



Interaccions planta-planta en gradients d'estrès en ecosistemes freds

Plant-plant interactions along stress gradients in cold ecosystems

Oriol Grau Fernàndez

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**INTERACCIONS PLANTA-PLANTA EN GRADIENTS
D'ESTRÈS EN ECOSISTEMES FREDS**

***PLANT-PLANT INTERACTIONS ALONG STRESS GRADIENTS
IN COLD ECOSYSTEMS***

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Departament de Biologia Vegetal
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oriol

Octubre de 2012, Arrós de Cardós, Pallars Sobirà

Què dius que fève aguell goiat? Estudiar es barjals?

Au, va! Maitant, carallot!

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Introducció general

Marc conceptual

Les interaccions planta-planta han estat un tema central per als ecòlegs vegetals i han cridat l'atenció d'un nombrós grup d'investigadors durant força temps. Tradicionalment la majoria dels estudis s'han centrat en les interaccions negatives entre plantes (i.e. competència), però les interaccions positives (i.e. facilitació) també han esdevingut de gran interès durant les darreres dècades (Grime 1979; Callaghan i Emanuelsson 1984; Brooker et al. 2008). Per exemple, diversos estudis han demostrat que la facilitació podria tenir un gran impacte en la productivitat de l'ecosistema (Callaway et al. 1991; Zhang i Li 2003), en la diversitat d'espècies (Raffaele i Veblen 1998; Cavieres et al. 2002; Valiente-Banuet i Verdú 2007) i en la invasió de plantes exòtiques (Lenz i Facelli 2003; Davis et al. 2004). Així, gràcies a la consideració de la facilitació com a element clau, actualment es reconeix àmpliament que no només les interaccions planta-planta negatives dirigeixen l'estructura i el funcionament dels ecosistemes, sinó que també les positives hi tenen molt de pes. Aquestes interaccionen amb les condicions ambientals locals i amb l'acció d'altres organismes com ara els herbívors, els pol·linitzadors i els descomponedors. Així doncs, la formació de comunitats vegetals a partir d'un grup d'espècies en un lloc donat ve determinada pel balanç de les interaccions entre tots aquests factors conjunts, ja que no hi ha un factor únic que pugui explicar de forma completa als patrons observats (Lortie et al. 2004).

Les interaccions planta-planta poden donar lloc a competència per aconseguir més llum, nutrients, espai, agents pol·linitzadors i aigua; també poden apaivagar els impactes dels herbívors, d'altres competidors potencials o de condicions climàtiques extremes; també permeten l'adquisició de recursos addicionals a través dels exudats vegetals, i la millora de la comunitat microbiana, de les xarxes micorríziques i de l'absorció hidràulica (Brooker et al. 2008). En qualsevol cas, és difícil preveure en quines condicions dominaran les interaccions negatives per sobre de les positives o viceversa. La 'hipòtesi del gradient d'estrès' (Brooker i Callaghan 1998) suggereix que el balanç net de les interaccions planta-planta podrien passar de la competència a la

facilitació com a resposta a un augment de la severitat ambiental (Bertness i Callaway 1994). Aquesta hipòtesi preveu que en ambients relativament productius, la competència esdevindrà un element clau en la limitació del l'èxit de les plantes, mentre que en ambients poc productius, espècies relativament sensibles a l'estrès tendiran a sobreviure pitjor sense la presència d'un facilitador tolerant a l'estrès (Brooker 2010). Malgrat que aquesta hipòtesi ha estat avalada per diversos estudis (e.g. Gómez-Aparicio et al 2004; Callaway 2007), alguns treballs recents revelen que fins i tot dins del mateix ecosistema no tots els casos mostren un patró constant (e.g. Hopzapfel et al. 2006); a més, en ambients en què l'estrès és extrem no sempre s'ha detectat facilitació (e.g. Michalet et al 2006). Per altra banda, la magnitud i la direcció de les interaccions entre plantes poden variar al llarg de l'ontogènia de les espècies que interaccionen (Miriti 2006). Sembla que les transicions de competència a facilitació depenen en gran mesura de les característiques de les espècies que interaccionen (Choler et al. 2001), de la naturalesa del gradient d'estrès en qüestió (Maestre i Cortina 2004; Kawai i Tokeshi 2007), i de la variable escollida per avaluar el desenvolupament de la planta (Klanderud 2005; Maestre et al. 2005). Per aquest motiu, Maestre et al. (2009) defensen la necessitat d'explorar la forma i l'aplicabilitat de la relació entre les interaccions i la severitat ambiental, i la conveniència d'analitzar la naturalesa d'aquesta relació en diferents gradients d'estrès en ecosistemes terrestres.

Els canvis ambientals actuals i els previstos per al futur justifiquen que es busqui un coneixement més acurat dels canvis que puguin tenir lloc en els ecosistemes, tenint en compte que aquest canvis poden venir condicionats per possibles canvis en les interaccions entre les espècies. Callaway et al. (2002) van suggerir que els canvis ambientals podrien afectar les interaccions entre plantes més que no pas afectar les plantes individualment, i van emfatitzar la importància d'investigar més sobre l'efecte dels canvis ambientals en les interaccions planta-planta, i les conseqüències que això pugui tenir en la composició, dinàmica i diversitat de les comunitats vegetals. Conseqüentment, hi ha hagut un interès creixent per avaluar com els canvis ambientals globals poden desembocar cap a canvis en la vegetació a través d'alteracions en les interaccions planta-planta (Klanderud 2005; Brooker 2010). En

qualsevol cas, ja hi ha evidències clares que demostren que aquestes interaccions tenen un paper important en modular l'impacte dels canvis ambientals en les comunitats naturals (Maestre i Reynolds 2006, 2007).

Els canvis ambientals globals són el resultat de la interacció de diversos factors, com ara el clima i els canvis d'usos del sòl. Molt abans d'iniciar-se la industrialització, ja s'havien enregistrat canvis considerables de temperatura a hemisferi nord en períodes de temps força curts (Johnsen et al. 2001) i també canvis cíclics que ocupaven períodes de milers d'anys (Petit et al. 1999). De totes maneres, durant les dècades recents les activitats industrials han contribuït molt probablement a l'escalfament global com a resultat de la combustió de combustibles fòssils i de canvis en els usos del sòl, que han implicat un increment net de les concentracions de CO₂ a l'atmosfera (IPCC 2007).

El canvi climàtic és un dels factors més estudiats entre els que contribueixen clarament al canvi global; s'ha vist que la tendència a l'escalfament de la superfície terrestre enregistrat entre els anys 1956 i 2005 ha estat el doble que pels cent anys transcorreguts entre 1906 i 2005 (IPCC 2007). Per altra banda, els canvis d'usos del sòl han provocat una fragmentació significativa dels hàbitats en la superfície terrestre en àrees amb pressió humana creixent, mentre que una disminució en l'activitat humana i de pressió d'herbívors sovint ha conduït cap a la reforestació en zones abandonades. En termes generals, però, la biodiversitat ha baixat substancialment en zones amb forts canvis d'ús del sòl durant les darreres dècades (Hooper et al. 2012). Actualment s'accepta àmpliament que el canvi climàtic i els canvis d'usos del sòl han portat a canvis de vegetació a escala mundial i que aquests canvis molt probablement continuaran en el futur. Alguns ecosistemes en regions fredes s'han mostrat especialment sensibles i vulnerables als canvis ambientals (Van Bogaert et al. 2007; Post et al. 2009; Bhatt et al. 2010; Callaghan et al. 2011; Elmendorf et al. 2012), especialment en les latituds més septentrionals, on l'escalfament climàtic s'ha vist clarament amplificat (ACIA 2005; IPCC 2007; AMAP 2011). Això s'ha observat per exemple a través de l'expansió de les comunitats arbustives tant en ecosistemes d'alta latitud com en els d'altituds elevades (Sturm et al. 2001; Tape et al. 2006; Hallinger i Wilmking 2011; Myers-Smith et al. 2011; Rundqvist et al 2011). És interessant destacar

que les arbustos sovint s'han descrit com a facilitadors i competidors potencials de la colonització vegetal en diversos ecosistemes en regions fredes (e.g. Shevtsova 1995; Doležal et al. 2004; Klanderud i Totland 2004; Klanderud 2005; Henríquez i Lusk 2005). De totes maneres, es coneix poc com aquesta potencialitat podria afectar les espècies concretes que interactuen o com les interaccions poden canviar sota tipus i règims d'estrès canviant.

Estructura de l'estudi i aportacions concretes

En aquesta tesi presento quatre capítols, en els quals es discuteix com diferents espècies subarbustives interactuen amb plantes coexistents, sota règims variables d'estrès. Aquesta recerca ha estat centrada en ecosistemes de gran valor ambiental, ecològic i de conservació, i alhora sensibles als canvis ambientals, en quatre regions distintes situades en zones de latitud o altitud elevades. Per ordre latitudinal, els ecosistemes estudiats han estat: 1) el límit supraforestal dels Pirineus Centrals, situats en una zona temperada; 2) el gradient de successió primària d'un ecosistema situat en una zona boreal, a Finlàndia; 3) el límit supraforestal situat a la zona subàrtica de Lapònia; i 4) la tundra situada en una zona de l'alt àrtic, al nord-est de Grenlàndia (vegeu la Figura 1).

Els dos primers capítols es basen en una aproximació experimental i se centren en investigar com els subarbustos més comuns trobats prop del límit supraforestal interactuen amb plançons d'arbres de les espècies que formen el límit del bosc en dos ambients contrastats, i.e. el límit supraforestal subàrtic, i el límit supraforestal dels Pirineus Centrals. A més, donat que els arbres que viuen prop del límit de distribució són molt sensibles als canvis ambientals, especialment a l'augment de temperatures durant l'estació de creixement (Körner 2003), també s'ha investigat com podrien afectar el creixement dels plançons d'arbre i la seva supervivència al llarg de l'ecotò, diferents escenaris de canvis ambientals. El primer capítol es titula 'Les interaccions arbre-arbust i els canvis ambientals dirigeixen la dinàmica supraforestal a la zona subàrtica', i s'hi exposa l'experiment de tres anys de durada que es va executar al 'Parc

Nacional d'Abisko', en els Scandes subàrtics, al nord de Suècia; les plantes estudiades varen ser plançons de *Betula pubescens* i el subarbust *Vaccinium myrtillus*. En aquest estudi es va certificar la importància de les interaccions arbust-arbre, tant facilitatives com competitives, com a elements clau en la dinàmica supraforestal subàrtica. A més, vàrem demostrar que la gran sensibilitat dels plançons a l'escalfament tenia fortes implicacions per la dinàmica supraforestal, tenint en compte l'escenari previst d'augment de temperatures en latituds elevades. També es va observar que les interaccions complexes entre arbusts i herbívors són claus per preveure canvis futurs.

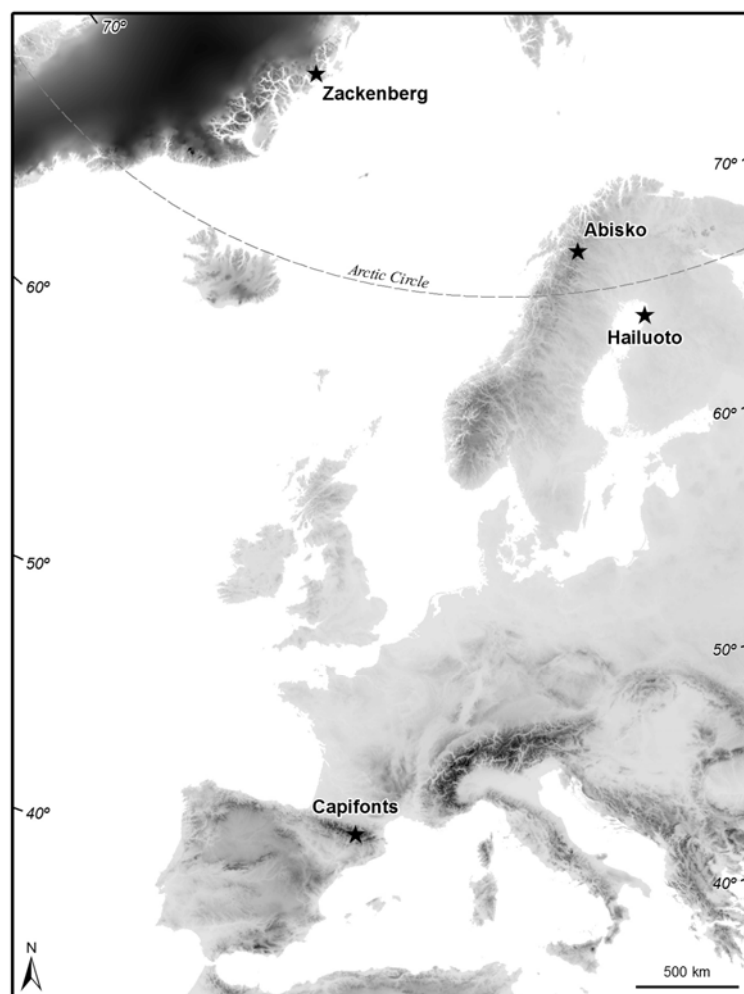


Figura 1. Situació de les àrees d'estudi: Capifonts (Pirineus Centrals, Catalunya); Hailuoto (Badia de Bòtnia, Finlàndia); Abisko (Scandes de Suècia, Lapònia); Zackenberg, (nord-est de Gronlàndia).

El segon capítol es titula 'Els plançons d'arbres situats als límits supraforestals Pirinencs i subàrtics mostren respostes semblants a la presència d'arbustos i a les simulacions de canvis ambientals'. Aquí es presenta una comparació entre els resultats de l'experiment presentat en el primer capítol i els obtinguts en un experiment paral·lel dut a terme durant un període de temps semblant prop del límit supraforestal en el 'Parc Natural de l'Alt Pirineu', als Pirineus Centrals catalans. Aquest experiment es va basar en el mateix disseny factorial que l'anterior estudi, però amb diferents espècies (i.e. plançons de l'arbre *Pinus uncinata* i l'arbust *Rhododendron ferrugineum*). Segons ens consta, és el primer estudi que avalua experimentalment les respostes de les plantes vers diferents escenaris ambientals en un ecosistema d'alta muntanya als Pirineus. En aquest capítol es presenten alguns mecanismes que ajudaran a comprendre la variabilitat recentment observada de les respostes locals de límits supraforestals de zones temperades i subàrtiques com a resultat del clima canviant, i també identifiquem alguns paral·lelismes que poden utilitzar-se per generalitzar les respostes a gran escala dels límits supraforestals al canvi climàtic.

El tercer capítol se centra en els efectes d'un subarbust dominant (i.e. *Empetrum nigrum*) en plançons de *Pinus sylvestris* al llarg del gradient de successió primària en un ecosistema boreal en una illa emergent de la badia de Bòtnia, a Finlàndia. Aquest capítol s'anomena 'Un arbust ericoide exerceix la doble funció de reclutar pins i els seus simbionts fúngics al llarg d'un gradient de successió primària'. Aquí hem mostrat que els efectes facilitadors i competidors dels subarbustos determinen fortament l'establiment de plançons i la seva colonització fúngica al llarg d'aquest gradient de successió. Segons ens consta, aquests són els primers resultats que demostren que un arbust ericoide micorrízic pot millorar tant el desenvolupament de l'arbre hoste ectomicorrízic com el dels simbionts fúngics de l'arbre.

L'estudi presentat al quart capítol es va realitzar al llarg d'un gradient de nivositat en un ecosistema extrem de tundra àrtica al 'Parc Nacional del nord-est de Grenlàndia', el Parc Nacional més gran del món. El capítol es titula 'Interaccions vegetals i composició de la vegetació àrtica al llarg d'un gradient de nivositat al nord-est de Grenlàndia'.

Aquest ecosistema és probablement el més sensible i fràgil de tots els ecosistemes estudiats en aquesta tesi, donat que s'espera que a la costa est de Groenlàndia s'hi esdevinguin canvis substancials en el clima com a resultat de canvis destacables en els règims de precipitació de neu i de les temperatures (Brown i Mote 2009). Aquí es va avaluar la riquesa d'espècies de plantes, així com els patrons d'establiment i composició de diverses formes de creixement existents en comunitats vegetals àrtiques associades a una variació de la cobertura de neu durant els mesos d'hivern. Aquest estudi ajudarà a preveure la diversitat potencial i els canvis en la vegetació a la zona de l'alt Àrtic, si els règims de precipitació de neu canvien en el futur com es preveu.

Objectius

Tal com s'indica en la introducció, en el context dels biomes freds és prioritari investigar com les interaccions entre arbustos i altres plantes, la hipòtesi del gradient d'estrès, i els canvis ambientals estan interrelacionats. És especialment rellevant poder determinar com aquests elements controlen el funcionament dels ecosistemes de zones de latituds i altituds elevades. Amb aquest objectiu, aquesta tesi es compon de quatre capítols, que tenen en compte aquests elements en diversos ecosistemes en regions fredes. Els objectius particulars de cadascun d'aquests capítols es presenten a continuació:

- 1) 'Les interaccions arbre-arbust i els canvis ambientals dirigeixen la dinàmica supraforestal a la zona subàrtica'

Aquest capítol es focalitza en un ecosistema supraforestal als Scandes subàrtics, una regió situada en una latitud septentrional que s'ha mostrat molt sensible als canvis ambientals durant les darreres dècades. Es pretén investigar: 1) si les interaccions arbust-arbre influencien la dinàmica supraforestal observada en l'ecotò bosc-tundra d'aquesta zona subàrtica; 2) la importància relativa dels factors causals biòtics i abiòtics potencials en la supervivència i el creixement dels plançons d'arbre al llarg d'aquest ecotò; 3) la resposta dels plançons d'arbre que creixen en aquest ecotò a diversos escenaris de canvis ambientals rellevants. Aquests objectius es van abordar mitjançant un estudi experimental de camp, de tipus multi-factorial. En concret, va consistir en exposar plançons transplantats de bedoll a manipulacions de temperatura de l'aire, de disponibilitat de nutrients i de cobertura de l'arbust dominant en dues localitats sotmeses a condicions d'estrès ambientals diferents al llarg de l'ecotò bosc-tundra.

