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Shrub–tree interactions and environmental changes drive treeline dynamics in the Subarctic

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Treelines have drawn persistent research interest as they can respond markedly to climate. However, the mechanisms that determine tree seedling recruitment and the response of the forest-tundra ecotone to environmental changes remain poorly understood. We hypothesise that treeline tree seedling performance depends on the interplay between climatic and soil nutritional changes and facilitative and competitive interactions between trees and shrubs. We conducted a seedling transplantation experiment with *Betula pubescens* at a subarctic treeline, in northern Sweden, which followed a full factorial design with four treatment factors relating to environmental regimes of stress and resource availability: site (forest vs treeline); temperature (+/– passive warming); shrub presence (+/– *Vaccinium myrtillus* removal); and nutrient availability (+/– NPK addition). During three growing seasons we assessed the establishment and performance of *Betula*. The experimental manipulations caused highly significant effects on seedling performance. Although *Vaccinium* enhanced seedling survival and reduced the effects of excessive solar radiation and insect herbivory, the seedlings growing with the shrub had a poorer performance by the end of the experimental period. Also, seedlings in the forest had a poorer performance than those at the treeline. *Betula* seedlings showed a very pronounced and positive response to passive warming and to nutrient addition, but such effects were more evident at the treeline site and often interacted with the presence of *Vaccinium*. This experiment shows that shrub–tree interactions are important drivers of subarctic treeline dynamics and that they vary with time and space. Facilitation, competition, herbivory and environmental changes at the tree seedling stage act as important filters in structuring the forest–tundra ecotone. We demonstrate that changes in this ecotone cannot be simply predicted from changing temperature patterns alone, and that complex interactions need to be considered, not only between shrubs and trees, but also with herbivores and between warming and soil nutrient availability.

The effect of global warming on recent vegetation dynamics has been widely studied during the last few years because increases in temperature are expected to modify the distribution of some major vegetation zones, especially at northernmost latitudes, where global warming is amplified (IPCC 2007). Two northern vegetation zones of particular importance in terms of extent, biological singularity and land–atmosphere interactions are the tundra and the boreal/subarctic forest (Callaghan et al. 2002).

Treelines have moved substantially during the Holocene in response to changes in climate (Tinner and Theurillat 2003). As the treeline has been associated with the mean July air isotherm of 11°C at subarctic latitudes (Körner 1998), we expect a northward (latitudinal) and upward (elevational) relocation of subarctic–alpine treelines in the future. However, some changes in treeline have been shown to depend more on herbivory and change in land use than on climate change (Cairns and Moen 2004, Van Bogaert et al. 2011). As vegetation dynamics are the result of the interaction of several of these factors, which are difficult to evaluate in the field or to reproduce experimentally, causal relationships behind observed treeline relocations are still poorly understood.

The impacts of global environmental change on the dynamics of treeline trees and shrubs have been investigated recently through observations, experiments and modelling with complex and often conflicting results (Dullinger et al. 2004). For instance, recent observations in some northern areas in Europe report a significant emergence of tree seedlings and saplings high above the treeline (Kullman 2002) as well as important treeline ecotone densification processes (Tømmervik et al. 2004, Rundqvist et al. 2011), although in some Scandinavian areas receding treelines have also been observed (Dalen and Hofgaard 2005), while in other nearby locations the treeline has remained static for about 200 years (Van Bogaert et al. 2011) despite recent climate warming. Similarly, several studies report an increase in shrub productivity and an advance in shrub communities across higher altitudes and latitudes due to warming climate, but again there is remarkable variability in responses from region to region and even locally (Sturm et al. 2001, Hallinger et al. 2010).

These observations of recent dynamics of treelines and shrublines are more heterogeneous than the results of numerous environmental manipulation experiments that create passive warming in the tundra or at treeline (Walker et al. 2006, Danby and Hik 2007). In general, these experiments show increased shrub growth in response to warming. Further, vegetation models generally predict significant displacement of tundra by boreal/subarctic forest in response to the projected warming (Euskirchen et al. 2009); however, the invasion of forest and shrubs into the tundra is further complicated by an array of possible drivers of the dynamics beyond recent climate warming. These include human disturbance (Vlassova 2002), extensive land use e.g. by reindeer herding (Moen et al. 2004), geomorphological characteristics (Holtmeier and Broll 2005), herbivory by vertebrates (Olofsson et al. 2009) and invertebrates (Van Bogaert et al. 2009) and extreme winter warming events (Bokhorst et al. 2009).

The role of shrubs as regulators of treelines has been poorly studied in alpine systems, although there are indications that both the forest boundary itself (Cuevas 2000) and the shrub community are key factors controlling tree seedling recruitment at treeline (Batllori et al. 2009). Shrubs facilitate seedling survival and/or performance in different ecosystems (Gómez-Aparicio et al. 2005, Grau et al. 2010), suggesting that they may have a strong effect on treeline dynamics in the Subarctic. However, as far as we know, this has not been demonstrated. In fact, dwarf shrubs have been frequently disregarded in treeline research despite their important occurrence across the forest–tundra ecotone.

Plant–plant interactions may steadily shift from competition to facilitation with increasing environmental severity (Brooker and Callaghan 1998), referred to as the stress-gradient hypothesis. Callaway et al. (2002) argued the need for more experiments on environmental change effects on individual plant–plant interactions and their consequences for community composition, dynamics and diversity, because environmental changes might affect plant–plant interactions rather than plant individuals directly. This study seeks to meet this need in the context of shrub–tree interactions at the treeline in the Subarctic, aiming to investigate: 1) whether shrub-tree interactions influence treeline dynamics observed in the forest–tundra ecotone; 2) the relative importance of potential abiotic and biotic drivers on tree seedling survival and growth across this ecotone; 3) the response of tree seedlings growing in this ecotone to distinct relevant environmental change scenarios. To address these aims we set up an experimental field study in the Subarctic; the approach was to expose transplanted tree seedlings to passive manipulations of air temperature, nutrient addition and shrub removal at two experimental locations across the forest–tundra ecotone with distinct environmental stress conditions.

