–plant interactions would shift to a reduction in competition to neutral, but not facilitation (He et al. 2013).

Challenges for seedling growth in the warming future

The magnitude of the increase in air temperatures caused by greenhouses was slightly larger in tundra sites than in forest sites (Fig. 3a, b, Table 2), suggesting that seedling biomass

may have decreased in greenhouses in the open tundra sites due to overheating. Spruce seedling photosynthesis has been shown to decrease at temperature above 22°C, perhaps due to excessive light and water stress (Grossnickle 2000). Although white spruce usually grows better in sunny sites (Burns and Honkala 1990), only 15% of full sunlight is enough for seedling survival up to three years after planting (Grossnickle 2000). In fact, more than 80% of full solar radiation can be a stress rather than a resource (McIntire et al. 2016). Excessive light can cause chlorosis directly due to photoinhibition and damage seedlings, and also induce high water vapor deficit or desiccation (Ronco 1970, Bader et al. 2007, McIntire et al. 2016). In our study, however, the light intensity in greenhouses was less than the intensity outside, so photoinhibition was probably not a significant hazard. On the other hand, water stress would be especially problematic for plants with poorly developed root systems such as seedlings (Grossnickle 2012). In our experiment, soil moisture was always lower in greenhouses than outside, especially in tundra sites (Fig. 3d). Seedlings in greenhouses had mostly less depleted $\delta^{13}\text{C}$ in their foliage than seedlings outside (Fig. 7c,d), suggesting more stomatal closure to avoid water stress. Therefore, it is likely that our seedlings in greenhouses in tundra suffered from heat-induced water stress. If water stress becomes more common as temperatures increase in the future, it is reasonable to conclude that unless improved root systems develop, exposed habitats such as tundra could confront spruce seedlings with moisture limitation.

Although seedling growth was affected by greenhouse warming, warming did not change plant–plant interactions. Competition was neither enhanced nor decreased by greenhouse for any variables in our analyses, because none of the greenhouse-by-neighbor interactions were significant (G \times N; Table 3, 4). It is possible that even though temperatures were warmer inside the greenhouse than outside, seedlings growing in a greenhouse did not decrease physical stress by temperature. It is also possible that the growth of neighbor plants was not changed enough by experimental warming to affect their interactions with seedlings.

Warming seedlings with greenhouses may not have altered their physical stress due to temperatures if the ambient temperatures were already not cold enough. Seedlings growing in this area in the mid-1900s likely experienced a higher frequency of cool summers than has been seen in recent years, since temperatures were 2°C lower than now (Karl et al. 2009). Hence, natural warming has had a positive effect on seedling growth at high elevations (Nicklen et al. 2016), as treelines have moved upward in the DNPP (Hamm 2007, Stueve et al. 2011), including near our highest site where a few naturally occurring saplings were present (K. Okano, *personal observation*). In our experiment, however, the ambient temperatures at high elevations (generally colder than at low elevations) were possibly already warm enough that they did not limit seedling growth; thus, greenhouse warming might not have reduced physical stress due to low temperatures for seedlings any further. Rather, as our foliar $\delta^{13}\text{C}$ result suggests, seedlings at exposed tundra at high elevations may suffer from water stress, so water could become the limiting factor for their growth in dry and warm environments (Nicklen et al. 2019). If the cold temperature used to be the strongest limiting factor but is not anymore, then we could predict that the treeline in DNPP in the near future would not be able to advance as fast as we saw in the past 50 yr.