- 2) 'Els plançons d'arbres situats als límits supraforestals Pirinencs i subàrtics mostren respostes semblants a la presència d'arbustos i a les simulacions de canvis ambientals'

En aquest segon capítol es compara l'experiment efectuat a la zona dels Scandes subàrtics presentat en el primer capítol amb els resultats d'un experiment multifactorial similar desenvolupat en un límit supraforestal típic dels Pirineus Centrals catalans. Es pretén investigar si els mecanismes que controlen la dinàmica supraforestal a través de les interaccions arbust-arbre i dels canvis ambientals actuen de forma semblant en aquestes dues regions tan diferenciades, com són els Pirineus i els Scandes. En aquest estudi es volen conèixer els rols dels factors causals generals i locals que dirigeixen la dinàmica supraforestal, així com la gran variabilitat de les respostes supraforestals al canvi climàtic recentment observat en les zones subàrtiques i alpines. També investiguem si la hipòtesi del gradient d'estrès ajuda a explicar els patrons observats en aquests dos ecosistemes situats al límit supraforestal.

- 3) 'Un arbust ericoide té el doble rol de reclutar pins i els seus simbionts fúngics al llarg de gradients de successió primària'

En el tercer capítol l'objectiu és avaluar les interaccions arbust-arbre al llarg del gradient de successió d'una zona costanera amb sòl sorrenc en un ecosistema boreal, sotmesa a un procés eustàtic emergent. Se centra en l'efecte del subarbust dominant *Empetrum nigrum* sobre plançons de *Pinus sylvestris*. En base a la hipòtesi del gradient d'estrès, es vol investigar si el reclutament i el desenvolupament dels plançons de pi, així com la seva simbiosi ectomicorrízica, es veuen millorades per la presència de l'arbust en estadis de successió joves, i si aquests efectes esperadament positius disminueixen vers els estadis de successió primària més madurs.

- 4) 'Interaccions vegetals i composició de la vegetació àrtica al llarg d'un gradient de nivositat al nord-est de Grenlàndia.'

L'objectiu del quart capítol és investigar si diferents tipus de vegetació dominant, i.e. amb alt recobriment de molses o de subarbusts com *Salix arctica* o *Dryas*

octopetala × *intermedia*, dirigeixen l'establiment de plantes vasculars i la composició d'espècies en tres comunitats vegetals àrtiques comunes al nord-est de Grenlàndia. Aquestes comunitats vegetals són la congestera de *Salix arctica*, la formació subarbustiva de *Dryas* i els erms amb vegetació esparsa, respectivament associades a un gradient altitudinal creixent i amb un gruix de neu alt, mitjà i baix, respectivament durant el període hivernal, i sotmès consegüentment a règims d'estrès canviants. També investiguem si la hipòtesi del gradient d'estrès es pot aplicar en el gradient de gruix de neu descrit, típic de l'ecosistema de tundra d'elevada latitud, i molt sensible als canvis ambientals.

Informe del director de tesi

Informe del director de tesi del factor d'impacte dels articles publicats

En Josep Maria Ninot Sugañes, Doctor en Biologia, com a director de la tesi que porta per títol 'Interaccions planta-planta en gradients d'estrès en ecosistemes freds', que ha dut a terme el doctorand Oriol Grau Fernández, emet el següent informe relatiu al factor d'impacte i a la participació del doctorand en cadascun dels articles inclosos en la memòria de l'esmentada Tesi.

A tots quatre apareix com a primer autor, el que reflecteix el seu paper principal a totes les fases d'elaboració dels mateixos, i molt especialment en el mostreig de camp, el tractament de mostres i dades, la preparació dels manuscrits, i la gestió del procés de publicació:

Article 1: *Shrub-tree interactions and environmental changes drive treeline dynamics in the Subarctic*. Publicat a la revista *Oikos* el 2012, amb índex d'impacte (2011) de 3,061, el núm. 38 de 134 en la categoria 'Ecology'. Correspon a una experiència en què s'analitza la resposta de plançons arboris a diferents factors creats en petites parcel·les experimentals i seguits durant tres períodes vegetatius, als Scandes septentrionals. El disseny fou conjunt amb els dos co-directors, i el doctorand va realitzar la part principal de totes les tasques (des del mostreig, fins a l'edició de l'article). La resta de co-autors van participar en les anàlisis químiques, en la interpretació de resultats, i en l'elaboració de la discussió.

Article 2: *Similar tree seedling responses to shrubs and to simulated environmental changes at Pyrenean and subarctic treelines*. Sotmès a la revista *Plant Ecology and Diversity*, amb índex d'impacte (2011) de 1,036, el núm. 111 de 190 en la categoria 'Plant Sciences', pendent de modificacions per a ser acceptat. En aquest treball es presenten els resultats d'una experiència paral·lela a la de l'article 1, però realitzada als Pirineus, també durant tres períodes vegetatius; i es fa una anàlisi comparativa de totes dues. El rol dels diferents co-autors és també similar.

Article 3: *An ericoid shrub plays a dual role in recruiting both pines and their fungal symbionts along primary succession gradients.* Publicat a la revista *Oikos* el 2010, amb índex d'impacte (2010) de 3,393, el núm. 33 de 130 en la categoria 'Ecology'. Aquest treball és fruit d'una anàlisi de les interaccions vegetals al llarg de la successió primària en una illa finlandesa, plantejat pels co-autors segon i sisè. Es deu al doctorand des del mostreig i la detinguda anàlisi de les mostres, fins a l'edició de l'article. La resta de co-autors van fer aportacions en l'elaboració de les dades i en l'acabat del manuscrit.

Article 4: *Plant interactions and high-arctic vegetation composition along a snow-depth gradient in NE Greenland.* Sotmès a la revista *Polar Research*, amb índex d'impacte (2011) de 1,616, el núm. 69 de 170 en la categoria 'Geosciences', pendent de modificacions per a ser acceptat. Es tracta de l'estudi d'un gradient ambiental en base a mostreig de camp i anàlisi de mostres i dades de vegetació en aquesta zona àrtica. També aquí el doctorand ha realitzat la major part de totes les tasques, i la resta de co-autors han participat en el disseny i mostreig de camp (qui això signa), o bé en la interpretació dels resultats i en la preparació del manuscrit (tots).

Barcelona, Octubre de 2012

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Discussió general i Conclusions finals

Discussió general

Durant les darreres dècades s'han obtingut moltes evidències que indiquen que les interaccions positives planta-planta dirigeixen l'estructura i el funcionament dels ecosistemes. Això ha quedat plasmat en un gran nombre d'estudis recents i de revisions de recerca (e.g. Pugnaire 2008). Callaway et al. (2002) argumenta que un cop inclosa la facilitació en els models ecològics i teòrics, cal avaluar com els canvis ambientals poden condicionar la relació entre les interaccions i la severitat ambiental, i alhora investigar les conseqüències d'aquestes interaccions en les comunitats vegetals (Maestre et al. 2009). En aquesta tesi he aprofundit en aquesta temàtica en diversos ecosistemes de zones fredes, tot investigant la importància de les interaccions planta-planta sotmeses a diferents règims d'estrès, i també avaluant els efectes potencials dels canvis ambientals en l'estructura de les comunitats vegetals.

Efectes de les interaccions planta-planta en l'estructura de l'ecosistema

Tal com teoritzen Lortie et al. (2004), en tots els ecosistemes analitzats en aquesta tesi s'ha observat que les interaccions planta-planta modulen clarament l'estructura de l'ecosistema (Taula 1). Els diversos capítols d'aquesta tesi mostren que la naturalesa i la implicació ecològica del balanç net derivat de les interaccions positives i negatives entre arbusts i plançons d'arbre són semblants en dos ecosistemes clarament diferenciats, com són el límit supraforestal dels Pirineus Catalans i el de la zona subàrtica de la Lapònia sueca. A més, es va veure que els diversos escenaris de canvis ambientals simulats experimentalment en relació a canvis en els règims de temperatura, en la disponibilitat de nutrients i en la cobertura dels subarbusts, donen lloc a respostes similars en els plançons d'arbre trasplantats en aquests dos ecosistemes tan allunyats. Aquests resultats indiquen que els impactes de les comunitats arbustives i dels canvis ambientals en la dinàmica del límit supraforestal poden tenir rellevància en ecosistemes del límit supraforestal amb diferents orígens biogeogràfics i amb espècies diferents, i que les interaccions planta-planta tenen

clarament un paper destacat en la modulació dels impactes que els canvis ambientals provoquen en les comunitats vegetals naturals (Maestre i Reynolds 2006).

Les interaccions arbust-arbre tenen també un paper rellevant en la successió primària de la vegetació en l'illa emergent de Hailuoto, situada en una zona boreal, a Finlàndia. L'efecte positiu de la comunitat arbustiva es tradueix en un augment del reclutament de plançons i de la biomassa fúngica del sòl en estadis avançats de la successió en claps on predomina el subarbust *Empetrum*. Aquests resultats reforcen la idea que la facilitació és un component vital en la successió primària de la vegetació (Callaway 1995; Lortie et al. 2004; Moora and Zobel 2008). Per altra banda, els efectes de la competència esdevenen més visibles en estadis més avançats de la successió, ja que el percentatge de plançons que creixen vora el subarbust disminueix en aquests estadis. Tot plegat, indica que tant les interaccions negatives com les positives són rellevants en la successió primària de la vegetació (Glenn-Lewin et al. 1992).

Les dades analitzades al llarg del gradient de gruix de neu a l'alt Àrtic, al nord-est de Grenlàndia, revelen que l'establiment de les plantes en diferents comunitats vegetals presents al llarg d'aquest gradient està fortament influenciat pels efectes positius i negatius dels tipus de vegetació dominant (subarbusts i molses). Els resultats suggereixen que els efectes positius i negatius dels tipus de vegetació dominant tindran un paper rellevant en modular els canvis que pugui tenir la vegetació, a través de les espècies co-existents, en resposta als canvis d'innivació i de règim de temperatura pronosticats per aquesta regió àrtica (IPCC 2007; Brown and Mote 2009).

La hipòtesi del gradient d'estrès (SGH)

La hipòtesi del gradient d'estrès preveu que les interaccions planta-planta variïn progressivament de competència a facilitació al llarg d'un gradient d'increment d'estrès ambiental. És a dir, les interaccions positives predominaran en condicions de poca disponibilitat de recursos i baixa productivitat, mentre que les interaccions negatives prevaldran allà on la disponibilitat de recursos sigui elevada (Brooker i

Callaghan 1998). Tal com s'ha comentat en la secció anterior, les interaccions positives i negatives tenen un paper rellevant en modular l'estructura de l'ecosistema. Per això, és clau avaluar l'aplicabilitat de la SGH en els casos d'estudi considerats en aquesta tesi (Taula 1).

No s'ha trobat un suport generalitzat per a la SGH en els ecosistemes estudiats. S'ha observat que en els límits supraforestals dels Pirineus i de la zona subàrtica, la facilitació i la competència co-existeixen al llarg de l'ecotò; en el gradient de successió primària de la zona boreal s'han trobat evidències a favor de la SGH pel que fa al reclutament de plançons, però no pel que fa a la micorrizació o al desenvolupament dels plançons d'arbre; en el gradient de gruix de neu de l'alt Àrtic no es va trobar un increment en les interaccions positives a mesura que l'estrès ambiental augmentava, sinó que, contràriament, vam observar que una espècie subarbastiva mostrava un efecte competitiu creixent al llarg del gradient envers les espècies (de menys port) que hi co-existien. Aquests resultats suggereixen que els gradients d'estrès analitzats no són tan simples d'estudiar com s'havia cregut inicialment. Un exemple ben clar pot ser el cas del gradient d'estrès vinculat al gruix de neu en l'ecosistema àrtic, ja que la neu pot ser un recurs de disponibilitat d'aigua i també un element positiu que afavoreix l'aïllament i la protecció de les plantes durant l'hivern, però també pot esdevenir un factor negatiu, ja que al mateix temps la neu escurça el període de creixement vegetal. Per tant, és difícil preveure a priori la força resultant de l'estrès ambiental i com aquesta canvia al llarg dels gradients. Els nostres resultats no neguen la SGH, però indiquen que cal més recerca sobre aquest tema per tal de preveure millor els efectes de la competència i la facilitació al llarg de gradients d'estrès, i millorar el coneixement dels factors específics implicats en aquestes interaccions.

Suggeriments per a futures investigacions

Els estudis desenvolupats als Pirineus, als Scandes i a Finlàndia presentats en aquesta tesi només mostren els resultats obtinguts a partir del treball realitzat amb espècies arbòries en la seva fase de plançó. Es va seleccionar aquest estadi de

desenvolupament per raons pràctiques i per la disponibilitat limitada de temps per a realitzar aquests estudis, però també per la innegable importància d'aquest estadi en la vida dels arbres. Per això, en els capítols que en resulten només es proporciona informació dels processos que dirigeixen la supervivència o el desenvolupament d'aquest únic estadi de la seva vida. No obstant això, en tots els capítols queda palès que els processos de selecció biòtics i abiòtics poden tenir lloc de forma natural abans de l'estadi de plançó, i també que hi ha altres processos de selecció i canvis en les interaccions entre plantes que tenen lloc en altres estadis més avançats del cicle de la vida de l'arbre. Per aquest motiu, seria interessant investigar com les interaccions planta-planta i els canvis ambientals condicionen el desenvolupament dels arbres durant l'estadi de germinació, i també un cop els individus s'han establert en l'ecosistema.

Per altra banda, aquest treball ha aportat moltes evidències de l'existència d'interaccions positives i competitives, i de com aquestes han tingut un paper rellevant en estructurar l'ecosistema a les regions fredes estudiades, que fins ara havien quedat força al marge d'aquest tipus d'anàlisi. De totes maneres, caldria seguir investigant per tal d'avaluar millor l'efecte net de la facilitació i la competència, i de poder quantificar millor els seus efectes en l'estructura i funcionament de l'ecosistema. Una manera de seguir aprofundint en el treball realitzat, ara que ja s'ha provat l'existència i la implicació potencial de les interaccions planta-planta en els ecosistemes estudiats, seria mesurar numèricament la importància relativa d'aquestes interaccions, utilitzant índexs que quantifiquin amb precisió la intensitat de la interacció entre plantes, tal com han suggerit Armas et al. (2004) i Kikvidze i Armas (2008).

Taula 1 (Vegeu la pàgina següent). Resum de la importància de les interaccions planta-planta, de l'aplicabilitat de la hipòtesi del gradient d'estrès, i dels efectes dels canvis ambientals i de les implicacions ambientals en l'estructura de l'ecosistema en els diferents ecosistemes estudiats en aquesta tesi.

Taula 1 (Vegeu el peu de la pàgina anterior).

Ecosistema estudiat	Importància de les interaccions planta-planta	Aplicabilitat de la hipòtesi del gradient d'estrès (SGH)	Efectes dels canvis ambientals (variació dels règims de temperatura, de la disponibilitat de nutrients i de la cobertura d'arbustos)	Implicacions potencials per a l'estructura de l'ecosistema
Capifonts Pirineus Catalans (Ecosistema de límit supraforestal en una zona temperada)	Les interaccions arbust-arbre modulen el desenvolupament dels plançons d'arbre	No hi ha evidències clares que la importància relativa de la facilitació i la competència varii a través de l'ecotò, com preveu la SGH	Efecte clarament positiu de l'augment de la temperatura i de l'augment de nutrients en el desenvolupament dels plançons d'arbre Millora del desenvolupament dels plançons d'arbre en absència de l'arbust	Els canvis de clima i d'ús del sòl que alterin la pressió de recursos, l'estrès abiòtic o la pressió d'herbívoros poden induir canvis en les interaccions arbust-arbre en ecosistemes del límit supraforestal de zones temperades Qualsevol canvi ambiental que alteri la cobertura arbustiva pot induir canvis importants en l'ecosistema que afectin el desenvolupament dels plançons d'arbre
Illa de Hailuoto Finlàndia (Ecosistema de successió primària en una zona boreal)	Les interaccions arbust-arbre modulen el reclutament de plançons d'arbre i llur micorrització	Clara conversió d'interaccions, de facilitació a competència, al llarg del gradient d'estrès tal com preveu la SGH respecte el reclutament de plançons, però no respecte llur micorrització	Els efectes de l'augment de la temperatura i de la disponibilitat de nutrients no han estat avaluats experimentalment al camp Augment de la biomassa fúngica en absència de l'arbust al llarg del gradient de successió. La presència de l'arbust té un paper canviant al llarg de la successió en el reclutament de plançons d'arbre: positiu en estadis joves i negatiu en estadis madurs	Qualsevol canvi ambiental que alteri la cobertura arbustiva pot induir canvis importants en l'ecosistema que afectin el reclutament de plançons d'arbre i la seva comunitat fúngica associada en ecosistemes de successió primària boreals
Abisko Scandes de Suècia (Ecosistema de límit supraforestal en una zona subàrtica)	Les interaccions arbust-arbre dirigeixen la supervivència dels plançons d'arbre i modulen el seu desenvolupament	No hi ha evidència que la importància relativa de la facilitació i la competència varii al llarg de l'ecotò, tal com preveu la SGH	Efecte clarament positiu de l'augment de la temperatura i de l'augment de nutrients en el desenvolupament dels plançons d'arbre En absència de l'arbust es detecta una davallada de la supervivència dels plançons d'arbre, i co-existeixen efectes positius i negatius pel que fa al seu desenvolupament	Els canvis de clima i d'ús del sòl que alterin la pressió de recursos, l'estrès abiòtic o la pressió d'herbívoros poden induir canvis en les interaccions arbust-arbre en ecosistemes del límit supraforestal de zones subàrtiques Qualsevol canvi ambiental que alteri la cobertura arbustiva pot induir canvis importants en l'ecosistema alterant el desenvolupament dels plançons d'arbre
Zackenber Nord-est de Grenlàndia (Ecosistema de l'alt Àrtic)	Les interaccions positives i negatives entre espècies co-existents condicionen l'establiment de les plantes vasculares	No hi ha canvi de facilitació a competència al llarg del gradient d'estrès, tal com preveu la SGH	Els efectes de l'augment de la temperatura i de la disponibilitat de nutrients no han estat avaluats experimentalment. Els diferents tipus de vegetació dominant originen establiment vegetal diferenciat. Els canvis observats en la composició vegetal al llarg del gradient de gruix de neu a l'alt Àrtic corresponen a la naturalesa canviant de les comunitats vegetals	Qualsevol canvi ambiental que alteri la proporció relativa dels diferents tipus de vegetació dominant a l'alt Àrtic pot tenir efectes marcats en la riquesa d'espècies i en el seu establiment en les diferents comunitats vegetals existents al llarg del gradient de gruix de neu

Conclusions finals

Les interaccions arbust-arbre són elements claus per entendre la dinàmica supraforestal, tal com es demostra en l'experiment a la zona subàrtica i als Pirineus. Per tant, aquestes interaccions han de ser incloses en els models que descriuen la dinàmica supraforestal del passat, actual i del futur; això permetria fer una estimació més acurada de la causalitat i de l'abast dels canvis altitudinals i latitudinals o del procés de densificació que s'ha observat en aquestes zones en les darreres dècades i que podrien donar-se també en el futur com a resposta a canvis de clima o d'usos del sòl.