We hypothesise that, at treelines with substantial shrub cover: 1) facilitation and competition are important drivers of treeline dynamics, and their relative importance will shift across the forest–tundra ecotone; 2) trees will respond positively to tree-shrub interactions at the seedling stage, as shrubs may facilitate tree seedling survival and development, especially under severe environmental conditions (we expect harsher conditions at the treeline compared to the forest); 3) a warmer and nutrient-rich environmental scenario will positively affect the survival and performance of tree seedlings at the forest–tundra ecotone.

Material and methods

Study area

The experiment was conducted on the eastern slope of Mount Nuolja (68°21′562″N, 18°44′504″E) in the Scandes mountain range, near the Abisko (Abeskovvu) National Park (Lapland, Sweden), which is near the Abisko Scientific Research Station (Abisko Naturvetenskapliga Station, ANS), about 200 km north of the Arctic Circle. We chose two neighbouring study sites: one at the treeline (661 m a.s.l.) and another in a closed forest immediately below the treeline (589 m a.s.l.), separated by approximately 140 m horizontally. The upper site was an open area, with a very low density of mountain birches *Betula pubescens* ssp. *czerepanovii*) higher than 5 m (about 0.7 trees 100 m^{-2}), whereas in the lower site the birches formed a rather closed forest with a denser canopy of higher trees (about 4.6 trees 100 m^{-2}). In both sites, *Vaccinium myrtillus* (bilberry) dominated the field layer ($>50\%$ cover), although there were also other abundant dwarf shrubs (mainly *Empetrum nigrum* ssp. *hermaphroditum*, *Betula nana*, *Vaccinium vitis-idaea, Juniperus communis*), shrubs (*Salix* sp.) and a few grasses and herbs (*Deschampsia flexuosa*, *Cornus suecica*).

The experimental site has a subarctic montane climate. Mean annual temperature measured at the ANS (388 m a.s.l.) for the period 1980–2009 is -0.09 ± 0.89°C, and the mean temperatures of the coldest and hottest months are -10.1 ± 3.7°C and $+11.7 \pm 1.4$°C, in February and July respectively (ANS data archive). Given the slightly higher elevation compared to ANS, we estimate the mean annual temperatures at the study sites to be generally about 3°C lower than these values, assuming a temperature decline of 1°C 100 m^{-1}. The study area is situated in the rain-shadow of Mount Nuolja, where precipitation is remarkably low compared to nearby areas. Mean annual precipitation at Abisko during 1980–2009 amounts to 335.9 ± 63.6 mm, about 40% of which is during the growing season, from June to August (ANS data archive). Climate warming in subarctic Sweden since 2000 has exceeded the warming recorded in the late 1930s and early 1940s and has accelerated. Smoothed mean annual temperatures have risen between 1913 and 2006 by 2.5°C, and have significantly exceeded the 0°C threshold for the first time in the 20th and 21st centuries. Spring warming is the strongest contributor to this long-term mean annual temperature rise and winter temperature the weakest. Summer precipitation increased over the second half of the 20th century (Callaghan et al. 2010); periods of heavy snow fall have doubled over the last century (Kohler et al. 2006). However, winters have been extremely dry with relatively shallow snow since the late 1990s (Callaghan et al. 2010) and there has been an increase in the length of the growing season (Andrews et al. 2011).

Study species

We used seedlings of *Betula pubescens* ssp. *czerepanovii* (*Betula* hereafter). *Betula* is generally restricted to upland areas, forming subarctic forests and altitudinal and latitudinal treelines in northern Fennoscandia and along the mountain chains in Norway and Sweden. It often forms polycormic (multi-stemmed) structures, especially in dry, oligotrophic heaths (Wielgolaski 2002), and it is believed to be a multi-origin hybrid between *B. pubescens* ssp. *pubescens* and *B. nana* L. (Thórsson et al. 2001). At the start of the growing season, the red leaf colouration of *Betula* seedlings indicates increased anthocyanin concentration in response to high solar radiation (Hughes et al. 2005), which is associated with lower photosynthetic capacity (Close and Beadle 2003).

The dominant agents of disturbance to the birch forest in the study area are the more or less cyclic pest outbreaks of the autumnal moth *Epirrita autumnata*, the winter moth *Operophtera brumata* (Babst et al. 2010) and disturbance by reindeer *Rangifer tarandus* (Cairns and Moen 2004, Van Bogaert et al. 2011).

Vaccinium myrtillus (*Vaccinium* hereafter) is an ericaceous deciduous dwarf shrub which forms very extensive carpets in subarctic heaths and it is also found in the understory of Nordic mountain birch forests, where it may constitute over 40% of the ground cover (Laakso et al. 1990). It is a semi-shade species, occurring in acidic soils. Some studies suggest that *Vaccinium* has a positive effect on tree growth (Chrimes et al. 2004), whereas others have shown its allelopathic effects on seedling recruitment and performance (Gallet 1994). *Vaccinium* is periodically disturbed by the rodent *Clethrionomys rufocanus* (grey-sided vole), which feeds on stems below the snow pack during winter causing huge damage to the shrub (Dahlgren et al. 2007).

Experimental design

The experiment simulated several environmental scenarios at the forest–tundra ecotone between August 2006 and September 2009. To initiate the experiment, we transplanted 384 *Betula* seedlings into the experimental units and after these three growing seasons they were collected for laboratory analyses.