Another possible explanation why we did not see significant interactions between having neighbors and growing in a greenhouse could be due to a lack of growth enhancement of neighbors by warming. Likely, our experiment was so brief that the neighboring plants did not increase their growth in response to warming enough to change the effects on seedlings. Over a longer time frame, however, warming may affect seedlings indirectly by affecting their neighbors. Common Alaskan deciduous shrubs such as birch (*Betula nana*) and willow (*Salix pulchra*) can acquire resources rapidly (Kattge et al. 2011, Díaz et al. 2016, Pierce et al. 2017) and respond to environmental changes strongly (Chapin et al. 1995, Bret-Harte et al. 2008); however, it took them several years to respond to fertilization or warming (Chapin and Shaver 1996, Bret-Harte et al. 2004). If our experimental period were longer, neighboring deciduous shrubs in the greenhouses would probably respond strongly

and increase biomass. In addition, at the highest site (AboveT:1170), competition has been hardly seen so far, and there are few deciduous shrubs (Figs. 2, 5a, c, e). Recent alpine research in interior Alaska, including DNPP, suggests that woody species have increased in abundance and are replacing forbs due to warming (Roland et al. 2019). If warming favors recruitment of deciduous shrubs that are currently low in abundance like our highest elevation (Fig. 2), competition could become more pronounced in a future climate.

In conclusion, we found competition to be the predominant plant–plant interaction regardless of elevation and habitat type. However, the patterns of seedling growth with elevation in forest and tundra were different. Growth of seedlings in tundra sites decreased with elevation as expected, suggesting that physical stress due to low temperature reduced growth as elevation increased. In contrast, growth and allocation of seedlings in forest sites suggested that resource stress due to low light availability was more important than physical stress due to low temperatures for these seedlings. Warming did not alter the patterns of plant–plant interactions in this experiment. Instead, warming induced water stress in seedlings growing in open tundra sites, especially at high elevations. Our study suggests that resource stress, primarily light and water limitation, control seedling growth and plant–plant interactions during the critical period of seedling establishment, across a wide range of environmental conditions, and in the climate of today and in a warmer future.

ACKNOWLEDGMENTS

We thank M. Wright and J. Alden for assistance with growing seedlings prior to planting in the field; C. Roland for providing seeds from DNPP; J. Foote and M. Okada for assistance with fieldwork; M. Sybert for assistance with laboratory work; L. Oliver for assistance with soil analyses; S. Laundry, V. Stevens, RJ Stevens, and A. Crawford for logistics; and J. McIntyre, R. Barry, A. Crawford, and G. Breed for statistical consultations. We appreciate P. Shearer and R. Lachelt for providing us with access to their properties in DNPP. Special thanks are extended to Denali National Park Wilderness Center LTD (Camp Denali) and its owner, J. Hamm, for allowing us to conduct this research on their property, for in-kind support, and for advice. We

thank A. Richardson, H. Rodenhizer, L. Gomez-Aparicio, D. Peters, and two anonymous reviewers for helpful comments. Funding was provided in part by the UAF Graduate School Thesis Completion Fellowship, the Institute of Arctic Biology Summer Research Graduate Fellowships, the UAF Global Change Student Research Grant, the Friends of UWA Scholarships to Kyoko Okano, and the NSF grants DEB 1556481 and PLR 1623461 to M. Syndonia Bret-Harte. We acknowledge there is no conflict of interest in this study to declare.