Malgrat les diferències en les característiques de les espècies presents en el límit supraforestal de zones subàrtiques i dels Pirineus i malgrat les respectives diferències en la disponibilitat de nutrients del sòl, hi ha una gran similitud en els factors i els processos que dirigeixen la dinàmica supraforestal en ambdues zones d'estudi. Aquest podria ser un motiu que ajudaria a explicar els patrons similars en la dinàmica supraforestal observada durant les darreres dècades en zones subàrtiques i pirinenques.

La facilitació, la competència, l'herbivorisme i els canvis ambientals actuen com a filtres determinants en l'estructuració de l'ecotò bosc-tundra a la zona subàrtica i a l'ecotò bosc-prats alpins als Pirineus. Qualsevol canvis en aquests ecotons homòlegs no es poden preveure simplement a partir de canvis en els patrons de temperatura, ja que s'ha de considerar també la gran complexitat derivada de les interaccions entre factors biòtics o abiòtics. Les interaccions poden donar-se, per exemple, entre arbusts i arbres, entre plantes i herbívors, o bé entre l'augment de temperatures i la disponibilitat de nutrients.

Els canvis de clima i d'usos del sòl que alterin la pressió de recursos, l'estrès abiòtic o la pressió d'herbívors són susceptibles a induir canvis en les interaccions arbust-arbre en el límit supraforestal de zones subàrtiques i de zones temperades. S'espera una sensibilitat superior dels plançons d'arbre a les variacions dels règims de temperatura i

de disponibilitat de nutrients que tinguin lloc a la zona subàrtica. Qualsevol canvi ambiental futur que faci variar la cobertura arbustiva pot originar canvis notables en el desenvolupament de plançons d'arbre en els límits supraforestals d'aquestes dues regions.

En l'ecosistema de successió primària boreal, les interaccions arbust-arbre dirigeixen el reclutament de plançons i la seva micorrizació. El subarbust micorríctic ericoide *Empetrum nigrum* fa de facilitador tant per l'hoste ectomicorríctic *Pinus sylvestris*, com pels simbionts fúngics d'aquest hoste. Qualsevol canvi ambiental futur que faci variar la cobertura arbustiva podria originar canvis marcats notables en el desenvolupament de plançons d'arbre i la seva comunitat fúngica associada en ecosistemes de successió primària boreals.

Les formes de creixement no són bons predictors de la co-existència d'espècies a l'alt Àrtic; diferents formes de creixement com ara les moltes i el subarbust *Salix arctica* mostren patrons molt similars de co-existència d'espècies al llarg del gradient de gruix de neu, i esdevenen punts calents de biodiversitat en l'extrem més sever del gradient. El subarbust *Dryas octopetala x intermedia* es mostra cada cop més competitiu amb les espècies que hi co-existien al llarg del gradient cap a l'extrem de més severitat ambiental. Els canvis observats en la composició vegetal al llarg del gradient de gruix de neu a l'alt Àrtic corresponen a la naturalesa canviant de les comunitats vegetals existents. Qualsevol canvi ambiental que alteri la proporció relativa dels diferents tipus de vegetació dominant a l'alt Àrtic pot tenir efectes marcats en la riquesa d'espècies i en l'establiment de plantes vasculares en les diferents comunitats vegetals existents al llarg del gradient de gruix de neu.

Tot i que s'ha confirmat l'existència de facilitació i competència en els ecosistemes estudiats, no s'ha pogut trobar una evidència generalitzada que confirmi que el predomini de les interaccions positives o negatives varien al llarg d'un gradient d'estrès de severitat ambiental, tal com prediu la SGH. Un cop ha estat provada l'existència d'aquestes interaccions, una possible via futura de recerca podria ser mesurar la importància relativa i la intensitat d'aquestes interaccions i entendre millor

els mecanismes associats a les interaccions per tal d'estimar millor el seu paper en l'estructura i el funcionament dels ecosistemes estudiats.

Investigacions futures haurien de considerar també que els efectes de les interaccions planta-planta i dels canvis ambientals en els arbres poden variar durant la seva ontogènia. Per tant, calen experiments futurs per tal d'avaluar com aquests factors causals afecten la germinació i els estadis de desenvolupament posteriors a l'establiment, per tal d'obtenir una visió més completa de la importància relativa de les interaccions planta-planta i dels canvis ambientals al llarg de la vida de l'arbre. Això permetrà fer projeccions de futur del límit supraforestal més completes i adaptades a cada localitat; sense aquest coneixement local i específic, els models generals de projecció futura d'aquest ecotò tan rellevant no tindran una validesa suficient.

Versió en anglès /English version

General introduction

Conceptual framework

Plant-plant interactions are a major focus for terrestrial plant ecologists and have attracted the attention of a multitude of researchers for many years. Traditionally, most studies have focused on negative interactions between plants (i.e. competition), but positive interactions (i.e. facilitation) have also become a major interest during the last decades (Grime 1979; Callaghan and Emanuelsson 1985; Brooker et al. 2008). For instance, several studies have shown that facilitation may have a great impact on ecosystem productivity (Callaway et al. 1991; Zhang and Li 2003), on species diversity (Raffaele and Veblen 1998; Cavieres et al. 2002; Valiente-Banuet and Verdú 2007) and on exotic plant invasibility (Lenz and Facelli 2003; Davis et al. 2004). Thus, thanks to the inclusion of facilitation into plant ecology research, it is now widely accepted that not only negative but also positive plant-plant interactions operate as key drivers of ecosystem structure and functioning. They interact with local environmental conditions and other organisms such as herbivores, pollinators and decomposers. The balance of the interaction among all these factors together determines the conversion from a global species pool at a given site into the realised extant plant communities, with no single factor alone providing a complete explanation for the observed patterns (Lortie et al. 2004).

Plant-plant interactions may result in competition for light, nutrients, space, pollinators and water; in protection from the impacts of herbivores, potential competitors or extremes of climate; and in provision of additional resources through canopy leaching, microbial enhancement, mycorrhizal networks and hydraulic lift (Brooker et al. 2008). However, knowing when and where negative or positive interactions will prevail is difficult to predict. It has been suggested for some time that net plant-plant interactions may switch from competitive to facilitative with increasing environmental severity (Bertness and Callaway 1994). This has been commonly referred to as the 'Stress-gradient hypothesis' (Brooker and Callaghan 1998), which predicts that in relatively productive environments, competition is a key factor potentially limiting plant success, while in unproductive environments, relatively stress-sensitive species would be less likely to survive in the absence of stress-tolerant

facilitators (Brooker 2010). Although this hypothesis has been supported by several studies (e.g. Gómez-Aparicio et al. 2004; Callaway 2007), some recent findings reveal that results within the same system may not always show a constant pattern (e.g. Holzapfel et al. 2006); in addition, facilitation may not always occur with extreme stress (e.g. Michalet et al. 2006) and the magnitude and direction of plant interactions may change through the ontogeny of the interacting species (Miriti 2006). Such transitions from competition to facilitation depend to a large degree on the characteristics of the species interacting (Choler et al. 2001), the nature of the stress gradient involved (Maestre and Cortina 2004; Kawai and Tokeshi 2007), and the performance measure considered (Klanderud 2005; Maestre et al. 2005). Therefore, Maestre et al. (2009) argued that there is a great need for further exploration of the shape and generality of the relationship between interactions and environmental severity, and a particular need to see if and how it applies to distinct stress gradients in terrestrial ecosystems.

Current and projected environmental change urgently requires a better practical understanding of ecosystem change that, according to the principles established above, relies on changes in species interactions. Callaway et al. (2002) suggested that environmental changes might affect plant-plant interactions rather than plant individuals directly and therefore claimed the importance of doing more research on how environmental changes may affect individual plant-plant interactions and their consequences for community composition, dynamics and diversity. Consequently, there has been growing interest in assessing if and how global environmental changes may lead to vegetation changes through altered individual plant-plant interactions (Klanderud 2005; Brooker 2010). Significant evidence is proving that such interactions play a role in mediating the impact of environmental change drivers on natural communities (Maestre and Reynolds 2006, 2007).

Global environmental changes are the result of the interaction of several factors, such as climate and land-use changes. Long before the human industrialisation started, considerable changes in Northern Hemisphere temperature had been recorded within time spans from a few years (Johnsen et al. 2001), to cyclic changes over thousands of

years (Petit et al. 1999). However, during recent decades human industrial activities have most likely contributed to a global warming by the burning of fossil fuels and land-use changes, which have resulted in net increase of CO₂ in the atmosphere (IPCC 2007).

Climate change is one of the most studied factors contributing to global change; it has been shown that the linear warming trend of global surface temperature for 1956 to 2005 is nearly double that for the 100 years from 1906 to 2005 (IPCC 2007). On the other hand, land-use changes have led to significant habitat fragmentation across the globe in areas with increasing human pressure, whereas a decrease in human activity and grazing pressure has often led to reforestation in abandoned areas. Overall however, biodiversity has been decreasing dramatically during the last decades in areas with marked land-use changes (Hooper et al. 2012). It is widely acknowledged that climate change and land-use changes have led to world-wide changes of vegetation and that these changes are likely to continue. Some ecosystems in cold regions have been shown to be especially vulnerable and sensitive to environmental changes (Van Bogaert et al. 2007; Post et al. 2009; Bhatt et al. 2010; Callaghan et al. 2011; Elmendorf et al. 2012), especially in the northernmost latitudes, where climate warming is amplified (ACIA 2005; IPCC 2007; AMAP 2011). This has been demonstrated for instance by the expansion of shrub communities both in high latitude and high altitude ecosystems (Sturm et al. 2001; Tape et al. 2006; Hallinger and Wilmking 2011; Myers-Smith et al. 2011; Rundqvist et al. 2011). Interestingly, shrubs have been commonly described as potential facilitators and competitors in several ecosystems in cold regions (e.g. Shevtsova 1995; Doležal et al. 2004; Klanderud and Totland 2004; Klanderud 2005; Henríquez and Lusk 2005). However, little is known about how this potentiality might be affecting interacting species (understorey plants or tree seedlings) or how the interactions may change under varying types and regimes of stress.

Structure of the study and major findings

In this thesis I present four chapters, and in all of them I discuss how dwarf shrubs interact with co-occurring plants under varying regimes of stress. This research

involved ecosystems of great environmental, nature conservation and ecological value, yet highly sensitive to environmental changes, in four contrasting cold regions at high altitude or high latitude. Following a latitudinal order, the selected ecosystems were: 1) a temperate alpine treeline in the Central Pyrenees; 2) a primary succession gradient in a boreal ecosystem in Finland; 3) a subarctic alpine treeline in Lapland; and 4) a high-arctic tundra in north-eastern Greenland (see Figure 1).

The first two chapters are based on an experimental approach and focus on how shrubs commonly found near the treeline interact with tree seedlings of treeline-forming species in two contrasting environments, i.e. in a subarctic forest-tundra ecotone in Lapland, northern Sweden, and in a more southern, temperate forest-alpine pasture ecotone in the Central Pyrenees. In addition, since trees living near their limit of distribution are very sensitive to environmental changes, especially to increased temperature during the growing season (Körner 2003), we also assessed how distinct environmental change scenarios may affect tree seedling growth and survival across the ecotone. The first chapter is entitled 'Shrub-tree interactions and environmental changes drive treeline dynamics in the Subarctic', where we explain the three-year-long experiment performed in the Abisko National Park, in the subarctic Scandes, Northern Sweden; the species studied were *Betula pubescens* tree seedlings and the shrub *Vaccinium myrtillus*. In this study we showed the importance of facilitative and competitive shrub-tree interactions as drivers of subarctic treeline dynamics. Furthermore, we demonstrated that the great sensitivity of tree seedlings to warming had strong implications for treeline dynamics under the predicted warmer scenario at high latitudes, and we identified that complex interactions between shrubs and herbivores are critical to predicting future changes.

The second chapter is entitled 'Similar tree seedling responses to shrubs and to simulated environmental changes at Pyrenean and subarctic treelines'. Here we presented a comparison between the results obtained in the experiment presented in the first chapter and those obtained in a parallel experiment performed during a similar period near the treeline in the Alt Pirineu Natural Park, in the Central Pyrenees, Catalonia. This experiment was based on the same factorial design but with different

species (i.e. *Pinus uncinata* tree seedlings and the shrub *Rhododendron ferrugineum*). To our knowledge, it is the first study which experimentally tests the responses of plants to distinct environmental scenarios in a high mountain ecosystem in the Pyrenees. In this chapter we presented some mechanisms for understanding the recently observed variability of local responses of both subarctic and alpine treelines to currently changing climate while identifying some commonalities that can be used to generalise large scale response of treelines to climate warming.

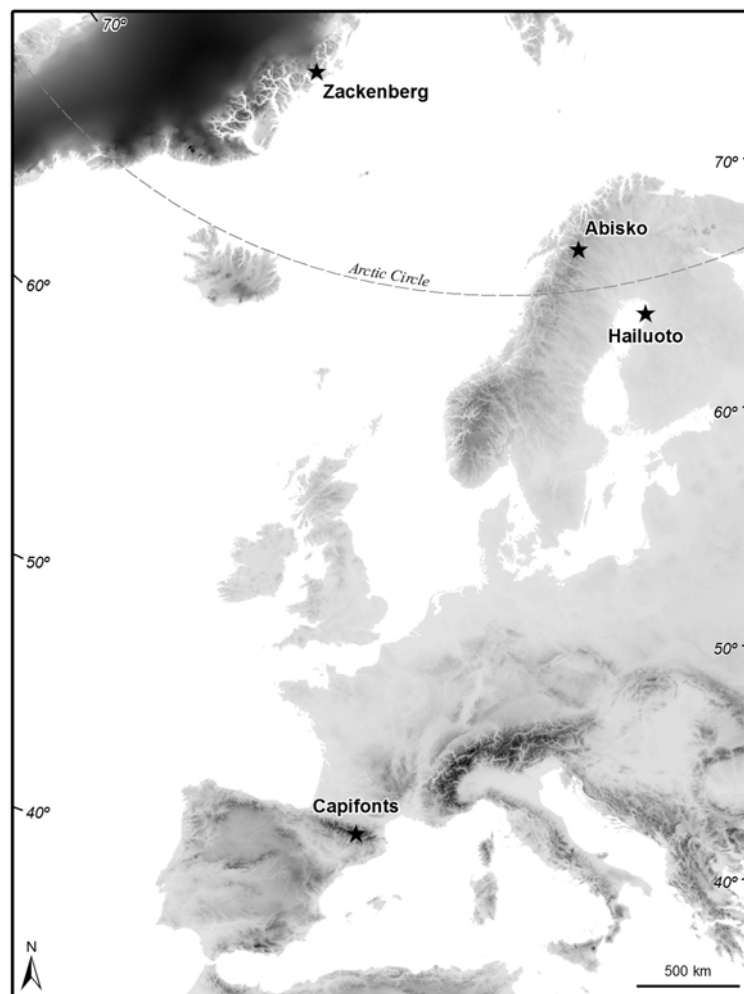


Figure 1. Location of the study areas: Capifonts (Central Pyrenees, Catalonia); Hailuoto (Bothnian Bay, Finland); Abisko (Swedish Scandes, Lapland); Zackenberg, (North-eastern Greenland).

The third chapter focuses on the effects of a dominant dwarf shrub (i.e. *Empetrum nigrum*) on *Pinus sylvestris* tree seedlings along a primary succession within a boreal

ecosystem on an uplifting island in Bothnian Bay, Finland. This chapter is called 'An ericoid shrub plays a dual role in recruiting both pines and their fungal symbionts along primary succession gradients'. Here we showed that facilitative and competitive effects of shrubs markedly determined tree seedling establishment and their fungal colonisation along this succession gradient, but in this chapter we did not relate these findings to any environmental changes. As far as we know, we presented the first finding that an ericoid mycorrhizal shrub may enhance both the performance of the ectomycorrhizal host tree and the tree's fungal symbionts.