The experiment followed a full factorial design with four binary factors, with a total of 16 distinct combinations of treatments. The factors were: 1) site (forest vs treeline); 2) temperature (± open top chambers to give passive warming, 'OTC' hereafter); 3) shrub presence (± *Vaccinium* removal); 4) nutrient availability (± NPK addition). The treatments were distributed among 16 experimental units of approximately 1.13 m^2 in each of the two sites; each unit was divided into two halves or microplots (with and without *Vaccinium* removal, $n = 32$ microplots in each site). Into each microplot (or replicate) we transplanted six *Betula* seedlings (pseudoreplicates). In total we had four replicates per treatment combination ($n = 6$ seedlings × 2 microplots × 16 experimental units × 2 sites, $n = 384$ seedlings in total).

The experimental units were installed in an area (about 150 m^2) where *Vaccinium* was the most dominant species, and where there would be sufficient space for them, both in the forest and at the treeline. The experimental units were situated on patches with the highest *Vaccinium* cover possible (over 50% in all cases) and the shrub density of the patches selected was very similar between sites and experimental units. *Vaccinium* was cut in the shrub removal microplots at the beginning of the growing season each year and again during summer to eliminate any re-growth. Roots were not removed so as not to cause excessive disturbance. Once the seedlings had been planted within each microplot we distributed OTC and/or NPK treatments randomly among the experimental units in each of both study sites. Re-growth of ramets and redistribution of nutrients from ramets outside the plots were prevented annually by severing all below-ground connections with a spade along the border of the removal plot.

This study was restricted to the tree seedling stage: experimentation with saplings or mature trees is logistically impractical in the natural environment and would cause unnecessary environmental impacts. On the other hand, the period of study required to sow tree seeds and to follow emerging seedlings would not be feasible. All the seedlings were therefore 2–3 years old, but their heights ranged from 1.5 to 4.5 cm. These tree seedlings for transplantation were collected from a large population in a road embankment at the border of the Abisko National Park, next to ANS. Only healthy seedlings with uniform appearance were chosen. They were transplanted soon after collecting them and a small amount of original soil was kept around the roots. In order to account for the differences in height and to uniformly distribute the seedlings into the experimental units, they were sorted into four groups (1.5–2; 2.1–3; 3.1–4; 4.1–4.5 cm) and distributed in a proportion of 1:2:2:1 respectively in each microplot. The seedlings were watered a few times during the first week after transplantation to avoid seedling mortality. Since the overall survival after transplantation was very high (over 80%) we did not replace the dead seedlings.

The OTCs were designed according to hexagonal ITEX models (Marion et al. 1997) in order to simulate an expected increase of air temperature (Sælthun and Barkved 2003). For uniformity, those experimental units without OTCs were also hexagonally shaped and they all had the same area (1.13 m^2). The ambient air temperature regime in the OTCs was measured by temperature loggers (two sensors per treatment); these indicated that the mean temperature increase during the growing season in the OTC compared to control plots was 0.99°C at the treeline and 0.90°C in the forest. This difference between treeline and forest was very constant throughout the summer despite monthly variation; for instance, in July, which was the hottest month, the temperature increase in the OTCs was 1.01°C at treeline and 0.99°C in the forest. The mean summer temperature at the treeline was approximately 2.6°C higher than in the forest (owing presumably to interception of radiation by *Betula* trees) and this difference was most marked in July, when it reached 3.4°C. At the treeline, microplots without OTCs but with *Vaccinium* had a considerably lower temperature than those without the shrub (2.5°C less), whereas the difference was much smaller in the forest (0.4°C).

The OTCs were put out in the field at the end of August 2006 and they were not removed during the experimental period. Snow depth in both study sites was much thicker than the height of the OTCs during most of the cold season, so we assume that the differences in snow depth on the microplots with and without chambers were not too important, although the OTCs could potentially affect the rate of thaw, the duration of the snow-free season and length of the growing season.

Fertilisation treatments started in early July 2007, when we added 175 g of slow-release NPK granules (10% N, 5% P_2O_5, 20% K) to each fertilised experimental unit, which simulated an assumed moderate fertilisation effect by 15 g N m^{-2}, 4.3 g P m^{-2} and 25 g K m^{-2}. The enhanced soil nutrient availability aimed at mimicking the effects of increased nutrient mineralisation that would be expected with soil warming (Rustad et al. 2001). The release of the NPK granules was not always as homogeneous as expected over the microplots, as some seedlings suffered from excessive fertilisation due to an over-aggregation of the granules, and this caused seedling mortality in some of the fertilised plots. Thus, we excluded any seedlings which had suffered from lethal or sublethal negative effects from the analyses. Consequently, four out of the 64 microplots were not considered in the analysis; the combination + NPK + OTC + *Vaccinium* + Forest was especially affected.

Data collection

The data were collected over three growing seasons, from 2007 to 2009 in two sampling periods each year: in early summer (mid or late June) and before leaf senescence (late August or early September). In each sampling period, and for each of the *Betula* seedlings, we measured several indicators of their performance: survival, size of all leaves, number of leaves, stem length and leaf injuries (damage due to high solar radiation and herbivory symptoms). We assessed the effects of solar radiation by recording the presence or absence of sun-induced red colouration on the leaves of *Betula* seedlings in June at the start of each season. The caterpillar herbivory symptoms on the seedlings were recorded by counting the amount of eaten leaves at the start and at the end of each summer season. The number of buds was also counted during the first two years. Also, in 2009 the leaf chlorophyll content was estimated non-destructively in the field using an absorbance-based Soil Plant Analysis Development chlorophyll meter. The relationship between chlorophyll concentration and SPAD values in *Betula* is expected to follow an exponential function with increasing chlorophyll concentration at higher SPAD values (Uddling et al. 2007). For these measurements we selected the first healthy leaf big enough to be properly measured with SPAD starting from the top of the seedling; four values were obtained and averaged for each leaf measured.

In late August 2009 the seedlings were collected and immediately processed in the lab, where we measured diameter of seedlings' stems and leaf weight. The samples were then dried to analyse C and N content. The leaves from all seedlings (pseudoreplicates) of each microplot (replicate) were mixed, ground and total carbon and nitrogen were determined by dry combustion with a elemental analyser.