LITERATURE CITED

- Annighöfer, P. 2018. Stress relief through gap creation? Growth response of a shade tolerant species (*Fagus sylvatica* L.) to a changed light environment. *Forest Ecology and Management* 415–416:139–147.
- Badano, E. I., E. Villarroel, R. O. Bustamante, P. A. Marquet, and L. A. Cavieres. 2007. Ecosystem engineering facilitates invasions by exotic plants in high-Andean ecosystems. *Journal of Ecology* 95:682–688.
- Bader, M. Y., I. Van Geloof, and M. Rietkerk. 2007. High solar radiation hinders tree regeneration above the alpine treeline in northern Ecuador. *Plant Ecology* 191:33–45.
- Batllori, E., J. J. Camarero, J. M. Ninot, and E. Gutiérrez. 2009. Seedling recruitment, survival and facilitation in alpine *Pinus uncinata* tree line ecotones. Implications and potential responses to climate warming. *Global Ecology and Biogeography* 18:460–472.
- Bertness, M. D., and R. Callaway. 1994. Positive interactions in communities. *Trends in Ecology and Evolution* 9:187–191.
- Bret-Harte, M. S., E. A. Garcia, V. M. Sacre, J. R. Whorley, J. L. Wagner, S. C. Lippert, and F. S. Chapin. 2004. Plant and soil responses to neighbour removal and fertilization in Alaskan tussock tundra. *Journal of Ecology* 92:635–647.
- Bret-Harte, M. S., M. C. Mack, G. R. Goldsmith, D. B. Sloan, J. DeMarco, G. R. Shaver, P. M. Ray, Z. Biesinger, and F. S. Chapin. 2008. Plant functional types do not predict biomass responses to removal and fertilization in Alaskan tussock tundra. *Journal of Ecology* 96:713–726.
- Bret-Harte, M. S., G. R. Shaver, J. P. Zoerner, J. F. Johnstone, J. L. Wagner, A. S. Chavez, R. F. Gunkelman IV, S. C. Lippert, and L. A. Laundre. 2001. Developmental plasticity allows *Betula nana* to dominate tundra subjected to an altered environment. *Ecology* 82:18–32.
- Burns, R. M., and B. H. Honkala. 1990. *Silvics of North America*. USFS Handbook 654. USDA Forest Service, Washington, D.C., USA.
- Callaway, R. M., et al. 2002. Positive interactions among alpine plants increase with stress. *Nature* 417:844–848.
- Callaway, R. M. 2007. Positive interactions and interdependence in plant communities. Springer, Dordrecht, The Netherlands.
- Cavieres, L. A., E. I. Badano, A. Sierra-almeida, S. Gómez-gonzález, and M. A. Molina-montenegro. 2006. Positive interactions between alpine plant species and the nurse cushion plant *Laretia acaulis* do not increase with elevation in the Andes of central Chile. *New Phytologist* 169:59–69.
- Chapin, F. S. 1980. The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics* 11:233–260.
- Chapin, F. S., and G. R. Shaver. 1996. Physiological and growth responses of arctic plants to a field experiment simulating climatic change. *Ecology* 77:822–840.
- Chapin, F. S., G. R. Shaver, A. E. Giblin, K. J. Nadelhoffer, and J. A. Laundre. 1995. Responses of arctic tundra to experimental and observed changes in climate. *Ecology* 76:694–711.
- Chapin, F. S., S. F. Trainor, P. Cochran, H. Huntington, C. Markon, M. McCammon, A. D. McGuire, and M. Serreze. 2014. Ch. Alaska. Pages 514–536 in J. M. Melillo, Terese (T.C.) Richmond, and G. W. Yohe, editors. *Climate change impacts in the United States: The third national climate assessment*. U.S. Global Change Research Program, Washington, D.C., USA.
- Chmura, D. J., J. Modrzyński, P. Chmielarz, and M. G. Tjoelker. 2017. Plasticity in seedling morphology, biomass allocation and physiology among ten temperate tree species in response to shade is related to shade tolerance and not leaf habit. *Plant Biology* 19:172–182.
- Choler, P., R. Michalet, and R. M. Callaway. 2001. Facilitation and competition on gradients in alpine plant communities. *Ecology* 82:3295–3308.
- Danby, R. K., and D. S. Hik. 2007. Responses of white spruce (*Picea glauca*) to experimental warming at a subarctic alpine treeline. *Global Change Biology* 13:437–451.
- Díaz, S., et al. 2004. The plant traits that drive ecosystems: evidence from three continents. *Journal of Vegetation Science* 15:295–304.
- Díaz, S., et al. 2016. The global spectrum of plant form and function. *Nature* 529:167–171.
- Dvorský, M., J. Doležal, M. Kopecký, Z. Chlumska, K. Janatková, J. Altman, F. De Bello, and K. Řeháková. 2013. Testing the stress-gradient hypothesis at the roof of the world: effects of the cushion plant *Thylacospermum caespitosum* on species assemblages. *PLOS ONE* 8:e53514.