The study presented in the fourth chapter was performed along a snow-depth gradient in an extreme arctic tundra ecosystem in the Northeast Greenland National Park, the largest national park in the world. The chapter is entitled 'Plant interactions and high-arctic vegetation composition along a snow-depth gradient in NE Greenland'. This ecosystem is probably the most sensitive and fragile among the ecosystems studied in this thesis as the eastern coast of Greenland is expected to experience substantial changes in climate due to marked changes in snow precipitation and temperature regimes (Brown and Mote 2009). Here we assessed plant species richness, establishment and composition patterns in distinct growth forms occurring in common arctic plant communities associated with varying snow-depth during the winter season. This study will help to predict potential diversity and vegetation changes in the high Arctic if snow precipitation regime changes in the future as anticipated.

Objectives

As indicated in the introduction, there is a need to gain insight into how the interactions between shrubs and other plants, the stress-gradient hypothesis, and the on-going environmental changes are inter-related. It is particularly timely and relevant to determine how these elements may control ecosystem functioning in cold regions, namely high latitude or high altitude ecosystems. To this aim, in this thesis we present four chapters, which deal with these elements in contrasting ecosystems in cold regions. The particular aims of each of these chapters are presented as follows:

1) 'Shrub-tree interactions and environmental changes drive treeline dynamics in the Subarctic'

This chapter focuses on a subarctic treeline ecosystem in the Scandes, a high latitude region which has been shown to be highly sensitive to environmental changes during the last decades. We aim to investigate: 1) whether shrub-tree interactions influence treeline dynamics observed in this subarctic 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; 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 contrasting environmental stress conditions.

2) 'Similar tree seedling responses to shrubs and to simulated environmental changes at Pyrenean and subarctic treelines'

In the second chapter we aim to compare the experiment in the subarctic Scandes presented in the first chapter with the results of a similar multi-factorial experiment in a typical treeline in the Central Pyrenees. We seek to investigate whether the mechanisms that control treeline dynamics, through shrub-tree interactions and environmental changes, are similar between contrasting regions, such as the Pyrenees and the Scandes. We aim to gain knowledge on the relative roles of general and local drivers of treeline dynamics, and on the high variability of treeline

responses to climate change recently observed across subarctic and alpine regions. We also investigate whether the stress-gradient hypothesis helps to explain the patterns observed in these two ecosystems near the treeline.

- 3) 'An ericoid shrub plays a dual role in recruiting both pines and their fungal symbionts along primary succession gradients'

In the third chapter we aim to assess shrub-tree interactions along a successional gradient in a coastal land uplift area on sandy soil in a boreal ecosystem, focusing on the effect of the dominant dwarf shrub *Empetrum nigrum* on *Pinus sylvestris* tree seedlings. On the basis of the stress-gradient hypothesis, we aim to investigate whether pine seedling recruitment, performance and ectomycorrhizal symbiosis are enhanced by the presence of the shrub in early successional stages, and whether these expectedly positive effects decrease towards more mature stages of the primary succession.

- 4) 'The role of plant interactions on vegetation composition along a snow-depth gradient in a high-arctic ecosystem (NE Greenland)'

The aim of the fourth chapter is to investigate whether distinct dominant arctic plant patch types, i.e. dominated by mosses or by the dwarf shrubs *Salix arctica* or *Dryas octopetala* × *intermedia*, drive the establishment of vascular plants and the species composition in three common arctic plant communities in NE Greenland. These plant communities are the *Salix* snowbed, the *Dryas* heath and the fell-field, respectively associated with an increasing altitudinal gradient and with high, medium and low snow-depth during winter, and thus with varying regimes of stress. We also investigate whether the stress gradient hypothesis applies over this snow-depth gradient in this highly sensitive high-latitude ecosystem.

General discussion and Final conclusions

General discussion

During the last decades, it has become widely accepted that positive plant-plant interactions act as key drivers of ecosystem structure and functioning. Evidence comes from a multitude of recent studies and research reviews (e.g. Pugnaire 2008). Callaway et al. (2002) argued that the next step after the inclusion of facilitation into ecological models and theory was to assess how environmental changes may affect individual plant-plant interactions, and to explore the shape and generality of the relationships between interactions and environmental severity, and the consequences of such interactions for plant communities (Maestre et al. 2009). In this thesis I have gained insight into these issues by assessing the importance of individual plant-plant interactions under varying regimes of stress and also by evaluating the potential effects of environmental changes on community structure in contrasting cold region ecosystems.

The effects of plant-plant interactions on ecosystem structure

As theorised by Lortie et al. (2004), in all the ecosystems studied we found that positive and negative plant-plant interactions were key drivers of ecosystem structure (Table 1). The papers presented in this thesis show that the nature and the ecological implications of the net balance derived from positive and negative shrub-tree interactions showed a converging response of tree seedling performance in contrasting ecosystems, such as in a temperate alpine treeline in the Catalan Pyrenees and in a subarctic alpine treeline in the Swedish Lapland. In addition, we found that the environmental change scenarios simulated experimentally in these two ecosystems in relation to varying temperature regimes, nutrient availability and dominance of shrubs, led to similar responses of the tree seedlings. These findings indicate that the impacts of shrub communities and of environmental changes on treeline dynamics may apply to contrasting treeline ecosystems with different biogeographic histories and species characteristics, and that plant-plant interactions play a role in mediating the impact of environmental change drivers on natural communities (Maestre and Reynolds 2006, 2007).

Shrub-tree interactions also played a major role in primary plant succession in the uplifting boreal island of Hailuoto, Finland. The positive effect of the shrub community was evidenced by an increase of tree seedling recruitment and of soil fungal biomass in *Empetrum* patches in early succession stages, and by higher seedling recruitment, greater diversity and increased number of mycorrhizal roots of tree seedlings in *Empetrum* patches in mid-succession stages. These findings support the idea that facilitation is a vital component of primary plant succession (Callaway 1995; Lortie et al. 2004; Moora and Zobel 2008). On the other hand, the effects of competition were increasingly visible towards later stages of succession, as the percentage of tree seedlings growing in shrub patches decreased towards mature stages, indicating that not only positive but also negative plant interactions drive primary plant succession (Glenn-Lewin et al. 1992).

The data collected along the snow-depth gradient in the high Arctic in north-eastern Greenland revealed that plant establishment in the distinct plant communities occurring along this gradient was also influenced by the positive and the negative effects of the dominant vegetation types (shrubs and mosses) on co-occurring species. Our findings suggest that such positive and negative effects of the dominant vegetation types will thus play a major role in driving the expected changes of vegetation in response to the predicted changes in snow precipitation and temperature regimes in this arctic region (IPCC 2007; Brown and Mote 2009).

The stress-gradient hypothesis (SGH)

The SGH predicts that plant-plant interactions may steadily shift from competition to facilitation with increasing environmental severity, i.e. positive interactions are expected to prevail in low resource, low productivity conditions and negative interactions where resource availability is high (Brooker and Callaghan 1998). As discussed in the previous section, positive and negative plant-plant interactions were found to be key drivers of ecosystem structure. Thus, it is relevant to evaluate the applicability of the SGH to these case studies (Table 1).

We did not find general support for the SGH. We observed that in the temperate and in the subarctic treeline ecosystems, facilitation and competition co-occurred across the ecotone; in the boreal primary succession gradient we found support for the SGH with regard to seedling recruitment but not to mycorrhization or seedling performance; in the snow-depth gradient in the high Arctic we did not find increasing facilitation with increasing environmental stress, but unexpectedly we found that a shrub species showed an increasingly competitive effect on co-occurring species. These results suggest that the stress gradients studied are not as simple as earlier thought; for instance, in a snow-depth gradient, snow may act as a resource of water, a positive factor in terms of shelter and insulation, but a negative factor in shortening growing season length. Thus, it is difficult to define how the overall strength of environmental stress changes along the gradients. Our findings do not necessarily negate the SGH, but indicate that further research is needed to better forecast the effects of competition and facilitation along stress gradients and to better understand the specific mechanisms involved in such interactions.

Suggestions for future research

In the studies performed in the Pyrenees, in the Scandes and in Finland I have only focused on the seedling stage, basically for practical reasons and because of the limited time available. Thus, the resulting papers only provided information on processes that select for the survival or performance of tree individuals for only one stage – although an important stage – of the long, tree life cycle. The papers acknowledged that abiotic and biotic selection processes occur naturally before seedlings reach the stage that was used and that further selection processes and changes in species interactions occur during later stages of the tree life cycle on older saplings and trees. Therefore, it would be interesting to investigate how plant-plant interactions and environmental changes may drive tree performance during germination and also once the individuals have established in the ecosystem.

On the other hand, our work provided strong evidence on the occurrence of facilitative and competitive interactions, which acted as important drivers of ecosystem structure

in the cold regions studied. However, I suggest that now some further research is needed to better estimate the net effect of facilitation and competition and better quantify their effect on ecosystem structure. A possible way to reach this next step would be to numerically measure the relative importance and intensity of such interactions by using plant interaction indexes, as suggested by Armas et al. (2004) and by Kikvidze and Armas (2008), which has not been done in this thesis.

Table 1 (See next page). Summary of the importance of plant-plant interactions, of the applicability of the stress-gradient hypothesis and of the effects of environmental changes and the potential implications for ecosystem structure in the distinct ecosystems studied in this thesis.

Table 1 (See previous page).

Ecosystem studied	Importance of plant-plant interactions	Applicability of the stress-gradient hypothesis (SGH)	Effects of environment changes (variation in temperature regime, in nutrient availability or in dominance of shrubs)	Potential implications for ecosystem structure
Capifonts Catalan Pyrenees (Temperate alpine treeline ecosystem)	Shrub-tree interactions drive tree seedling performance	No clear evidence that the relative importance of facilitation and competition shifted across the ecotone as predicted by the SGH	Marked positive effect of temperature and nutrient increase on tree seedling performance. Enhanced seedling performance if shrub was not present	Climate and land-use changes altering resource, non-resource or herbivore pressure are likely to induce changes in shrub-tree interactions in temperate alpine treeline ecosystems. Any future environmental changes causing variations in shrub cover may lead to marked ecosystem changes by altering tree seedling development
Hailuoto island Finland (Primary succession boreal ecosystem)	Shrub-tree interactions drive tree seedling recruitment and mycorrhization	Clear shift from facilitation to competition along the stress gradient as predicted by the SGH with regard to seedling recruitment but not to mycorrhization	The effects of increased temperature or nutrient availability have not been assessed experimentally in the field. Higher fungal biomass without shrubs across the succession gradient. The presence of shrubs played a varying role in tree seedling recruitment along the succession: it was positive in early stages and negative in mature stages	Any future environmental changes causing variations in shrub cover may lead to marked ecosystem changes by modifying both tree seedling recruitment and the associated fungal community in primary succession boreal ecosystems
Abisko Swedish Scandes (Subarctic alpine treeline ecosystem)	Shrub-tree interactions drive tree seedling survival and performance	No clear evidence that the relative importance of facilitation and competition shifted across the ecotone as predicted by the SGH	Marked positive effect of temperature and nutrient increase on tree seedling performance. Shrub removal impaired survival and it had simultaneous positive and negative effects on tree seedling performance	Climate and land-use changes altering resource, non-resource or herbivore pressure are likely to induce changes in shrub-tree interactions in subarctic alpine treeline ecosystems. Any future environmental changes causing variations in shrub cover may lead to marked ecosystem changes by altering tree seedling development
Zackenbergl NE Greenland (High-arctic ecosystem)	Positive and negative interactions between co-occurring species drive vascular plant establishment	No shift from facilitation to competition along the gradient as predicted by the SGH	The effects of increased temperature or nutrient availability have not been assessed experimentally in the field. Distinct dominant vegetation types showed contrasting patterns of plant establishment. Changes in plant composition responded to the distinct nature of the plant communities occurring along the snow-depth gradient	Any future environmental altering the relative proportion of the distinct dominant vegetation types in the high Arctic may have a marked effect on plant species richness and establishment in the distinct plant communities occurring along snow-depth gradients

Final conclusions

Shrub-tree interactions are important drivers of treeline dynamics, as shown in the experiment in the Subarctic and in the Pyrenees. Thus, such interactions must be included when modelling past, current and future treeline dynamics to better estimate any potential altitudinal or latitudinal shift or densification process as a response to climatic or land-use changes.

Notwithstanding the differences in species characteristics and soil nutrient status between subarctic and Pyrenean treelines, the factors and the underlying processes that drive treeline dynamics in both regions share many similarities. This could be a reason for the similar pattern of treeline dynamics observed during recent decades.

Facilitation, competition, herbivory and environmental changes act as crucial filters in structuring the forest-tundra ecotone in the Subarctic and the forest-alpine grassland ecotone in the Pyrenees. Any future changes in these homologous ecotones cannot be simply predicted from changing temperature patterns alone, as complex biotic and abiotic interactions need to be considered. These include interactions between shrubs and trees, between plants and herbivores, and between warming and soil nutrient availability.

Future climate and land-use changes altering resource, non-resource or herbivore pressure are likely to induce changes in shrub-tree interactions in temperate and in subarctic alpine treeline ecosystems; in the Subarctic, a comparatively higher sensitivity of tree seedlings to variations in temperature regimes and nutrient availability is suggested. Any future environmental changes causing variations in shrub cover may lead to marked ecosystem changes by altering tree seedling development in these two regions.

In the boreal primary succession ecosystem studied, shrub-tree interactions drive tree seedling recruitment and mycorrhization. The ericoid mycorrhizal shrub *Empetrum nigrum* acts as a facilitator for both the ectomycorrhizal host *Pinus sylvestris* and its

fungal symbionts. Any future environmental changes causing variations in shrub cover may lead to marked ecosystem changes by modifying both tree seedling recruitment and the associated fungal community in primary succession boreal ecosystems.

The growth forms are not good predictors of species co-occurrence in the high Arctic; contrasting growth forms such as the mosses and the shrub *Salix arctica* showed very similar patterns of species co-occurrence along the snow-depth gradient and acted as biodiversity hotspots in the harsher end of the gradient. The shrub *Dryas octopetala x intermedia* showed an increasingly competitive effect on co-occurring species along the gradient. The observed changes in plant composition along the snow-depth gradient in the high Arctic responded to the distinct nature of the extant plant communities. Any future environmental changes altering the relative proportion of the distinct dominant vegetation types in the high Arctic may have a marked effect on plant species richness and establishment in the distinct plant communities occurring along snow-depth gradients.

By assessing the occurrence of facilitation and competition in the ecosystems studied we found no general evidence that the prevalence of positive and negative interactions shifted as expected by the SGH. However, measuring the relative importance and intensity of such interactions and better understand the associated underlying mechanisms could be a next step to better estimate their role in ecosystem structure and functioning.

Future research should take into account that the effects of plant-plant interactions and environmental changes on trees may vary during the ontogeny of trees. Thus, further experiments are needed to assess how these driving forces affect both the germination and the stages following establishment to get a more complete view of their relative importance throughout tree life. Only then can location-specific projections of future treelines be made and without such location-specific knowledge, general models of this critically important ecotone will have impaired relevance.

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Publicacions / *Publications*

Capítol I

**Les interaccions arbre-arbust i els canvis ambientals dirigeixen la
dinàmica supraforestal a la zona subàrtica**

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

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Resum

Els límits supraforestals han estat objecte d'estudi durant força temps per la seva forta resposta a les fluctuacions climàtiques. Tot i així, els mecanismes que determinen el reclutament de plançons d'arbres en aquest ecosistema i la resposta de l'ecotò bosc-tundra als canvis ambientals han estat encara poc investigats. En aquest estudi partim de la hipòtesi que el desenvolupament dels plançons depèn en bona mesura de la interrelació entre les variacions del règim climàtic, les propietats nutricionals del sòl, i les interaccions facilitatives i competitives entre arbustos i arbres presents al límit supraforestal. Vàrem dur a terme un experiment de transplantament de plançons de *Betula pubescens* al límit supraforestal d'una zona subàrtica, al nord de Suècia. L'experiment es va basar en un disseny factorial amb quatre factors vinculats al règim d'estrès ambiental i a la disponibilitat de nutrients: posició al llarg de l'ecotò bosc-tundra (bosc *versus* límit de l'arbre); temperatura (amb o sense escalfament passiu mitjançant cambres tèrmiques); presència d'arbust (amb o sense *Vaccinium myrtillus*); i disponibilitat de nutrients (amb o sense addició de NPK). Al llarg de tres períodes de creixement es va avaluar l'establiment i el desenvolupament dels plançons de *Betula*.

Les manipulacions experimentals van causar efectes clarament significatius en el desenvolupament dels plançons. Tot i que *Vaccinium* va afavorir la supervivència d'aquests i va reduir els efectes de l'excés de radiació solar i de l'herbivorisme per part d'insectes, els plançons que creixien en presència de l'arbust van tenir un desenvolupament final més pobre en acabar el període experimental. A més, els plançons situats dins del bosc es van desenvolupar pitjor que els que creixien a la part superior de l'ecotò. Els plançons de *Betula* van mostrar una resposta marcadament positiva a l'augment de temperatures i a l'addició de nutrients, però aquests efectes van ser més evidents a la part superior de l'ecotò que dins del bosc, i es va detectar que la presència de *Vaccinium* modulava aquests efectes. Aquest experiment mostra que les interaccions arbust-arbre són factors causals claus en la dinàmica supraforestal de la zona subàrtica i que aquestes interaccions poden variar amb el temps i en l'espai. La facilitació, la competència, l'herbivorisme i els canvis ambientals en estadi de plançó actuen com a filtres importants de l'estructura de l'ecotò bosc-tundra. En aquest estudi s'ha demostrat que els canvis en aquest ecotò no es poden preveure només a partir de canvis en el règim de temperatures, sinó que cal considerar una gran

complexitat d'interaccions biòtiques i abiòtiques, com les que hi pot haver entre arbustos i arbres, però també amb herbívors o entre l'augment de temperatures i la disponibilitat de nutrients del sòl.