Statistical analyses

Each measured variable for a given sampling period was first analysed in a linear mixed model as implemented in R 2.11.0, using the 'nlme' and 'lme4' packages (R Development Core Team 2010). To account for the grouping structure of the data, 'microplots' and 'shrubs' (nested within 'microplots') were considered as random factors. Given a certain variable, the interaction terms and factors which had no statistical support ($p\text{-value} > 0.05$) were removed from the model. The significance of the remaining interactions and factors was recalculated every time that a term was excluded in the analyses provided that the new model obtained was improved ($p\text{-value} < 0.05$) when compared with the more complex model by means of a likelihood ratio test. The significance of each factor was based on the minimal adequate model. This allowed us to avoid the multi-term interactions in most cases and to simplify the interpretation of the results. This analysis identified the effect of a given factor (i.e. site, shrub presence, temperature increase and fertiliser addition) in each sampling period as summarised in Table 1, and provides information about the underlying processes which determine the short-term growth of *Betula* seedlings.

Secondly, in order to simplify the interpretation of Table 1 and to assess the temporal trends observed for the variables measured, we ran another analysis with those variables that had been sampled during the whole experimental period and that were expected to change gradually through time (i.e. stem length, number of leaves and leaf length; Table 2). This analysis revealed the overall balance of the effects derived from each experimental factor on the growth rate considering the whole experimental period. The analysis was based on the same mixed model described above, but including time as a covariable. Furthermore, this model also included an autocorrelated error term which took into account the fact that the measures were repeated on the same individuals throughout the experimental period (Pinheiro and Bates 2000). We also obtained the minimal adequate model as explained earlier.

Results

The experimental manipulations caused very significant and fast changes to seedling performance, initiating distinct responses between treatments with regard to biotic and abiotic drivers. *Vaccinium* had both important positive and negative effects on *Betula* seedling performance and survival, which changed over the course of the experiment. The treeline site offered better growing conditions than the forest site; environmental manipulations also caused a very significant effect on seedling development (Table 1, 2).

Positive effects of *Vaccinium* on *Betula* seedlings

The most crucial positive effect of *Vaccinium* on *Betula* seedlings is that seedling survival was significantly higher in *Vaccinium* patches both at the treeline and in the forest; this effect was more evident in 2007 than at the end of the experiment. The overall survival was lower in 2009 than in 2007 (Fig. 1).

The presence of *Vaccinium* was also associated with a significant reduction in the proportion of red *Betula* leaves in 2007 and 2009, in both the forest and the treeline sites (Fig. 2). There was no difference in the proportion of red

Table 1. Summary table including the statistically significant results (p-value < 0.05) found in each sampling period for all the variables measured in the experiment. The symbols > and < indicate a statistically significant increase and decrease of a given variable, respectively. The results correspond to one of the two states of each factor (site, shrub, temperature and fertiliser); the effects observed for the other state of each factor have the opposite signal. Statistical interactions between factors are marked in brackets, indicating the significant state of a given factor. See values of means and standard deviations in Supplementary material Appendix 1 Table A1. *Note: more variables were recorded in August 2009.

	June 2007	August 2007	June 2008	August 2008	June 2009	August 2009*
Forest site	> longest leaf		> longest leaf		> longest leaf	< longest leaf
	> stem length				< stem length	< stem length
			< number leaves	< number leaves	< number leaves	< number leaves
	< red damage		< red damage		< red damage	
		> herbivory	> herbivory	> herbivory	< herbivory	< herbivory
						< diameter
					< SPAD	< SPAD
						< % N in leaves
						< % C in leaves
						> CN ratio
						< total leaf biomass
						< total N
						> [N]/total N ratio
+ *Vaccinium*						< longest leaf
		> stem length (forest)	> stem length (forest)	> stem length (forest)	> stem length (forest)	
				< stem length (treeline)		
		< number leaves (treeline)	< number leaves	< number leaves	< number leaves	< number leaves (treeline)
	< red damage (treeline)			< red damage	< red damage	< diameter (treeline)
		< herbivory			< herbivory	
		< number buds				
	> survival		Rodent outbreak (*Clethrionomys rufocanus* grazing on *Vaccinium*)			
+ OTC	> longest leaf		> longest leaf (treeline)		> longest leaf (treeline)	> longest leaf (treeline)
				> stem length (treeline)	> stem length (treeline)	> stem length (treeline)
					> number leaves	
			< red damage (treeline)	< red damage	> red damage	
		< herbivory				> SPAD (treeline)
						> total leaf biomass (w/o *Vaccinium*)
						> total N (w/o *Vaccinium*)
						< [N]/total N ratio (w/o *Vaccinium*)
+ NPK		> longest leaf	> longest leaf			> longest leaf (treeline)
						< longest leaf (forest)
		> stem length (treeline)	> stem length (treeline)	> stem length (treeline)	> stem length (treeline)	> stem length (treeline with OTC)
	(not applied yet)	> number leaves (treeline, w/o *Vaccinium*)	> number leaves	> number leaves (with OTC)	> number leaves	> number leaves (treeline, w/o *Vaccinium*)
			> red damage		< red damage	
		> number buds				> diameter (with OTC or in forest)
						> % N in leaves
						> total leaf biomass (w/o *Vaccinium*)
						> total N (w/o *Vaccinium*)
						< [N]/total N ratio (w/o *Vaccinium*)

Table 2. Summary table of the results obtained for the whole experimental period from a mixed model analysis using time as a covariable and an autocorrelated error term. This table summarises the temporal trends observed on the seedlings derived from each of the experimental factors after three growing seasons (2007–2009). Only the variables that were measured repeatedly on the same seedlings throughout the experimental period and that could show a temporal trend have been considered in this analysis. See caption of Table 1 for more details about the symbols in the table; n.s. indicates 'not significant'.