- Ehleringer, J., A. E. Hall, and G. Farquhar, editors. 1993. Stable isotopes and plant carbon-water relations. Academic Press, San Diego, California, USA.
- Ettinger, A., and J. HilleRisLambers. 2017. Competition and facilitation may lead to asymmetric range shift dynamics with climate change. *Global Change Biology* 23:3921–3933.
- Farquhar, G., J. Ehleringer, and K. Hubick. 1989. Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Biology* 40:503–537.
- Graglia, E., S. Jonasson, A. Michelsen, I. K. Schmidt, M. Havström, and L. Gustavsson. 2001. Effects of environmental perturbations on abundance of subarctic plants after three, seven and ten years of treatments. *Ecography* 24:5–12.
- Grau, O., J. M. Ninot, J. H. C. Cornelissen, and T. V. Callaghan. 2013. Similar tree seedling responses to shrubs and to simulated environmental changes at Pyrenean and subarctic treelines. *Plant Ecology & Diversity Issues* 64:329–342.
- Graves, S., H. Piepho, L. Selzer, and S. Dorai-Raj. 2019. multcompView: visualizations of Paired Comparisons. R package version 0.1-8. <https://cran.r-project.org/web/packages/multcompView/multcompView.pdf>
- Greenwood, S., and A. S. Jump. 2014. Consequences of treeline shifts for the diversity and function of high altitude ecosystems. *Arctic, Antarctic, and Alpine Research* 46:829–840.
- Grime, J. P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist* 111:1169–1194.
- Grossnickle, S. C. 2000. Ecophysiology of northern spruce species: the performance of planted seedlings. NRC Research Press, Ottawa, Ontario, Canada.
- Grossnickle, S. C. 2012. Why seedlings survive: influence of plant attributes. *New Forests* 43:711–738.
- Hamilton, J. A., W. El Kayal, A. T. Hart, E. E. Runcie, A. Arango-Velez, and J. E. K. Cooke. 2016. The joint influence of photoperiod and temperature during growth cessation and development of dormancy in white spruce (*Picea glauca*). *Tree Physiology* 36:1432–1448.
- Hamm, J. 2007. Recent tree line advance and the influence of shrub and tundra communities on white spruce (*Picea glauca*) establishment in Denali National Park, Alaska. Thesis. Antioch University, Keene, New Hampshire, USA.
- He, Q., M. B. Bertness, and A. H. Altieri. 2013. Global shifts towards positive species interactions with increasing environmental stress. *Ecology Letters* 16:695–706.
- Hobbie, J. E., E. A. Hobbie, H. Drossman, M. Conte, J. C. Weber, J. Shamhart, and M. Weinrobe. 2009. Mycorrhizal fungi supply nitrogen to host plants in Arctic tundra and boreal forests: ^{15}N is the key signal. *Canadian Journal of Microbiology* 55:84–94.
- IPCC [Intergovernmental Panel on Climate Change]. 2014. Climate Change 2014: Synthesis report. Pages 1–151 in Core writing team, R. K. Pachauri, and L. A. Meyer, editors. Contribution of working groups I, II and III to the fifth assessment report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland.
- Juday, G. P., V. Barber, S. Rupp, and J. Zasada. 2003. A 200-year perspective of climate variability and the response of white spruce in interior Alaska. Pages 226–250 in D. Greenland, D. G. Goodin, and R. C. Smith, editors. Climate variability and ecosystem response at long-term ecological research (LTER) sites. Oxford University Press, Oxford, UK.
- Karl, T. R., J. M. Melillo, and T. C. Peterson. 2009. Global climate change impacts in the United States. Cambridge University Press, New York, New York, USA.
- Kattge, J., et al. 2011. TRY – a global database of plant traits. *Global Change Biology* 17:2905–2935.
- Klanderud, K., and Ø. Totland. 2007. The relative role of dispersal and local interactions for alpine plant community diversity under simulated climate warming. *Oikos* 116:1279–1288.
- Körner, C. 2003. Alpine plant life: Functional ecology of high mountain ecosystems. Springer, Basel, Switzerland.
- Körner, C. 2012. Alpine treelines: Functional ecology of the global high elevation tree limits. Springer, Basel, Switzerland.
- Kudo, G., and S. Suzuki. 2003. Warming effects on growth, production, and vegetation structure of alpine shrubs: a five-year experiment in northern Japan. *Oecologia* 135:280–287.
- Lambers, H., F. Chapin III, and T. Pons. 2008. Plant physiological ecology. Second edition. Springer, New York, New York, USA.
- Lenth, R., H. Singmann, J. Love, P. Buerkner, and M. Herve. 2020. Emmeans: estimated marginal means, aka least-squares means. R package version 1.4.8. <https://cran.r-project.org/web/packages/emmeans/emmeans.pdf>
- Liancourt, P., Y. Le Bagousse-Pingret, C. Rixen, and J. Dolezal. 2017. SGH: Stress or strain gradient hypothesis? Insights from an elevation gradient on the roof of the world. *Annals of Botany* 120:29–38.
- Maestre, F. T., R. M. Callaway, F. Valladares, and C. J. Lortie. 2009. Refining the stress-gradient hypothesis for competition and facilitation in plant communities. *Journal of Ecology* 97:199–205.
- Marion, G., et al. 1997. Open-top designs for manipulating field temperature in high-latitude ecosystems. *Global Change Biology* 3:20–32.