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 $-0.09 \pm 0.89^\circ\text{C}$, and the mean temperatures of the coldest and hottest months are $-10.1 \pm 3.7^\circ\text{C}$ and $+11.7 \pm 1.4^\circ\text{C}$, 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 (\pm open top chambers to give passive warming, ‘OTC’ hereafter); 3) shrub presence (\pm *Vaccinium* removal); 4) nutrient availability (\pm NPK addition). The treatments were distributed among 16 experimental units of approximately 1.13 m² 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 \times 2 microplots \times 16 experimental units \times 2 sites, $n = 384$ seedlings in total).

The experimental units were installed in an area (about 150 m²) 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 (Sæthun and Barkved 2003). For uniformity, those experimental units without OTCs were also hexagonally shaped and they all had the same area (1.13 m²). 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% P₂O₅, 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 -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 -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 tree-line 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 > stem length < red damage	> herbivory	> longest leaf < number leaves < red damage > herbivory	< number leaves > herbivory	> longest leaf < stem length < number leaves < red damage < herbivory < SPAD	< longest leaf < stem length < number leaves < herbivory < diameter < SPAD < % N in leaves < % C in leaves > CN ratio < total leaf biomass < total N > [N]/total N ratio < longest leaf
+ <i>Vaccinium</i>		> stem length (forest) < number leaves (treeline) < herbivory < number buds	> stem length (forest) < number leaves	> stem length (forest) < stem length (treeline) < number leaves < red damage	> stem length (forest) < number leaves < red damage < herbivory	> number leaves (treeline) < diameter (treeline)
+ OTC	> survival > longest leaf	< herbivory	Rodent outbreak (<i>Clethrionomys rufocanus</i> grazing on <i>Vaccinium</i>) > longest leaf (treeline) < red damage (treeline)	> stem length (treeline) > longest leaf (treeline)	> longest leaf (treeline) > stem length (treeline) > number leaves > red damage	> longest leaf (treeline) > stem length (treeline)
+ NPK	(not applied yet)	< herbivory > longest leaf > stem length (treeline) > number leaves (treeline, w/o <i>Vaccinium</i>) > number buds	> longest leaf > stem length (treeline) > number leaves > red damage	< red damage > stem length (treeline) > number leaves (with OTC)	> stem length (treeline) > number leaves < red damage	> SPAD (treeline) > total leaf biomass (w/o <i>Vaccinium</i>) > total N (w/o <i>Vaccinium</i>) < [N]/total N ratio (w/o <i>Vaccinium</i>) > longest leaf (treeline) < longest leaf (forest) > stem length (treeline with OTC) > number leaves (treeline, w/o <i>Vaccinium</i>) > diameter (with OTC or in forest) > % N in leaves > total leaf biomass (w/o <i>Vaccinium</i>) > total N (w/o <i>Vaccinium</i>) < [N]/total N ratio (w/o <i>Vaccinium</i>)

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	+ <i>Vaccinium</i>	+ OTC	+ NPK
< stem length	stem length (n.s.)	> stem length (treeline)	> stem length (treeline, w/o <i>Vaccinium</i>)
< 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).

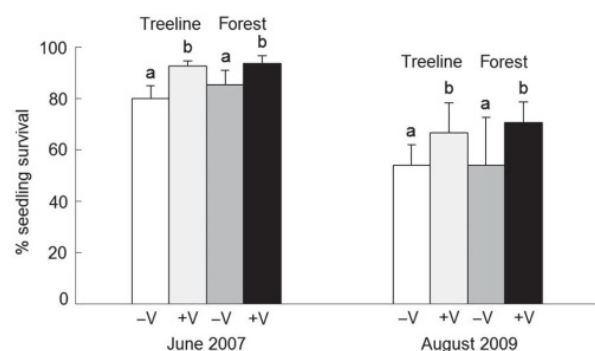


Figure 1. Percentage of surviving *Betula* seedlings at the beginning (June 2007) and at the end (August 2009) of the experiment. As only shrub presence and site across the ecotone gave consistent results in relation to survival, the mixed model analysis applied here considered only the factors 'site' 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

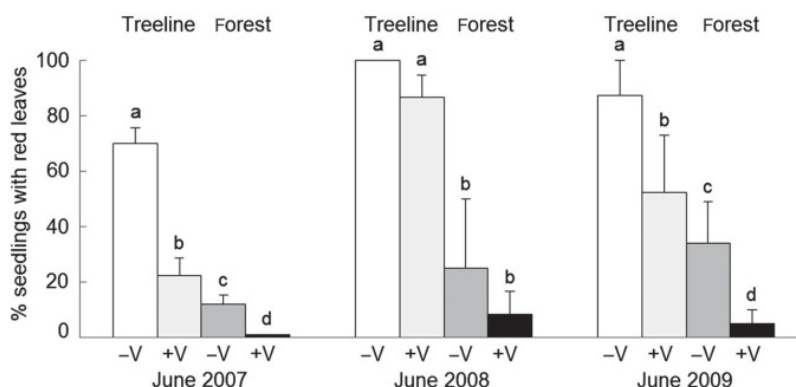


Figure 2. Percentage of *Betula* seedlings with leaves damaged by solar radiation over the course of the experiment. As only shrub presence and site across the ecotone gave consistent results in relation to solar radiation, the mixed model analysis applied here considered only the factors 'site' and '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

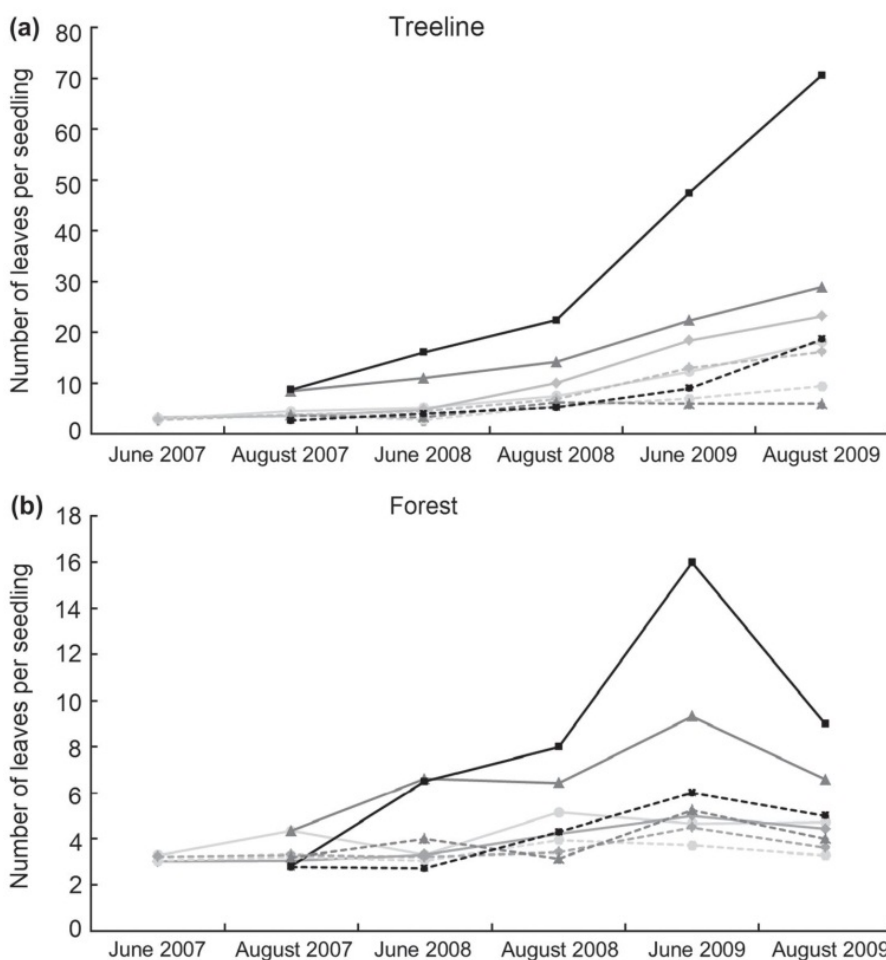


Figure 3. Mean number of leaves of *Betula* seedlings during the experimental period (a) at the treeline and (b) at the forest site for each of the treatments. Note that the y-axes have very different scales for treeline and forest. The statistical significances of each factor in each period are summarised 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*.

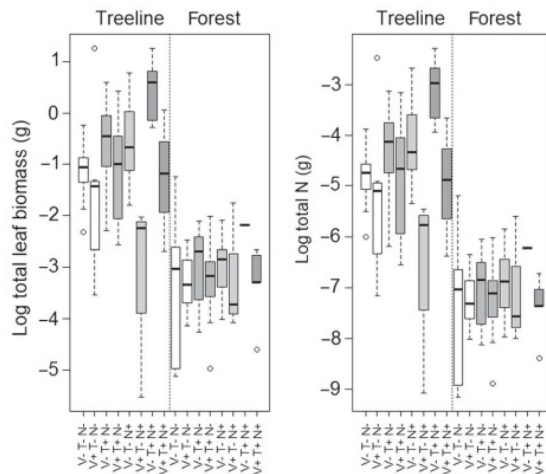


Figure 4. Left: log of total leaf dry biomass of *Betula* seedlings in August 2009. Right: log of total N content in the leaves of *Betula* seedlings in August 2009. The statistical significance of each factor is summarised in Table 1. The boxes in the figure correspond to the 1st and 3rd quartiles and 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 Sveinbjörnsen 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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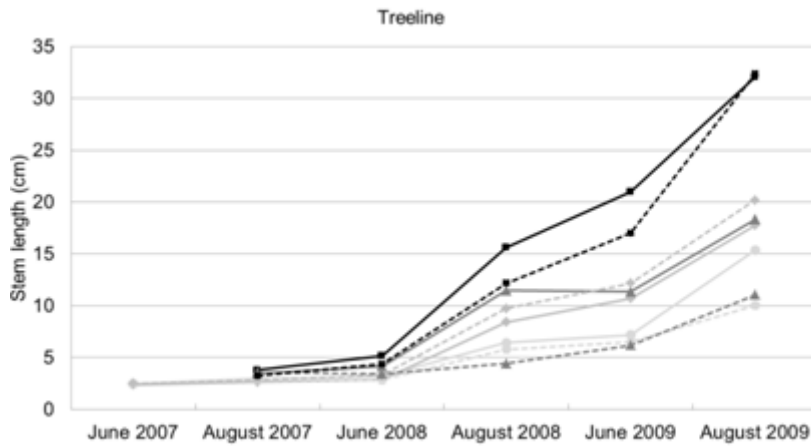
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Supplementary material (available as Appendix O20032 at <www.oikosoffice.lu.se/appendix>). Appendix 1.

Appendix 1.

a)



b)

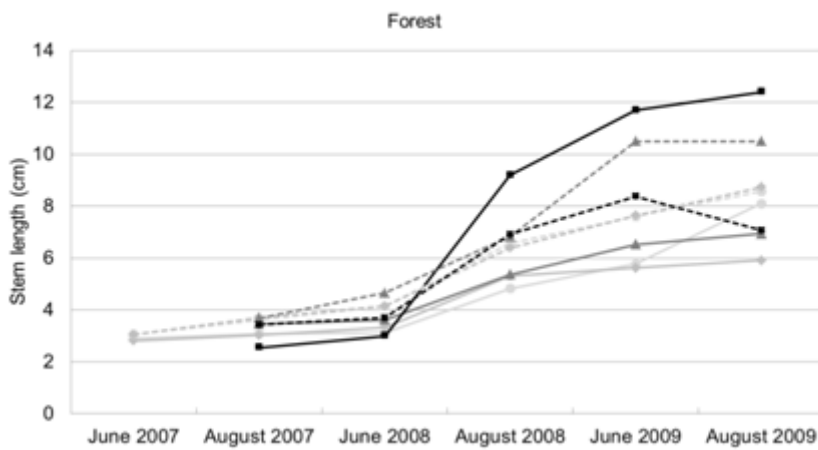
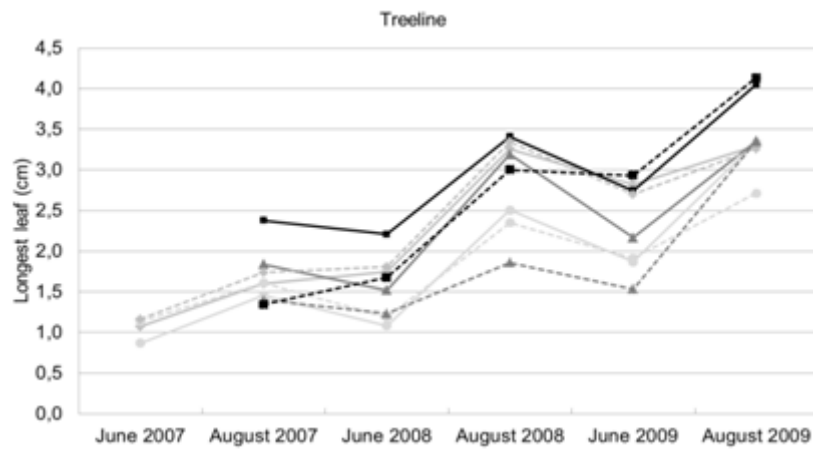


Fig. A1. Mean stem length of *Betula* seedlings during the experimental period **a)** at the treeline and **b)** at the forest site for each of the treatments. The statistical significances of each factor in each period are summarised in Table 1.

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*.

a)



b)

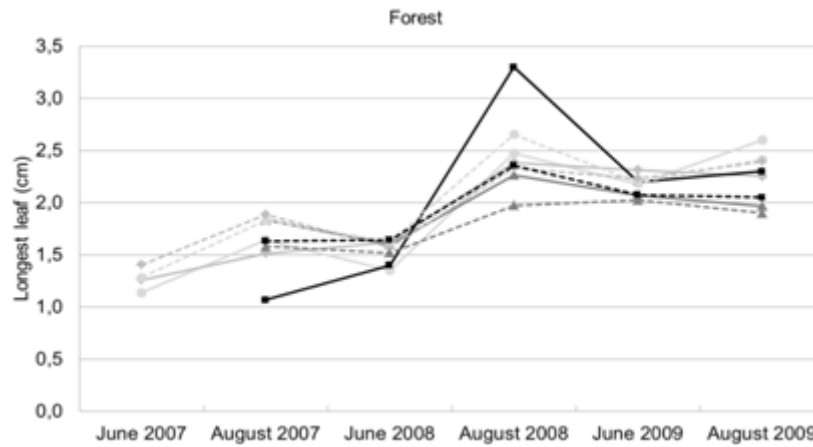


Fig. A2. Mean length of the longest leaf of *Betula* seedlings during the experimental period **a)** at the treeline and **b)** at the forest site for each of the treatments. The statistical significances of each factor in each period are summarised in Table 1.

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*.

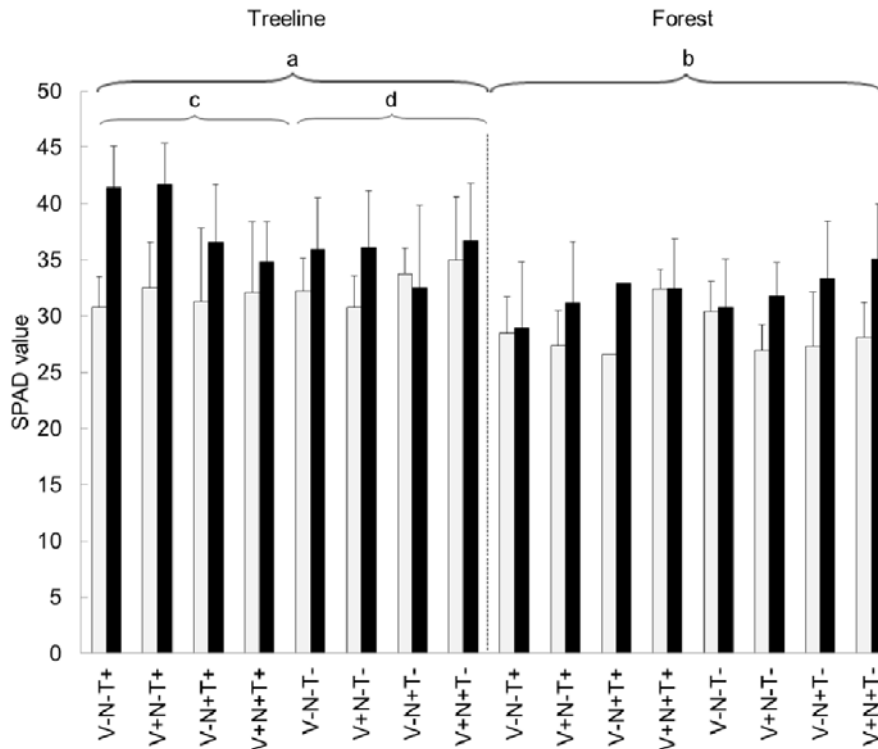


Fig. A3. Estimation of chlorophyll content in leaves of *Betula* seedlings, measured with a SPAD-502 device, an absorbance-based chlorophyll meter (see Material and Methods) in summer 2009. White and black boxes correspond to the measurements made in June 2009 and August 2009, respectively. The bars indicate standard deviation of the means. Treatments with different letters are significantly different (p -value < 0.05) and refer to the values from August 2009. The details about the statistical significances of each factor for both periods are summarised in Table 1.