Forest site	+ *Vaccinium*	+ OTC	+ NPK
< stem length	stem length (n.s.)	> stem length (treeline)	> stem length (treeline, w/o *Vaccinium*)
< number leaves	< number leaves (treeline)	> number leaves (treeline)	> number leaves
< longest leaf	< longest leaf (treeline)	> longest leaf (treeline)	< longest leaf (forest)

Betula leaves between treatments with and without *Vaccinium* in June 2008, probably due to the decreased *Vaccinium* cover associated with intense herbivory of the vole *Clethrionomys rufocanus*.

In addition, *Vaccinium* presence was related to less herbivory on *Betula* seedlings by caterpillars (probably mainly *Epirrita autumnata*) in two of six sampling periods (Table 1). Site was an important factor in five of six sampling periods; from August 2007 until August 2008 there was more herbivory in the forest site, whereas in 2009 herbivory was stronger at the treeline (Table 1).

Negative effects of *Vaccinium* on *Betula* seedlings

Despite the positive effects of *Vaccinium*, *Betula* seedlings gradually showed poorer development in response to *Vaccinium*. At the end of the first growing season and also at later stages of development, both positive and negative effects co-occurred. However, towards the end of the experiment, lower number of leaves, smaller leaf size and thinner stems indicated progressively reduced performance of seedlings growing in *Vaccinium*, sometimes interacting with 'site' (Fig. 3, Table 1, Supplementary Material Appendix 1 Fig. A2, Table A1). When considering the whole experimental period, the number and length of *Betula* leaves decreased at the treeline site if *Vaccinium* was present (Table 2).

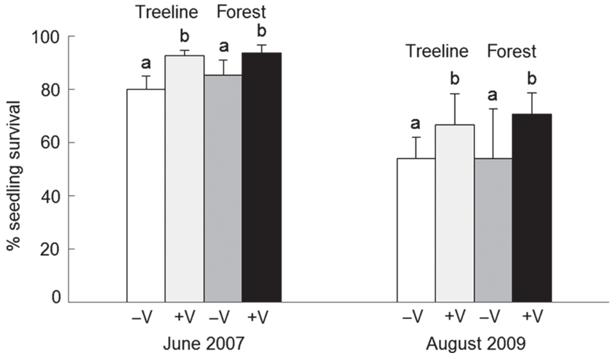

Figure 1. Percentage of surviving *Betula* seedlings at the beginning (June 2007) and at the end (August 2009) of the experiment. As only shrub presence and site across the ecotone gave consistent results in relation to survival, the mixed model analysis applied here considered only the factors 'site' and '*Vaccinium*' in control treatments (without temperature increase nor fertiliser addition). Treatments with different letters are significantly different (p-value = 0.027) in 2007 and marginally significantly different (p-value = 0.082) in 2009. The error bars indicate the standard error.

Forest versus treeline

Survival did not differ significantly between sites (Fig. 1). However, seedlings in the forest switched from a better development at the beginning of the experiment to a reduced performance at the end, when lower number of leaves, smaller leaf size, shorter and thinner stems, lower chlorophyll content, lower total leaf biomass and total N, and lower percentage of N and C in the leaves were found at the forest site compared to that at treeline (Fig. 3, Table 1, Supplementary Material Appendix 1 Fig. A1–A3, Table A1). However, the proportion of seedlings with red leaves due to solar radiation was always lower in the forest (Fig. 2, Table 1), and the C/N ratio and the N concentration/total N ratio ([N]/total N ratio hereafter) were higher (Table 1, Supplementary Material Appendix 1 Table A1).

Abiotic manipulations

OTCs exerted a very positive effect on the seedlings as most performance variables responded to increased temperature with time (Table 1, 2). However, this positive response was almost exclusive to those seedlings growing at the treeline, indicating a strong interaction between 'site' and 'OTC' throughout the whole experiment. The effect of increased temperature did not result in any effect on seedling survival during the period of study.

During the last growing season, the most vigorous seedlings were found at the treeline, suggesting that the position across the ecotone strongly determines seedling development; in June 2009 they had higher number of leaves, longer stems and larger leaves (Fig. 3, Table 1, Supplementary Material Appendix 1 Fig. A1–2) and in August 2009 they had longer stems, bigger leaves and also higher chlorophyll content (Table 1, Supplementary Material Appendix 1 Fig. A1–3). In August 2009, the *Betula* seedlings growing in OTCs showed a strong interaction with the presence of *Vaccinium*, since the total leaf biomass and the total N content were higher, and the [N]/total N ratio lower, in microplots with OTCs but without the shrub (Fig. 4, Table 1, Supplementary Material Appendix 1 Fig. A4–5, Table A1).

Nutrient addition quickly stimulated seedling development (Table 1, 2). However, fertilisation interacted very often with the other treatments. NPK generally resulted in longer stems and larger leaves at the treeline during most of the experimental period (Table 1, Supplementary Material Appendix 1 Fig. A1–2); the number of leaves in fertilised plots was always significantly higher but it also showed interactions with other factors (Fig. 3, Table 1). The treatment + OTC + NPK also resulted in a very significant