- McBean, G., G. Alekseev, D. Chen, E. Førland, J. Fyfe, P. Y. P. Groisman, R. King, H. Melling, R. Vose, and P. H. Whitfield. 2005. Chapter 2: Arctic climate: past and present. Pages 21–60 in J. Berner and O. W. Heal, editors. Arctic Climate Impact Assessment-Scientific Report. Cambridge University Press, Cambridge, UK.
- McDonald, J. H. 2014. Handbook of biological statistics. Third edition. Sparky House Publishing, Baltimore, Maryland, USA.
- McIntire, E. J. B., F. I. Piper, and A. Fajardo. 2016. Wind exposure and light exposure, more than elevation-related temperature, limit tree line seedling abundance on three continents. *Journal of Ecology* 104:1379–1390.
- Michalet, R., R. W. Brooker, L. A. Cavieres, Z. Kikvidze, C. J. Lortie, F. I. Pugnaire, A. Valiente-Banuet, and R. M. Callaway. 2006. Do biotic interactions shape both sides of the humped-back model of species richness in plant communities? *Ecology Letters* 9:767–773.
- Michalet, R., C. Schöb, C. J. Lortie, R. W. Brooker, and R. M. Callaway. 2014a. Partitioning net interactions among plants along altitudinal gradients to study community responses to climate change. *Functional Ecology* 28:75–86.
- Michalet, R., Y. Le Bagousse-Pinguet, J. P. Maalouf, and C. J. Lortie. 2014b. Two alternatives to the stress-gradient hypothesis at the edge of life: the collapse of facilitation and the switch from facilitation to competition. *Journal of Vegetation Science* 25:609–613.
- Nicklen, E. F., C. A. Roland, A. Z. Csank, M. Wilmking, R. W. Ruess, and L. A. Muldoon. 2019. Stand basal area and solar radiation amplify white spruce climate sensitivity in interior Alaska: evidence from carbon isotopes and tree rings. *Global Change Biology* 25:911–926.
- Nicklen, E. F., C. A. Roland, R. W. Ruess, J. H. Schmidt, and A. H. Lloyd. 2016. Local site conditions drive climate-growth responses of *Picea mariana* and *Picea glauca* in interior Alaska. *Ecosphere* 7:e01507.
- Nitschke, N., A. Ebeling, T. Rottstock, C. Scherber, C. Middelhoff, S. Creutzburg, A. Weigelt, T. Tscharntke, M. Fischer, and W. W. Weisser. 2010. Time course of plant diversity effects on *Centaurea jacea* establishment and the role of competition and herbivory. *Journal of Plant Ecology* 3:109–121.
- Okano, K. 2018. Roles of neighboring plant and temperature on growth and survival of white spruce seedlings along elevational gradient in Alaska. Thesis. University of Alaska Fairbanks, Fairbanks, Alaska, USA.
- Okano, K., and M. S. Bret-Harte. 2015. Warming and neighbor removal affect white spruce seedling growth differently above and below treeline. *SpringerPlus* 4:79.
- Pierce, S., et al. 2017. A global method for calculating plant CSR ecological strategies applied across biomes world-wide. *Functional Ecology* 31:444–457.
- R Development Core Team. 2020. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rixen, C., and C. H. Mulder. 2009. Species removal and experimental warming in a subarctic tundra plant community. *Oecologia* 161:173–186.
- Roland, C. A., G. Sadot, E. F. Nicklen, S. A. McAfee, and S. E. Stehn. 2019. A structural equation model linking past and present plant diversity in Alaska: a framework for evaluating future change. *Ecosphere* 10:e02832.
- Roland, C. A., J. H. Schmidt, and J. F. Johnstone. 2014. Climate sensitivity of reproduction in a mast-seeding boreal conifer across its distributional range from lowland to treeline forests. *Oecologia* 174:665–677.
- Roland, C. A., and C. Stehn. 2014. Denali repeat photography project reveals dramatic changes: a drier, woodier, and more densely vegetated park. *Alaska Park Science* 12:64–65.
- Ronco, F. 1970. Influence of high light intensity on survival of planted Engelmann spruce. *Forest Science* 16:331–339.
- Schmid, B., A. Hector, M. A. Huston, P. Inchausti, I. Nijs, P. W. Leadley, and D. Tilman. 2002. The design and analysis of biodiversity experiments. Pages 61–75 in M. Loreau, S. Naeem, and P. Inchausti, editors. *Biodiversity and ecosystem functioning: synthesis and perspectives*. Oxford University Press, Oxford, New York, New York, USA.
- Shipley, B., and R. H. Peters. 1990. A test of the Tilman model of plant strategies: relative growth rate and biomass partitioning. *American Naturalist* 136:139–153.
- Sistla, S. A., J. C. Moore, R. T. Simpson, L. Gough, G. R. Shaver, and J. P. Schimel. 2013. Long-term warming restructures Arctic tundra without changing net soil carbon storage. *Nature* 497:615–618.
- Soliveres, S., and F. T. Maestre. 2014. Plant – plant interactions, environmental gradients and plant diversity: a global synthesis of community-level studies. *Perspectives in Plant Ecology Evolution and Systematics* 16:154–163.
- Stueve, K. M., R. E. Isaacs, L. E. Tyrrell, and R. V. Densmore. 2011. Spatial variability of biotic and abiotic tree establishment constraints across a treeline ecotone in the Alaska Range. *Ecology* 92:496–506.
- Stuiver, B. M., D. A. Wardle, M. J. Gundale, and M. C. Nilsson. 2014. The impact of moss species and biomass on the growth of *Pinus sylvestris* tree seedlings