Legend: V-: without *Vaccinium*; V+: with *Vaccinium*; N-: without fertiliser; N+: with fertiliser; T-: without OTC; T+: with OTC.

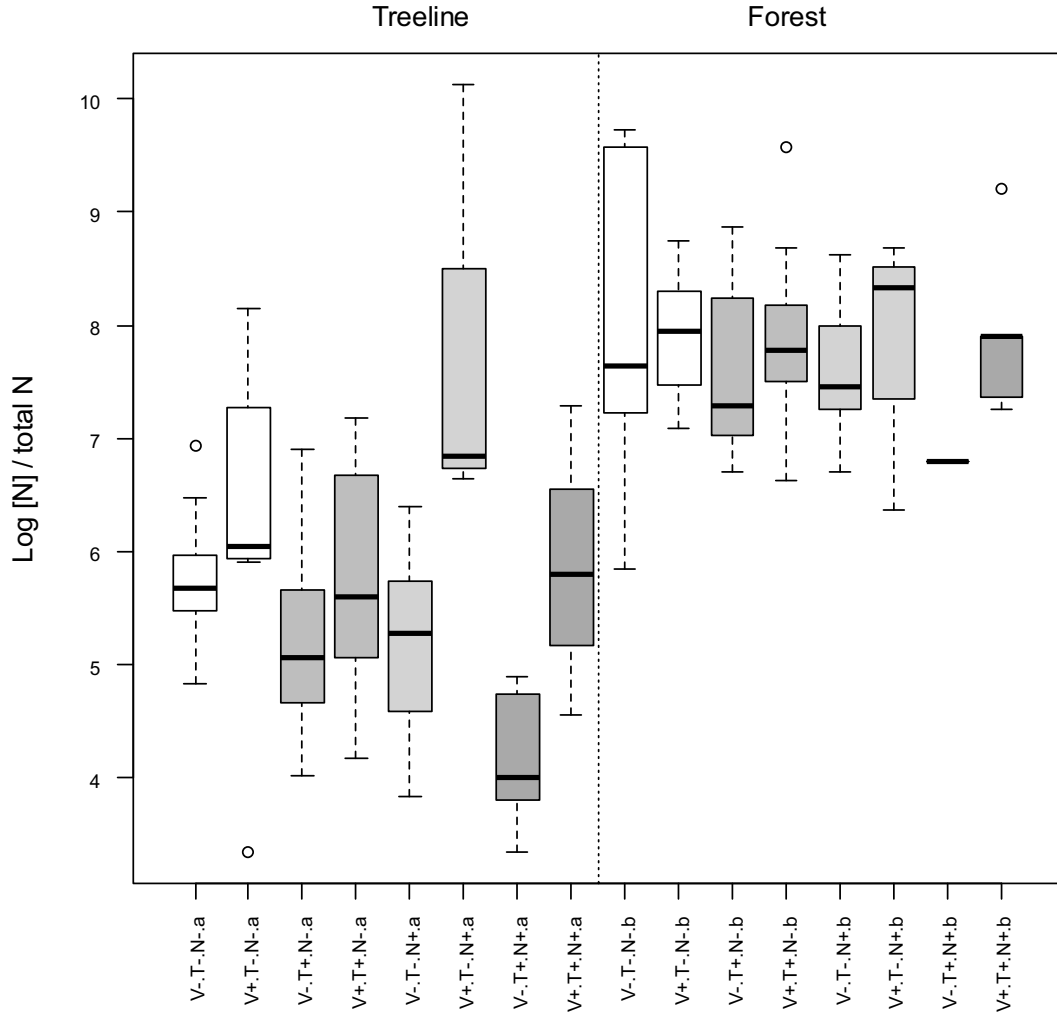


Fig. A4. Log [N] versus total N found in *Betula* seedlings in August 2009. Empty and filled boxes correspond to unfertilised and fertilised plots, respectively. The statistical significances of each factor in each period is summarised in Table 1. Legend: a: treeline; b: forest; V-: without *Vaccinium*; V+: with *Vaccinium*; T-: without OTC; T+: with OTC; N-: without fertiliser; N+: with fertiliser.

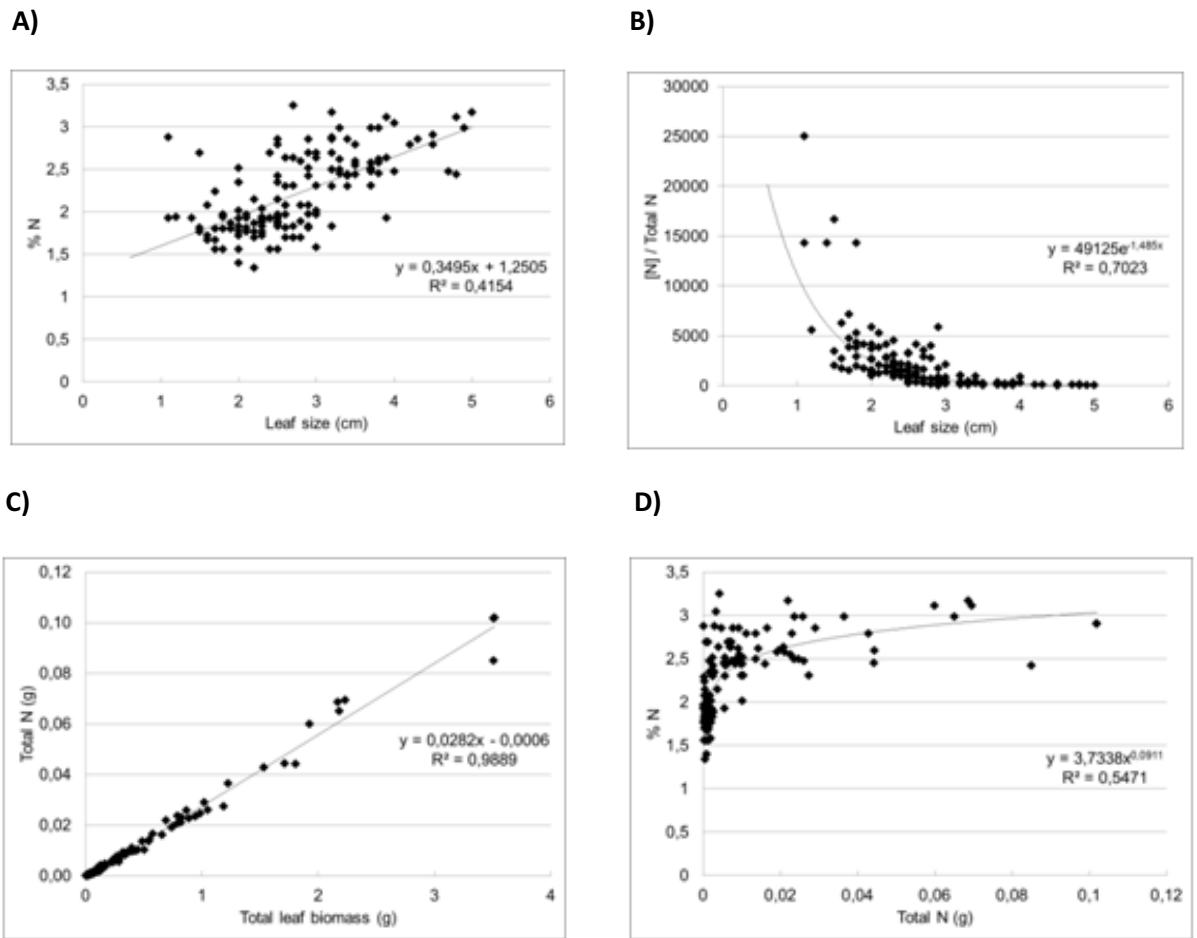


Fig. A5. Regressions showing the relationship between: **A)** % N vs Leaf size; **B)** Total N vs Total Leaf biomass; **C)** [N]/Total N vs Leaf size; **D)** % N vs Total N. Each point represents a *Betula* seedling.

Table A1. Mean value \pm standard deviation of the variables measured on each sampling occasion: **a)** Stem length; **b)** Number of leaves; **c)** Length of the longest leaf; **d)** Width of the widest leaf; **e)** SPAD value; **f)** Number of buds; **g)** Total leaf biomass and stem diameter; **h)** Leaf % C, % N, CN ratio and Total N of *Betula* seedlings. The abbreviations of the treatments correspond to: a: treeline; b: forest; V-: without *Vaccinium*; V+: with *Vaccinium*; N-: without fertiliser; N+: with fertiliser; T-: without OTC; T+: with OTC.

a) Mean stem length (cm) per group of *Betula* seedlings growing in a given treatment

Treatment	June 2007	August 2007	June 2008	August 2008	June 2009	August 2009
aV-N-T-	2,47 \pm 0,62	2,98 \pm 0,73	3,18 \pm 0,58	6,44 \pm 2,15	7,19 \pm 2,28	15,34 \pm 5,43
aV-N-T+	2,38 \pm 0,65	2,62 \pm 0,78	2,88 \pm 0,79	8,42 \pm 3,52	10,70 \pm 4,43	17,76 \pm 8,22
aV-N+T-		3,48 \pm 0,64	4,13 \pm 0,81	11,45 \pm 3,74	11,33 \pm 4,03	18,31 \pm 8,56
aV-N+T+		3,80 \pm 1,53	5,16 \pm 1,41	15,61 \pm 4,67	20,98 \pm 6,09	32,05 \pm 9,49
aV+N-T-	2,48 \pm 0,73	2,77 \pm 0,68	2,86 \pm 0,69	5,78 \pm 2,41	6,48 \pm 2,48	10,02 \pm 4,78
aV+N-T+	2,50 \pm 0,64	2,78 \pm 0,79	3,29 \pm 0,87	9,74 \pm 4,74	12,17 \pm 4,91	20,19 \pm 8,61
aV+N+T-		3,59 \pm 1,10	3,40 \pm 0,87	4,42 \pm 2,09	6,17 \pm 1,15	11,05 \pm 1,77
aV+N+T+		3,23 \pm 1,29	4,35 \pm 2,61	12,15 \pm 8,70	17,00 \pm 3,46	32,33 \pm 16,58
bV-N-T-	2,91 \pm 0,62	3,08 \pm 0,60	3,12 \pm 0,91	4,83 \pm 2,35	5,78 \pm 2,63	8,10 \pm 4,67
bV-N-T+	2,82 \pm 0,59	3,04 \pm 0,67	3,33 \pm 0,79	5,31 \pm 2,67	5,62 \pm 2,03	5,91 \pm 2,29
bV-N+T-		3,47 \pm 0,78	3,62 \pm 0,82	5,36 \pm 1,97	6,52 \pm 2,05	6,94 \pm 2,04
bV-N+T+		2,55 \pm 0,39	3,00 \pm 0,28	9,20 \pm 0,00	11,70 \pm 0,00	12,40 \pm 0,00
bV+N-T-	3,07 \pm 0,76	3,61 \pm 1,03	4,15 \pm 1,24	6,56 \pm 2,25	7,61 \pm 2,24	8,56 \pm 2,56
bV+N-T+	3,05 \pm 0,66	3,71 \pm 1,24	4,15 \pm 1,55	6,39 \pm 2,94	7,64 \pm 3,64	8,73 \pm 4,35
bV+N+T-		3,69 \pm 1,07	4,66 \pm 1,39	6,83 \pm 5,01	10,50 \pm 5,63	10,50 \pm 7,49
bV+N+T+		3,43 \pm 0,57	3,70 \pm 0,56	6,91 \pm 2,04	8,38 \pm 2,70	7,08 \pm 2,67

b) Mean number of leaves per group of *Betula* seedlings growing in a given treatment

Treatment	June 2007	August 2007	June 2008	August 2008	June 2009	August 2009
aV-N-T-	2,90 \pm 1,16	4,50 \pm 1,29	5,15 \pm 2,41	7,50 \pm 3,08	12,29 \pm 6,07	18,18 \pm 6,57
aV-N-T+	3,28 \pm 1,40	3,60 \pm 0,97	4,80 \pm 3,05	10,00 \pm 6,00	18,40 \pm 9,41	23,20 \pm 15,58
aV-N+T-		8,40 \pm 4,90	11,00 \pm 7,07	14,20 \pm 9,11	22,33 \pm 12,49	28,89 \pm 19,27
aV-N+T+		8,70 \pm 4,30	16,10 \pm 8,21	22,40 \pm 12,81	47,44 \pm 26,97	70,63 \pm 39,05
aV+N-T-	3,07 \pm 1,25	3,79 \pm 1,55	2,80 \pm 1,37	5,56 \pm 1,67	6,93 \pm 3,34	9,43 \pm 3,69
aV+N-T+	2,84 \pm 0,75	3,85 \pm 1,28	4,55 \pm 2,34	6,91 \pm 3,21	13,00 \pm 8,62	16,20 \pm 12,31
aV+N+T-		3,71 \pm 1,60	3,33 \pm 2,52	6,20 \pm 4,21	6,00 \pm 3,00	6,00 \pm 1,41
aV+N+T+		2,67 \pm 1,22	4,00 \pm 2,71	5,25 \pm 2,22	9,00 \pm 2,65	18,67 \pm 10,79
bV-N-T-	3,28 \pm 1,56	4,35 \pm 2,40	3,30 \pm 1,25	5,14 \pm 1,46	4,67 \pm 1,87	4,71 \pm 2,14
bV-N-T+	3,02 \pm 0,96	3,05 \pm 1,65	3,31 \pm 1,58	4,22 \pm 1,83	5,00 \pm 2,39	4,43 \pm 2,31
bV-N+T-		4,35 \pm 3,01	6,62 \pm 3,40	6,43 \pm 3,44	9,33 \pm 3,54	6,58 \pm 3,80
bV-N+T+		2,83 \pm 2,79	6,50 \pm 4,95	8,00 \pm 0,00	16,00 \pm 0,00	9,00 \pm 0,00
bV+N-T-	3,05 \pm 0,96	3,22 \pm 0,67	3,06 \pm 0,43	3,95 \pm 0,97	3,71 \pm 1,31	3,29 \pm 1,07
bV+N-T+	3,22 \pm 0,97	3,32 \pm 1,17	3,21 \pm 1,44	3,43 \pm 1,50	4,50 \pm 2,12	3,61 \pm 1,40
bV+N+T-		3,20 \pm 1,48	4,00 \pm 2,83	3,14 \pm 1,21	5,25 \pm 4,57	4,00 \pm 4,53
bV+N+T+		2,78 \pm 1,30	2,71 \pm 1,25	4,29 \pm 0,95	6,00 \pm 2,00	5,00 \pm 1,83

c) Mean length of the longest leaf (cm) per group of *Betula* seedlings growing in a given treatment

Treatment	June 2007	August 2007	June 2008	August 2008	June 2009	August 2009
aV-N-T-	0,87 ± 0,26	1,46 ± 0,33	1,08 ± 0,31	2,51 ± 0,51	1,87 ± 0,31	3,34 ± 0,42
aV-N-T+	1,07 ± 0,26	1,60 ± 0,29	1,75 ± 0,33	3,26 ± 0,76	2,82 ± 0,41	3,30 ± 0,54
aV-N+T-		1,84 ± 0,45	1,52 ± 0,37	3,19 ± 0,60	2,17 ± 0,33	3,36 ± 0,85
aV-N+T+		2,38 ± 0,56	2,21 ± 0,48	3,41 ± 1,01	2,74 ± 0,53	4,05 ± 0,57
aV+N-T-	1,14 ± 0,26	1,61 ± 0,53	1,19 ± 0,35	2,35 ± 0,61	1,91 ± 0,17	2,71 ± 0,51
aV+N-T+	1,16 ± 0,34	1,74 ± 0,52	1,81 ± 0,30	3,35 ± 0,98	2,70 ± 0,40	3,26 ± 0,73
aV+N+T-		1,40 ± 0,14	1,23 ± 0,55	1,86 ± 0,54	1,53 ± 0,21	3,35 ± 0,92
aV+N+T+		1,34 ± 0,82	1,68 ± 0,84	3,00 ± 0,91	2,93 ± 0,12	4,13 ± 0,51
bV-N-T-	1,14 ± 0,35	1,64 ± 0,49	1,35 ± 0,23	2,47 ± 0,67	2,18 ± 0,60	2,60 ± 0,65
bV-N-T+	1,26 ± 0,30	1,51 ± 0,26	1,62 ± 0,33	2,38 ± 0,47	2,31 ± 0,36	2,25 ± 0,33
bV-N+T-		1,84 ± 0,8	1,60 ± 0,52	2,26 ± 0,36	2,07 ± 0,20	1,97 ± 0,31
bV-N+T+		1,07 ± 0,65	1,40 ± 1,13	3,30 ± 0,00	2,20 ± 0,00	2,30 ± 0,00
bV+N-T-	1,29 ± 0,35	1,83 ± 0,44	1,62 ± 0,32	2,65 ± 0,54	2,19 ± 0,29	2,41 ± 0,36
bV+N-T+	1,41 ± 0,45	1,89 ± 0,62	1,57 ± 0,51	2,33 ± 0,53	2,23 ± 0,39	2,39 ± 0,40
bV+N+T-		1,58 ± 0,46	1,52 ± 0,64	1,97 ± 1,10	2,03 ± 0,36	1,90 ± 0,54
bV+N+T+		1,63 ± 0,55	1,64 ± 0,73	2,36 ± 0,30	2,08 ± 0,32	2,05 ± 0,33

d) Mean width of the widest leaf (cm) per group of *Betula* seedlings growing in a given treatment