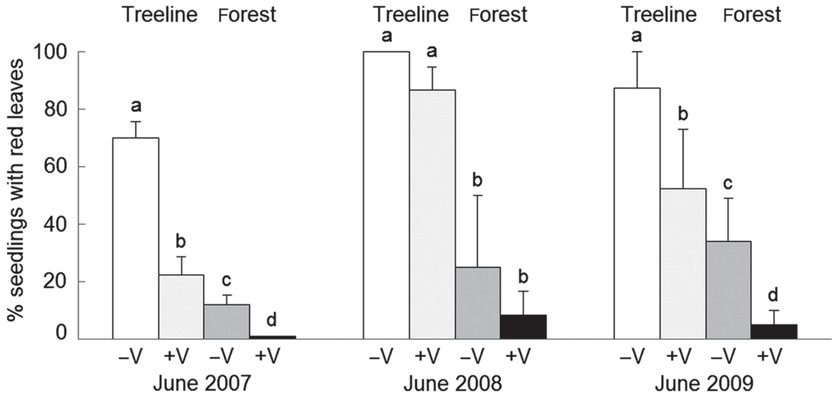

Figure 2. Percentage of *Betula* seedlings with leaves damaged by solar radiation over the course of the experiment. As only shrub presence and site across the ecotone gave consistent results in relation to solar radiation, the mixed model analysis applied here considered only the factors 'site' and '*Vaccinium*' in control treatments (neither temperature increase nor fertiliser addition). We only show the results at the start of each growing season, when the effects of solar radiation were more evident. Treatments with different letters are significantly different (p-value < 0.05) within each year. The error bars indicate the standard errors.

improvement in seedling vigour at the end of the experiment, denoted by significantly thicker and longer stems (Table 1, Supplementary Material Appendix 1 Table A1).

The N content in *Betula* leaves was significantly higher in fertilised plots and there was no interaction with other treatments (Table 1, Supplementary Material Appendix 1

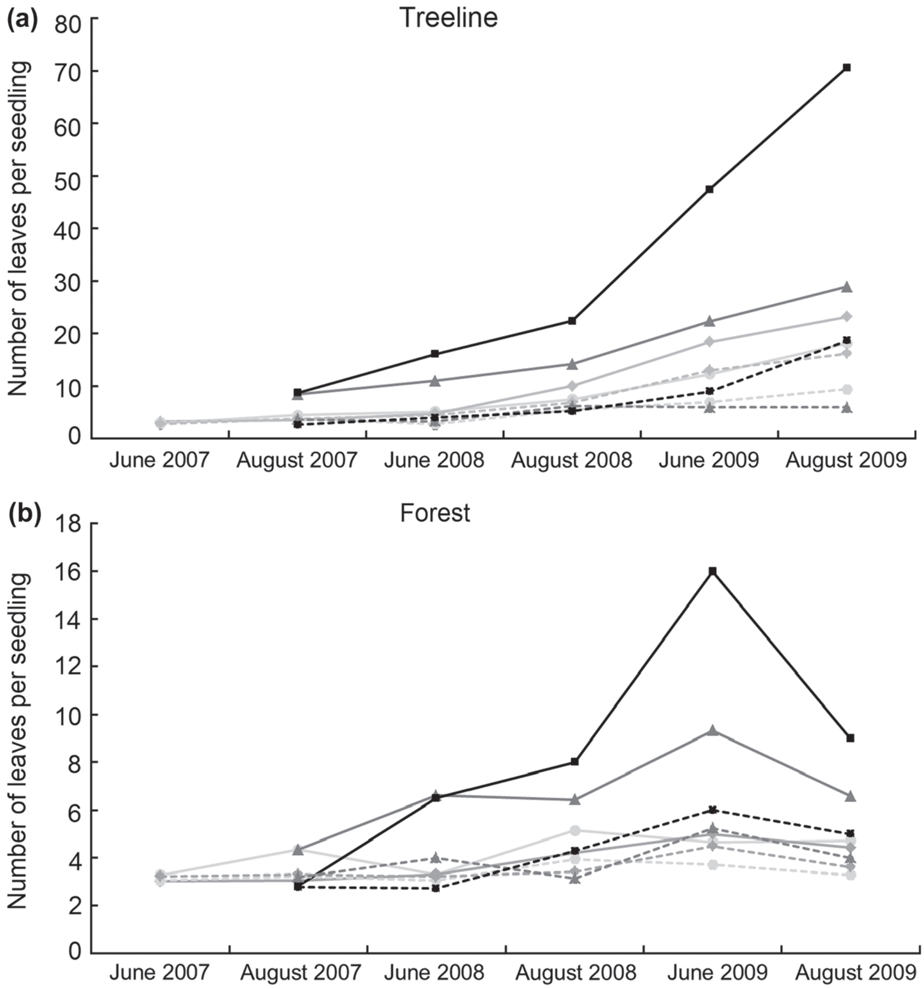

Figure 3. Mean number of leaves of *Betula* seedlings during the experimental period (a) at the treeline and (b) at the forest site for each of the treatments. Note that the y-axes have very different scales for treeline and forest. The statistical significances of each factor in each period are summarised in Table 1. Mean values and standard deviations of each value are detailed in the Supplementary material Appendix 1 Table A1. Treatments: light grey circles: –NPK –OTC; grey rhombus: –NPK +OTC; dark grey triangles: + NPK –OTC; black squares: + NPK +OTC. Dashed lines correspond to seedlings growing in *Vaccinium* and solid lines to seedlings without *Vaccinium*.

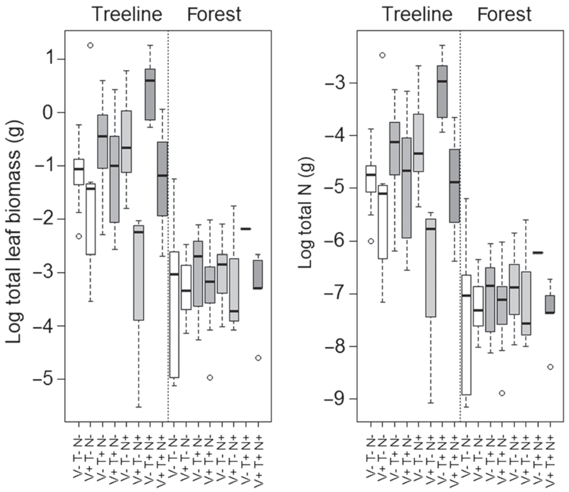

Figure 4. Left: log of total leaf dry biomass of *Betula* seedlings in August 2009. Right: log of total N content in the leaves of *Betula* seedlings in August 2009. The statistical significance of each factor is summarised in Table 1. The boxes in the figure correspond to the 1st and 3rd quartiles and the line inside the boxes defines the median. The whiskers indicate the highest and the lowest values within one and a half interquartile range from the 3rd and 1st quartile respectively. The circles indicate the outliers. Legend: V–: without *Vaccinium*; V + : with *Vaccinium*; T–: without OTC; T+ : with OTC; N–: without fertiliser; N+ : with fertiliser.