- at different precipitation frequencies. *Forests* 5:1931–1951.
- Sullivan, P. F., S. B. Z. Ellison, R. W. McNow, A. H. Brownlee, and B. Sveinbjörnsson. 2015. Evidence of soil nutrient availability as the proximate constraint on growth of treeline trees in northwest Alaska. *Ecology* 96:716–727.
- Welshofer, K. B., P. L. Zarnetske, N. K. Lany, and L. A. E. Thompson. 2018. Open-top chambers for temperature manipulation in stature plant communities. *Methods in Ecology and Evolution* 9:254–259.
- Woods, N. N., R. McCarthy, and M. N. Miriti. 2019. Non-hierarchical competition among co-occurring woody seedlings in a resource-limited environment. *Ecosphere* 10:e02751.
- Young, D. 2007. Estimating aboveground net primary production in shrub-dominated ecosystems. Pages 49–62 in T. J. Fahey and A. K. Knapp, editors. *Principals and standards for measuring primary production*. Oxford University Press, New York, New York, USA.
- Zamin, T. J., M. S. Bret-Harte, and P. Grogan. 2014. Evergreen shrubs dominate responses to experimental summer warming and fertilization in Canadian mesic low arctic tundra. *Journal of Ecology* 102:749–766.

SUPPORTING INFORMATION

Additional Supporting Information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3508/full>