Treatment	June 2007	August 2007	June 2008	August 2008	June 2009	August 2009
aV-N-T-	0,90 ± 0,28	1,46 ± 0,36	1,02 ± 0,28	2,52 ± 0,52	1,91 ± 0,32	3,45 ± 0,49
aV-N-T+	1,04 ± 0,26	1,43 ± 0,24	1,63 ± 0,31	3,02 ± 0,68	2,60 ± 0,34	3,26 ± 0,63
aV-N+T-		1,96 ± 0,55	1,47 ± 0,46	3,22 ± 0,74	2,20 ± 0,35	3,44 ± 0,94
aV-N+T+		2,43 ± 0,68	2,17 ± 0,48	3,21 ± 0,80	2,60 ± 0,44	4,21 ± 0,65
aV+N-T-	1,14 ± 0,28	1,57 ± 0,50	1,13 ± 0,36	2,29 ± 0,64	1,93 ± 0,23	2,79 ± 0,60
aV+N-T+	1,15 ± 0,34	1,64 ± 0,50	1,76 ± 0,38	3,12 ± 0,87	2,52 ± 0,36	3,30 ± 0,73
aV+N+T-		1,30 ± 0,17	1,17 ± 0,58	1,76 ± 0,55	1,70 ± 0,20	3,30 ± 0,99
aV+N+T+		1,23 ± 0,76	1,63 ± 0,66	2,78 ± 1,11	3,10 ± 0,56	4,23 ± 0,50
bV-N-T-	1,18 ± 0,39	1,62 ± 0,41	1,34 ± 0,30	2,23 ± 0,68	2,33 ± 0,59	2,73 ± 0,75
bV-N-T+	1,27 ± 0,29	1,41 ± 0,20	1,62 ± 0,32	2,16 ± 0,42	2,33 ± 0,32	2,33 ± 0,46
bV-N+T-		1,73 ± 0,74	1,58 ± 0,50	2,26 ± 0,28	1,98 ± 0,16	1,98 ± 0,26
bV-N+T+		1,10 ± 0,73	1,25 ± 1,06	2,90 ± 0,00	2,10 ± 0,00	2,20 ± 0,00
bV+N-T-	1,30 ± 0,36	1,75 ± 0,41	1,63 ± 0,31	2,51 ± 0,58	2,32 ± 0,24	2,39 ± 0,31
bV+N-T+	1,34 ± 0,42	1,59 ± 0,48	1,57 ± 0,52	2,18 ± 0,47	2,27 ± 0,54	2,33 ± 0,43
bV+N+T-		1,43 ± 0,42	1,44 ± 0,68	1,74 ± 1,02	2,05 ± 0,56	1,58 ± 1,09
bV+N+T+		1,56 ± 0,61	1,61 ± 0,67	2,14 ± 0,24	2,00 ± 0,35	1,95 ± 0,44

e) Mean leaf SPAD value per group of *Betula* seedlings growing in a given treatment

Treatment	June 2009	August 2009
aV-N-T-	32,24 ± 2,90	35,92 ± 4,65
aV-N-T+	30,77 ± 2,79	41,45 ± 3,66
aV-N+T-	33,74 ± 2,33	32,54 ± 7,28
aV-N+T+	31,28 ± 6,59	36,55 ± 5,10
aV+N-T-	30,81 ± 2,76	36,09 ± 5,07
aV+N-T+	32,55 ± 3,96	41,65 ± 3,71
aV+N+T-	34,97 ± 5,61	36,70 ± 5,09
aV+N+T+	32,07 ± 6,32	34,80 ± 3,58
bV-N-T-	30,36 ± 2,69	30,80 ± 4,32
bV-N-T+	28,45 ± 3,27	28,94 ± 5,88
bV-N+T-	27,34 ± 4,77	33,38 ± 5,06
bV-N+T+	26,60 ± 0,00	32,90 ± 0,00
bV+N-T-	26,88 ± 2,34	31,79 ± 2,96
bV+N-T+	27,40 ± 3,09	31,15 ± 5,45
bV+N+T-	28,13 ± 3,07	35,12 ± 4,85
bV+N+T+	32,43 ± 1,71	32,45 ± 4,42

f) Mean number of buds per group of *Betula* seedlings growing in a given treatment

Treatment	June 2007	August 2007	August 2008
aV-N-T-	1,26 ± 0,64	1,36 ± 0,64	1,36 ± 0,74
aV-N-T+	1,31 ± 0,61	1,10 ± 0,32	2,11 ± 1,83
aV-N+T-		2,60 ± 1,84	2,80 ± 1,93
aV-N+T+		2,50 ± 1,84	4,00 ± 1,88
aV+N-T-	1,22 ± 0,59	1,11 ± 0,31	1,13 ± 0,34
aV+N-T+	1,12 ± 0,32	1,08 ± 0,28	1,36 ± 0,67
aV+N+T-		1,43 ± 0,79	2,40 ± 2,19
aV+N+T+		1,22 ± 0,67	1,00 ± 0,00
bV-N-T-	1,46 ± 0,97	1,41 ± 0,94	1,29 ± 0,61
bV-N-T+	1,12 ± 0,39	1,23 ± 0,53	1,50 ± 1,46
bV-N+T-		1,65 ± 1,46	2,50 ± 1,91
bV-N+T+		1,50 ± 0,84	2,00 ± 0,00
bV+N-T-	1,16 ± 0,37	1,13 ± 0,34	1,00 ± 0,00
bV+N-T+	1,29 ± 0,59	1,32 ± 0,57	1,24 ± 1,09
bV+N+T-		1,10 ± 0,32	1,00 ± 0,00
bV+N+T+		1,11 ± 0,33	1,00 ± 0,00

g) Mean total leaf biomass (g) and stem diameter (mm) per group of *Betula* seedlings growing in a given treatment, measured in August 2009

Treatment	Leaf biomass (g)	Diameter (cm)
aV-N-T-	0,37 ± 0,19	4,00 ± 1,00
aV-N-T+	0,70 ± 0,49	5,00 ± 1,83
aV-N+T-	0,73 ± 0,60	5,00 ± 2,33
aV-N+T+	1,87 ± 1,03	7,85 ± 2,27
aV+N-T-	0,59 ± 1,18	3,14 ± 0,77
aV+N-T+	0,47 ± 0,45	3,40 ± 1,58
aV+N+T-	0,09 ± 0,06	2,50 ± 0,71
aV+N+T+	0,43 ± 0,43	5,67 ± 2,08
bV-N-T-	0,07 ± 0,85	2,00 ± 0,58
bV-N-T+	0,06 ± 0,04	1,86 ± 0,77
bV-N+T-	0,06 ± 0,23	2,50 ± 0,80
bV-N+T+	0,11 ± 0,00	4,00 ± 0,00
bV+N-T-	0,04 ± 0,22	1,66 ± 0,65
bV+N-T+	0,05 ± 0,30	1,88 ± 0,78
bV+N+T-	0,07 ± 0,88	2,13 ± 1,31
bV+N+T+	0,04 0,24	2,25 ± 0,50

h) Mean leaf % C, % N, CN ratio and Total g N content per group of *Betula* seedlings growing in a given treatment in August 2009

Treatment	% C	% N	CN ratio	Total g N
aV-N-T-	47,737 ± 0,768	2,537 ± 0,087	18,718 ± 0,456	0,009 ± 0,005
aV-N-T+	49,077 ± 0,758	2,408 ± 0,211	20,743 ± 2,209	0,017 ± 0,012
aV-N+T-	48,185 ± 0,292	2,753 ± 0,332	17,654 ± 2,844	0,021 ± 0,019
aV-N+T+	48,457 ± 0,559	2,915 ± 0,184	16,803 ± 1,459	0,055 ± 0,030
aV+N-T-	47,550 ± 0,843	2,594 ± 0,140	18,640 ± 1,721	0,014 ± 0,029
aV+N-T+	48,745 ± 0,250	2,449 ± 0,345	20,902 ± 3,659	0,012 ± 0,013
aV+N+T-	47,480 ± 0,441	3,012 ± 0,177	15,564 ± 0,819	0,003 ± 0,002
aV+N+T+	47,713 ± 0,000	2,475 ± 0,000	16,438 ± 0,000	0,011 ± 0,011
bV-N-T-	46,821 ± 1,159	1,845 ± 0,119	26,824 ± 3,301	0,001 ± 0,119
bV-N-T+	46,636 ± 0,752	1,754 ± 0,227	27,120 ± 5,387	0,001 ± 0,001
bV-N+T-	47,095 ± 0,724	1,938 ± 0,240	24,679 ± 3,481	0,001 ± 0,001
bV-N+T+	47,650 ± 0,000	1,768 ± 0,000	26,955 ± 0,000	0,002 ± 0,000
bV+N-T-	45,921 ± 2,771	1,881 ± 0,168	24,533 ± 1,902	0,001 ± 0,000
bV+N-T+	46,715 ± 0,439	1,888 ± 0,062	25,024 ± 1,131	0,001 ± 0,001
bV+N+T-	47,895 ± 0,564	2,088 ± 0,100	23,306 ± 1,666	0,002 ± 0,002
bV+N+T+	46,505 ± 1,719	1,815 ± 0,308	25,909 ± 5,914	0,001 ± 0,000

Capítol II

Respostes semblants dels plançons d'arbres a la presència d'arbustos i a les simulacions de canvis ambientals en límits supraforestals Pirinencs i subàrtics

Similar tree seedling responses to shrubs and to simulated environmental changes at Pyrenean and subarctic treelines

Oriol Grau, Josep M. Ninot, Johannes H.C. Cornelissen & Terry Callaghan

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Resum

Un experiment desenvolupat recentment a la zona subàrtica revela que les interaccions arbust-arbre, juntament amb els canvis ambientals, són determinants en el desenvolupament dels plançons d'arbre en l'ecotò bosc-tundra. Però s'ignora si aquest és un exemple d'un fenomen més general. L'objectiu d'aquest segon estudi és investigar si aquests resultats tenen aplicabilitat en altres regions amb històries biogeogràfiques i espècies diferents. Amb aquesta finalitat, es va realitzar durant tres anys un experiment semblant en una zona més temperada i meridional, a l'ecotò bosc-prats alpins dels Pirineus Centrals catalans. Els dos experiments es basaven en un disseny multifactorial amb quatre factors relacionat amb els règims de severitat ambiental i amb la disponibilitat de recursos, utilitzant plançons de *Betula pubescens* i el subarbust *Vaccinium myrtillus* a la zona dels Scandes subàrtics, i plançons de *Pinus uncinata* i l'arbust *Rhododendron ferrugineum* als Pirineus.

S'ha observat que els processos que determinen la dinàmica del límit supraforestal en aquestes dues zones tenen molts punts en comú; la facilitació i la competència entre arbusts i arbres són claus per entendre el desenvolupament dels plançons d'arbre en el límit supraforestal sota escenaris de canvis ambientals. Tot i les particularitats pròpies de cada zona, les semblances entre els dos ecosistemes homòlegs estudiats eren coherents amb els patrons generals de la dinàmica supraforestal observada durant les darreres dècades a escala regional als Scandes i als Pirineus. La convergència funcional en la resposta d'aquests ecotons ajudarà a construir un marc predictiu que tingui en consideració els efectes de la comunitat arbustiva en la dinàmica supraforestal d'altres regions.

Similar tree seedling responses to shrubs and to simulated environmental changes at Pyrenean and subarctic treelines

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Abstract

Background: A recent experiment in the Subarctic showed that shrub-tree interactions additional to environmental changes control tree seedling performance in a treeline ecotone. However, it remains uncertain whether this is an example of a more general phenomenon.

Aim: We set up a similar experiment in a more southern, temperate treeline ecotone in the Central Pyrenees to investigate whether this also applies to other regions with different biogeographic histories and species characteristics.

Methods: Both experiments featured a multi-factorial design with four treatment factors relating to environmental regimes of stress and resource availability, applied to transplanted tree seedlings (*Betula pubescens* in the subarctic Scandes and *Pinus uncinata* in the Pyrenees) during three growing seasons.

Results: The underlying processes that drive treeline dynamics in both regions shared many similarities; facilitation and competition between shrubs and trees are crucial to

understand tree seedling performance at the treeline ecotone under environmental change scenarios.

Conclusions: Although some particularities were exclusive to each region, the similarities were consistent with general patterns of treeline dynamics observed during recent decades at a regional scale in the Scandes and in the Pyrenees. This convergence of response will help the development of a robust mechanistically founded predictive framework for scaling up shrub impacts on treeline dynamics to other regions.

Keywords: competition, environmental change, facilitation, herbivory, plant-plant interaction, Pyrenees, shrub, Subarctic, treeline dynamics

Introduction

Treeline dynamics are strongly regulated by temperature since the locations of latitudinal and altitudinal treelines are very sensitive and potentially responsive to heat deficiency. However, although the ongoing mean annual temperature increase in arctic and subarctic regions is well documented (IPCC 2007), its effects on treeline dynamics seem variable and hard to predict (Mamet and Kershaw 2011). A confounding factor is that many temperature effects are indirect. For example, increasing temperatures increase soil nutrient mineralisation, and thereby nutrient availability (Rustad et al. 2001) while numerous papers describe the effects of herbivory - often temperature related - on treelines (Tenow 1996). Despite these indirect effects and although treeline responses may be asynchronous with the rate of warming (Rupp et al. 2001), northern hemisphere climate warming is still expected to cause treelines to advance to higher elevations and more northerly latitudes (Holtmeier and Broll 2005; Harsch et al. 2009). These expectations are based on models of projected future treeline dynamics (e.g. Wolf et al. 2008) and the patterns observed in the past (Tinner and Theurillat 2003; Cunill 2010).

A worldwide meta-analysis of treeline responses to climate warming during the last century shows that about 52% of the ecotones advanced, 46% remained static and only a few cases reported treeline recession (Harsch et al. 2009). However, there is

also a marked local and regional variability of treeline dynamics across the globe, so these percentages may not necessarily apply to smaller scales. An example of such regional and local variability was described by Van Bogaert et al. (2011) in the subarctic Scandes. In this northern region, some treelines have advanced, and some others have either become denser, remained static, or even retreated (Kullman 2002; Tømmervik et al. 2004; Dalen and Hofgaard 2005). In the European Alps however, several authors have reported a rather widespread densification and also an upslope tree expansion in the subalpine belt during the last decades (Motta and Nola 2001; Gehrig-Fasel et al. 2007; Chauchard et al. 2010). At a more southern latitude, in the Pyrenees, a regional densification of the treelines occurred during the second half of the 20th century, but it has been coupled with treeline rise only at the local scale (Batllori and Gutiérrez 2008). However, the variability of treeline dynamics observed within different regions and among mountain ranges across Europe is often not explained solely by climate. For instance, changes in land-use or in disturbance regime (i.e. slope processes, livestock and human pressure, insect outbreaks) may determine when treeline advance will occur (Harsch et al. 2009; Ameztegui et al. 2010) or if it will occur at all (Cairns and Moen 2004; Van Bogaert et al. 2011; but see also Batllori et al. 2010). These non-linear responses to climate make it challenging to predict if and how treelines will respond to the increasing temperatures (Daniels and Veblen 2004; Stueve et al. 2009; Elliot 2011). Indeed, treeline expansion predictions will be accurate only if the spatial variability of influences from abiotic and biotic site conditions are integrated at the local level (Stueve et al. 2011).

Knowledge of treeline dynamics has been dominated by abiotic factors. However, knowledge of mechanisms behind biotic interactions is also fundamental to our understanding of the dynamics of ecological communities such as the phenomenon of treeline. The role of competition has been widely debated, but facilitation has only more recently received particular attention (Callaway 2010). Besides, only a few studies have considered plant facilitation as an important driver of the functioning and fate of plant communities under varying environmental conditions (Brooker 2010). Plant facilitation has been shown to control tree seedling establishment at treeline by ameliorating microsite conditions; for instance, the positive effects of tree islands

(Alftine and Malanson 2004), individual krummholz trees (Batllori et al. 2009), tussocks (Sullivan and Sveinbjörnsson 2010), grass cover (Germino et al. 2002) or mosses (Wheeler et al. 2011) have been found to enhance tree seedling recruitment in this harsh environment. Also, facilitative shrub-tree interactions play a key role in determining the dynamics of alpine treelines (Henríquez and Lusk 2005; Akhalkatsi et al. 2006; Batllori et al. 2009). However, little is known about the underlying mechanisms that control tree seedling establishment and development at the treeline under the influence of shrubs, in spite of their current expansion at higher altitudes and latitudes (Sturm et al. 2001; Tape et al. 2006; Myers-Smith et al. 2011; Elmendorf et al. 2012). Moreover, there is little knowledge on how shrub-tree interactions could be affected by environmental change. Grau et al. (2012) experimentally showed the importance of shrubs and some of the mechanisms by which shrub-tree interactions control tree seedling survival and development across the forest-tundra ecotone in the subarctic Scandes. The results point to a key role for the nutrient and temperature stress regimes related to the position across and within the upper and lower parts of the ecotone. However, the nature of the forest-tundra ecotone and the shrub community differ between subarctic and temperate regions; therefore, we do not currently know if it is possible to extrapolate knowledge gained from the experiment in the Subarctic to shrub-tree interactions and to responses to environmental changes in more temperate, southern treelines, such as in the Pyrenees.