Table A1). Similarly to the temperature treatment, the NPK treatment showed a strong interaction with the presence of *Vaccinium*, and in this case the total leaf biomass and the total N content were also higher, and the [N]/total N ratio lower, in microplots without the shrub (Fig. 4, Table 1, Supplementary Material Appendix 1 Fig. A4, Table A1).

Discussion

This experiment comprehensively shows that shrub–tree interactions are important drivers of subarctic treeline dynamics, and these interactions change over the ontogeny of a tree (Soliveres et al. 2010). As hypothesised, our results indicate that facilitation and competition (but also herbivory) act, together with other factors, as important filters in structuring the extant plant communities at a given site, as suggested by Lortie et al. (2004), and that *Betula* seedlings responded clearly to shrub–tree interactions at the seedling stage. These filters may operate separately or interact with each other.

Facilitative interactions between *Vaccinium* and *Betula* seedlings

Vaccinium enhanced seedling survival, especially at the start of the experimental period (Fig. 1); this positive effect was irrespective of the environmental severity regimes occurring in the forest versus the treeline (against our hypothesis 2). Other performance variables varied with time and site, leading to very dynamic responses. We observed that a rodent outbreak in winter-spring 2008 indirectly had a rapid impact on *Betula* seedling performance. After a high population peak of the herbivore *Clethrionomys rufocanus*, the density of *Vaccinium* stems decreased about 2.5-fold on average compared to that of the previous summer at the treeline site (data not shown). Interestingly, when *Vaccinium* still had its original density in summer 2007, and also after full recovery from that grazing event by the end of summer 2008, the presence of *Vaccinium* strongly affected the performance of seedlings (Table 1). For instance, *Vaccinium* reduced the symptoms of high solar radiation in both the forest and the treeline site (Fig. 2), which may hinder tree seedling survival in alpine treelines (Bader et al. 2007). In contrast, in June 2008 (shortly after the outbreak which reduced *Vaccinium* density), the presence of shrub hardly affected the performance of the seedlings (Fig. 2, Table 1). The effects derived from this rodent outbreak confirmed the importance of *Vaccinium* on *Betula* seedlings when the shrub is present.

Caterpillar herbivory on birch leaves also showed a very dynamic pattern. *Betula* seedlings in the forest generally suffered more from herbivory during most of the experiment (Table 1), presumably because shade made their leaves bigger in the forest than at the treeline at the start of each season. Larger leaves usually have higher levels of N and lower C/N ratios which make them more susceptible to herbivory (Olofsson et al. 2007), as was confirmed in our study (Supplementary Material Appendix 1 Fig. A5). Interestingly, during 2009, at the treeline site, there was a significant increase in leaf size, with higher N concentration and lower C/N ratio in the leaves compared to the forest site, which coincided with a relative shift in herbivory from forest to treeline. The presence of *Vaccinium* also significantly reduced herbivory of *Betula* seedlings on two sampling periods. In this case we did not find any relationship between herbivory intensity and C/N ratio. Seedlings growing without *Vaccinium* could have been less protected against caterpillars, but the reason remains uncertain.

Hence, seedlings growing with *Vaccinium* were not only less frequently affected by herbivory as described above, but also suffered visibly less from the effects of high solar radiation, a combination which might have enhanced their survival.

At the treeline site we also detected other facilitative interactions. The mean air temperature in microplots with *Vaccinium* was 2.5°C lower than where the shrub was absent, indicating that seedlings growing with the shrub were probably less drought-prone in this low precipitation area. The lack of such a temperature difference in the forest indicates that *Vaccinium* will probably not significantly ameliorate soil moisture in the moister forest site. Thus, seedling survival in the forest will depend more on other processes, as discussed earlier. Hence, although overall survival did not differ between sites, seedling mortality seems to have resulted from different factors in both sites.

Negative impacts of adult birch trees and *Vaccinium* on *Betula* seedlings

Some variables showed a positive response to forest conditions at the start of the experiment and a negative response towards the end of the experiment. However, when the different sampling periods are analysed separately, we detect that the adult birch trees outcompeted the small seedlings transplanted into the forest, as evidenced by reduced growth and lower nutrient and chlorophyll content at the end of the experiment (Table 1). The competition was presumably for light and nutrients. The high C/N ratio measured in seedlings growing in the forest is probably due to low N availability as related to high concentrations of humic acids which immobilise nutrients (Hättenschwiler and Vitousek 2000). Contrary to what we expected, the growing conditions at the treeline site were more benign than in the forest site. The better performance at the treeline could also be associated to less shade from adult birches and higher temperatures, which may accelerate decomposition and mineralisation rates. However, [N]/total N was lower in seedlings at the treeline than in the forest. This could indicate that the greater N availability and total per-seedling N uptake at the treeline could not keep pace with the direct positive temperature effect on seedling biomass, resulting in a net N dilution effect (Supplementary Material Appendix 1 Fig. A5).

Despite the facilitative effects of *Vaccinium* on *Betula* seedlings, and contrary to our expectations, we observed a negative ontogenetic shift in the interaction, denoted by a poorer performance of the seedlings growing with the shrub in both sites, compared to those in shrub-free patches (Table 1). These results differ from those obtained in a study with conifer seedlings at an alpine treeline in Snowy Range (USA), where surrounding vegetation did not cause any negative effect on seedling development (Maher et al. 2005). Other studies have also shown both positive and negative effects of *Vaccinium* on tree seedlings, confirming the general importance of the balance between facilitation and competition (Gallet 1994, Chrimes et al. 2004). For instance, phenolic compounds released from *Vaccinium* leaf litter into the soil may be the actual agents of the allelopathic influence, e.g. through inhibiting the growth of associated ectomycorrhizal fungi (Pellissier 1993), which could explain the slower growth of *Betula* seedlings growing in *Vaccinium* microplots. Within the period of our study, it is difficult to assess whether facilitation of *Vaccinium* on *Betula* seedling survival dominated over its subsequent negative, competitive effects on seedling growth once they had survived. Overall, there was an ontogenetic shift of the shrub–tree interactions from more facilitative to more competitive in both sites. Therefore, facilitation and competition are important drivers of treeline dynamics but there is no clear evidence that the relative importance of facilitation and competition shifted across the ecotone as expected by the stress-gradient hypothesis and stated in our hypothesis 1. In addition, we did not observe more facilitative interactions under more severe environmental conditions, contrary to our hypothesis 2.

Effects of abiotic manipulations on *Betula* seedlings

The OTCs increased mean air temperature equally in both sites, but the temperature at the treeline site was above the mean 11°C threshold for July that is associated with treeline location (Körner 1998). In the forest, the OTCs did not increase temperatures beyond this threshold, which could explain the less pronounced seedling response to OTCs compared to the treeline site. Trees growing at the treeline are indeed very sensitive to soil temperature (Körner and Paulsen 2004), and our results demonstrate that such sensitivity is already detected at the seedling stage. Contrary to our expectations, the effect of temperature per se on seedling development did not result in any effect on seedling survival during the period of study. Within the period of the experiment therefore, drivers other than temperature (i.e. presence of a shrub) act as a bottleneck for seedling survival. However, we predict that those seedlings that survive at the treeline could be strongly influenced by increased temperature and may be more likely to survive until maturity if the 11°C mean July air temperature threshold is reached.

As hypothesised (hypothesis 3), the combination of increased temperature and nutrients produced substantial positive effects on *Betula* seedling development (especially at the treeline site; Table 1), evidenced by thicker and longer stems, as observed in other treeline species (Hobbie and Chapin 1998). This indicates that nutrient mineralisation and uptake by *Betula* seedlings are strongly influenced by temperature, consistent with the findings of Sveinbjörnsson et al. (1993) and Karlsson and Nordell (1996) and with findings for boreal trees (Jarvis and Linder 2000). Körner and Hoch (2006) argue that it is the thermal limitation of plant metabolism which controls the growth at the treeline, rather than the availability of nutrients in the substrate. In our study though, fertilisation alone also caused a very dramatic response at the treeline site, indicating that nutrients also limit the performance of seedlings. If N limitation at the lower forest site is due to strong nutrient immobilisation as discussed earlier, the less visible response to fertilising there could be due to the rapid sequestration of the surplus N in the soil by microbes (Jonasson et al. 1999).

Implications of the experimental findings for treeline dynamics

Seedlings at the treeline site benefitted from more benign abiotic conditions during the growing season than those in the forest site, with more light, higher temperatures and greater nutrient availability (in control plots). This may explain why *Betula* seedlings developed better in the treeline site compared to the forest site, against our expectations. However, growing conditions at seedling stage do not necessarily apply to later stages of tree development. Hence, although the recruitment of new seedlings is crucial for treeline dynamics and tree regeneration, the fate and performance of those seedlings that reach maturity will also determine the development of the forest–tundra ecotone. For instance, taller *Betula* saplings or trees, which protrude above the snow cover in winter, may be more susceptible to extreme winter weather events. They may also be more susceptible to moth outbreaks, which usually affect mature trees the most. Such factors may partly explain why seedlings but not mature trees are frequently found above the treeline (Truong et al. 2007).

We found that *Vaccinium* influences *Betula* seedling survival across the forest–treeline ecotone and thus, the potential capacity of birch for colonising the area. In our experiment, *Betula* seedlings growing in *Vaccinium*-free areas had lower survival, but the performance of those that did survive was significantly improved. Therefore, seedlings which manage to survive in shrub-free patches during their first stages of development will most likely persist and this will have a noticeable effect on tree recruitment in the area. Hence, the proportion of areas occupied by *Vaccinium* (and presumably by other shrubs) and by empty patches in the forest–treeline ecotone seems to be a crucial factor in tree regeneration and treeline dynamics. However, the intraspecific competition between adult birch trees and seedlings described earlier will reduce the recruitment of new trees in the lower part of the forest–tundra ecotone.

During the last decades, the mean annual temperature in the study area has risen very markedly (Material and methods; Callaghan et al. 2010). Such an increase may also help to explain the treeline densification and upslope recruitment of tree seedlings detected in some areas in the subarctic Scandes, as mentioned above (Kullman 2002, Tømmervik et al. 2004, Rundqvist et al. 2011). Furthermore, the responses of the seedlings to projected future increased temperature, especially at high latitudes (IPCC 2007), could have strong implications for treeline dynamics in the study area, since the temperature treatment promoted a consistently better performance with only a relatively small air temperature increase, especially at the upper treeline site. Similar results have also been obtained with *Picea glauca* in the Canadian subarctic tundra (Hobbie and Chapin 1998, Danby and Hik 2007).

Importantly, our study has demonstrated that changes in the forest–tundra ecotone cannot be simply explained or predicted from changing temperature patterns alone, and that complex interactions need to be considered, not only between shrubs and trees, but also with herbivores and between warming and soil nutrient availability.

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Supplementary material (available as Appendix O20032 at <www.oikosoffice.lu.se/appendix>). Appendix 1.