Here we report the results of a multi-factorial experiment in a typical treeline in the Central Pyrenees, and compare it with the experiment in the subarctic Scandes (Grau et al. 2012). In order to improve general predictions of future treeline dynamics, we seek to investigate whether the mechanisms that control treeline dynamics, through shrub-tree interactions and environmental changes, are similar between contrasting regions, viz. the Pyrenees and the Scandes. Similarly to the hypotheses that had been stated in Grau et al. (2012) in relation to the experiment in the Scandes, here we hypothesise (1) that, tree seedlings in the Pyrenees will: a) grow better in the forest below the treeline than at the treeline; b) benefit from growing with shrubs which will provide safe sites; c) grow more under raised temperatures and increased nutrient availability. Experimentally exploring these hypotheses will allow us to test whether

the potential drivers of seedling recruitment, and thus of treeline dynamics, act similarly in the two different regional treelines considered in this study. In other words, we aim to identify if the selected general drivers of treeline dynamics over-ride the specific species characteristics and biogeographic histories in the two regions. Although there are many differences in abiotic and biotic regimes between the two treeline ecotones (Table 1), our null expectation is that there are fundamental similarities in response to the treatments, given the overall temperature driver at the treelines in both regions. We also hypothesise (2) that, on the basis of the refined stress gradient hypothesis (Maestre et al. 2009 refined from Brooker and Callaghan 1998), plant-plant interactions will be neutral, positive and negative under low, medium and high resource stress, respectively, but will shift from negative to positive under increasing abiotic stress (Maestre et al. 2009) and/or herbivore pressure. The results from testing these hypotheses and understanding the relative roles of general and local drivers of treeline dynamics will lead to a better understanding of the high variability of treeline responses to climate change recently observed across arctic and alpine regions. Furthermore, understanding the mechanisms behind drivers of treeline change will lead to more accurate model projections of climate change driven impacts on treelines in general.

Methods

The two parallel experiments were conducted simultaneously in homologous ecosystems: one was located in the forest-tundra ecotone in a subarctic region in the Scandes (Abisko, Northern Sweden) and the other in the forest-alpine grassland ecotone in a more temperate region in the Central Pyrenees (Fig. 1). Hereafter, 'treeline' will be referred to as the upper part of these ecotones, where there is a very low density of trees, and these are not taller than 5 m (Smith et al 2003). We use the term 'forest' to denote the lower end of these ecotones, with a uniform, closed, dense canopy of taller trees. We define the 'treeline ecotone' as the whole area extending from 'forest' to 'treeline', i.e. the transition from the lower to the upper part of the ecotone.

SUMMARY OF THE EXPERIMENT IN THE SUBARCTIC

The experiment was conducted on the eastern slope of Mount Nuolja in the Scandes mountain range, near the Abisko (Abeskovvu) National Park (Lapland, Sweden), which is near the Abisko Scientific Research Station (Abisko Naturvetenskapliga Station), about 200 km north of the Arctic Circle. The study species were the tree *Betula pubescens* ssp. *czerepanovii* (Orlova) Hämet-Ahti and the shrub *Vaccinium myrtillus* L. *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. *Vaccinium* 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.

384 *Betula* seedlings were transplanted in a full factorial design experiment with four treatment factors: 1) site (forest *versus* treeline; Fig. 1, right); 2) temperature (+/- passive warming by open top chambers, OTCs hereafter); 3) shrub presence (with/without *Vaccinium* removal); and 4) nutrient availability (+/- NPK addition). During three growing seasons several variables were assessed to estimate the establishment and performance of the *Betula* seedlings. For more details of the study area, study species, vegetation composition, experimental design, data collection and statistical analyses of the experiment in the Subarctic, see Grau et al. (2012) and also Tables 1 and 2.

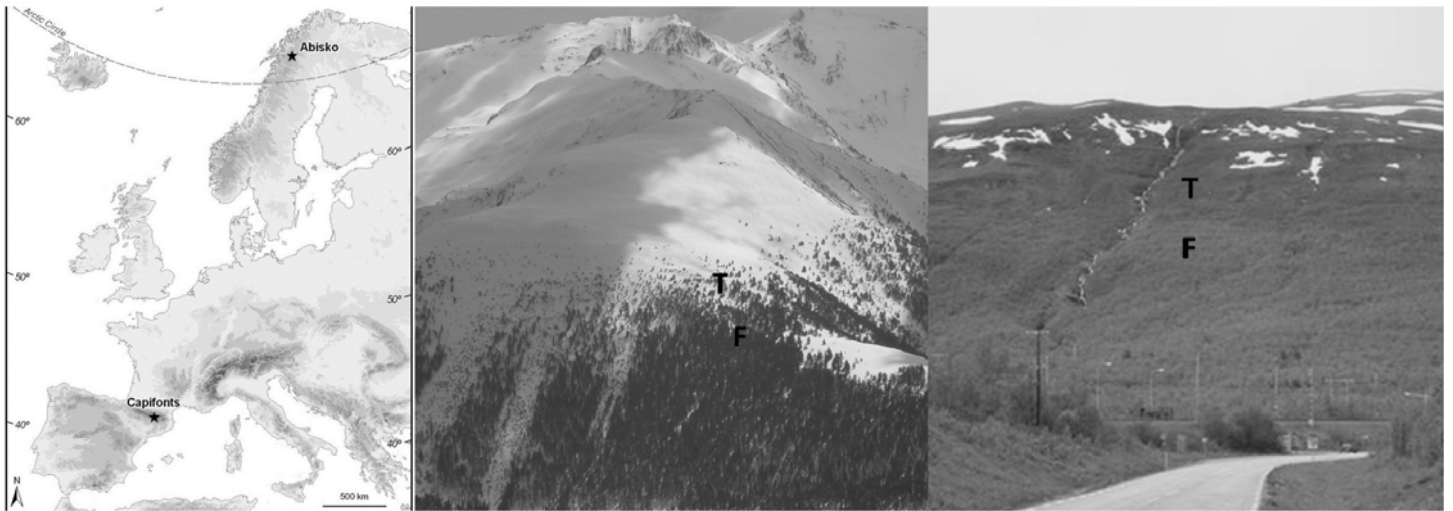


Figure 1. Left: Map of Western Europe showing the location of the experimental sites in both study regions: Serrat de Capifonts in the Central Pyrenees and Abisko in the subarctic Scandes; Middle: selected study sites at Serrat de Capifonts; Right: selected study sites at Abisko. (Photos by Oriol Grau). Abbreviations: T=treeline site, F=forest site

Table 1. Comparative table with main characteristics of the experiment in both study regions.

	Pyrenees	Scandes
Latitude, longitude	42° 33' N, 1° 23' E	68° 21.562' N, 18° 44.504' E
Altitude of upper site (treeline)	2350 m a.s.l.	589 m a.s.l.
Altitude of lower site (forest)	2400 m a.s.l.	661 m a.s.l.
Experimental unit size	1.32 m ²	1.13 m ²
Experimental period	October 2006-October 2009	August 2006-September 2009
Start of fertilisation and warming treatments	June 2007	July 2007
Mean annual temperature	2.0°C ^b	-0.9°C ^a
Length of growing season	5 months	3 months
Mean temperature of the coldest month	-5.2°C ^b	-10.1°C ^a
Mean temperature of the hottest month	11.7°C ^b	11.7°C ^a
Mean annual precipitation	1000-1100 mm ^c	336 mm ^a
Season with strongest contribution to mean annual temperature rise	Summer	Spring
Temperature difference between forest and treeline	2.1°C higher at treeline	2.6°C higher at treeline
Mean summer temperature increase in OTC (treeline)	2.1°C *	0.9°C
Mean summer temperature increase in OTC (forest)	1.2°C *	0.9°C
Temperature decrease in control plots with shrub (treeline)	2.8°C	2.5°C
Temperature decrease in control plots with shrub (forest)	2.1°C	0.4°C

^a obtained from Abisko Research Station data archive for the period 1980-2009 (closest station to the study site)

^b obtained from Salòria Meteorological Station (SMC 2010) for the period 2007-2010 (closest station to the study site, at 2451 m a.s.l.)

^c obtained from the Digital Climatic Atlas of Catalonia (Ninyerola et al. 2000)

* no temperature increase was detected if the shrub was present

Table 2. Comparative description of the potential factors affecting treeline dynamics in the Pyrenees and in the Scandes.

Potential factors affecting treeline dynamics		Pyrenees	Scandes
Herbivore pressure on treeline forming species and presence of rodents		Low livestock grazing pressure if land use abandonment persists (Lasanta 1990; Ameztegui, et al. 2010); impact of wild herbivores expected only at local scale (e.g. capercaillies). Absence of rodents	Variable reindeer grazing pressure at regional scale, slowly increasing during the last decade (Van Bogaert et al. 2011). Great importance of cyclic outbreaks of the autumn moth (<i>Epirrita autumnata</i> ; Van Bogaert et al. 2009) and the winter moth (<i>Operophtera brumata</i> ; Jepsen et al. 2011). Snow hare (<i>Lepus arcticus</i>) feeding on <i>Betula</i> ; cyclic outbreak of the grey-sided vole (<i>Clethrionomys rufocanus</i>) feeding on <i>Vaccinium</i>
Extreme climatic events and climatic trends		Periods with little snow cover in winter with very strong winter damage on needles; Increase of mean annual temperatures; no clear trend in precipitation changes (Esteban et al. 2009)	Extreme thaw events with negative consequences on vegetation (Bokhorst et al. 2008; 2009) ; Increase of mean annual temperatures and summer precipitation but decreases in snow depth (Callaghan et al. 2010)
Pollution and logging pressure		Potential damage to subalpine <i>P. uncinata</i> forests due to high ozone mixing ratios (Díaz-de-Quijano et al. 2009); very low logging pressure near the treeline at present	Fuelwood gathering about 100 years ago during the construction of a railway; tourism
Recent treeline dynamics		Regional densification of the treelines at regional scale during the second half of the 20 th century, often coupled with tree limit rise (Batllori and Gutiérrez 2008)	No clear trend at regional scale: some treelines have advanced whereas other treelines have become denser, remained static or even retreated (Kullman 2002; Tømmervik et al. 2004; Dalen and Hofgaard 2005; Hedenås et al. 2011; Rundqvist et al. 2011; Van Bogaert et al. 2011)
Growth rates and plasticity of treeline forming species		Slow growth; low phenological plasticity within one season (Ameztegui and Coll 2011)	Fast growth, high phenological plasticity within one season (Ovaska et al. 2005)
Family, life form and growth form of treeline forming species		Pinaceae; evergreen monocormic tree	Betulaceae; deciduous polycormic tree
Growth rates of the shrub community at the treeline ecotone		Slow growth, low phenological plasticity within one season	Fast growth, high phenological plasticity within one season
Family, life form and growth form of the shrub community at the treeline ecotone		Ericaceae; evergreen, clonal low shrub	Ericaceae; deciduous, clonal dwarf shrub
Light regime and solar radiation		Strong contrast day / night; high solar radiation	24h light in summer, less day / night variation in light level; low solar radiation
Seedling leaf [N] and C/N ratio at the treeline site (control plots)	Without shrub	1.40; 35.8	2.55; 18.7
	With shrub	1.52; 32.6	2.55; 18.6
Seedling leaf [N] and C/N ratio at the forest site (control plots)	Without shrub	1.99; 25.0	1.76; 26.8
	With shrub	2.03; 24.3	1.88; 24.5

EXPERIMENT IN THE PYRENEES

The experiment conducted in the Pyrenees was based on the same number of seedlings and on the same factors and experimental design used in the Scandes, but the particularities and full details of this parallel experiment are presented in the following sections:

Main study area

The experiment in the Pyrenees was conducted at Serrat de Capifonts (Pallars Sobirà, 42° 33' N, 1° 23' E), in the Alt Pirineu Natural Park, located in NW Catalonia in the Central Pyrenees (NE Iberian Peninsula). Two sites across the ecotone on a NW slope were selected (Fig. 1, middle), one at the treeline (2400 m a.s.l.) and the other in a closed forest right below the treeline (2350 m a.s.l.). We selected these two sites as they appropriately represent marked differences between the upper and the lower parts of the ecotone within a short distance. These two contrasting sites showed an abrupt change in biotic and abiotic conditions, mostly related to the density and nature of adult trees, and to the proximity of adult trees to younger individuals, which strongly determine tree establishment across the ecotone (Stueve et al. 2011). The lower site consisted almost entirely of mountain pine trees (*Pinus uncinata* Ramond ex DC.) (about 7 trees with stem diameter greater than 7,5 cm/ 100 m²), whereas in the upper site there were only some scattered pine individuals surrounded by open grasslands. In both sites, the shrub *Rhododendron ferrugineum* L. dominated the field layer, although it was especially abundant in the lower part of the treeline ecotone (around 70 % cover) and decreased gradually with higher altitude. There were also other abundant dwarf shrubs (*Vaccinium myrtillus* L., *Vaccinium uliginosum* L. ssp. *microphyllum* (Lange) Tolm., *Calluna vulgaris* L., *Loiseleuria procumbens* L. and *Juniperus communis* L. ssp. *alpina* (Suter) Celak.) and a few grasses (*Festuca nigrescens* Lam., *Festuca airoides* Lam. and *Deschampsia flexuosa* L.).

The experimental area has a high-mountain climate and the growing season usually lasts about 5 months (considering that the growing season starts when daily temperatures remain above 5°C longer than five days and finishes when temperatures are below 5°C longer than 5 days; Frich et al. 2002); however, the length of the

growing season varies between 4 and 6 months (personal observations, measured with data logger CR10X, Campbell Scientific equipment). Northerly winds prevail and reach the highest mean speed in the study area (SMC 2012) (see Table 1 for more details on climate). Smoothed mean annual temperatures have risen by 0.63-0.70 °C during the last seven decades (1934-2008) in the neighbouring Pyrenean meteorological stations of Andorra (Esteban et al. 2009), mainly due to summer warming. Mean annual precipitation for the period 1950-2008 does not show a clear trend, although summers appear to be getting slightly drier.

Study species

We used seedlings of *P. uncinata*; this species dominates the subalpine belt and forms the treeline in the Eastern and Central Iberian Pyrenees. *P. uncinata* is a long-lived, slow-growing and shade-intolerant evergreen conifer greatly adapted to cope with harsh climate and poor soils in the high mountains in these regions (Cantegrel 1983). The potential treeline is situated at approximately 2200-2450 m a.s.l. in the Central Pyrenees (Carreras et al. 1996).

R. ferrugineum is an ericaceous evergreen shrub which dominates the understory of siliceous subalpine Pyrenean *P. uncinata* forests and forms scattered patches above the treeline. In the forest site it was about 40-50 cm tall, and around 30-40 cm near the treeline; it is abundant in areas with long-lasting snow cover, where it remains protected against low temperatures. It usually expands clonally and a single genotype may occupy a large surface area (Pornon et al. 1997).

Experimental design

The experiment in the Pyrenees started in autumn 2006. Similarly to the experiment described in Grau et al. (2012), 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. Moreover, the period of study required to sow tree seeds and to follow emerging seedlings would not be feasible. However, we acknowledge that abiotic and biotic selection processes occur naturally before seedlings reach the stage that we used and that further selection processes occur during later stages of the tree life cycle on older saplings and

trees. We therefore provide information on processes that select for the survival and performance of tree individuals for only one stage – albeit an important stage – of the long, tree life cycle. These tree seedlings had been grown in a nursery before transplantation and their heights ranged from 8-10 cm; seeds had been collected from *P. uncinata* individuals from the Catalan Pyrenees.

Four main treatments were used: the presence or absence of shrubs to explore the importance of facilitation of tree seedlings by shrubs; increase or ambient summer air temperature by adding open top chambers (OTCs) to simulate climate warming; addition or no addition of plant nutrients to simulate the effects of warming soils on nutrient mineralisation; and location within and above the forest to explore the possible roles of competition between adult and seedling trees. Interactions were anticipated among the treatments.

We set up 16 experimental units both in the forest and at the treeline; to circumvent anomalies in the distribution of snow cover, we avoided big microtopographic differences between the experimental units; all of them were located at the same orientation and were similarly exposed to wind. In each unit we transplanted six *P. uncinata* seedlings at the leeward, southern side of a *Rhododendron* patch ('shrub presence' treatment) and six seedlings in an unprotected position, 50 cm southwest from the sheltered seedlings ('shrub absence' treatment). At the start of the experiment we cut all the grass growing in the microplots without shrubs to avoid any competition with the *P. uncinata* seedlings. The grass was then cut each subsequent spring to eliminate any re-growth. Again, to avoid snow cover anomalies, the OTCs were removed during winter and thus the seedlings growing in the unsheltered position were all totally exposed to the strong and dominant northerly winter winds which may be especially damaging if there is little snow cover. Therefore, we simulated increased temperature during the growing season but not in winter. The OTCs were designed according to hexagonal ITEX models (Marion et al. 1997) in order to simulate an expected increase of air temperature. For uniformity, all experimental units with or without OTCs had the same area (1.32 m²). Fertilisation treatments started in early June 2007, when we added 200 g of slow-release NPK granules (10% N, 5% P₂O₅, 20% K) to each fertilised experimental unit, which simulated an assumed moderate fertilisation effect by approximately 15 g N/m², 4.3 g P/m² and 25 g K/m².

Data collection and analysis

The data were collected from 2007 to 2009 in two sampling periods each year: early summer (soon after snow-melt) and the end of the growing season. In the first sampling period we evaluated the effects of winter damage on the *P. uncinata* seedlings (i.e. presence/absence of red needles from the previous winter season). We also assessed herbivory symptoms on needles or stems which had been browsed by the wild herbivore *Tetrao urogallus* L. subsp. *aquitanicus* (Ingram) (capercaillie). This bird of the grouse family inhabits the subalpine forested areas during winter and feeds on *P. uncinata* seedlings when there is little snow cover and seedlings remain unprotected. Also, at the end of the growing season, we measured stem length, number of branches (the first two years) and stem diameter just above the ground (only the last year) as plant size indicators. In autumn 2009 we collected a few needles of each seedling for laboratory analyses. The needles from all seedlings of an individual microplot (replicate) were mixed and ground, and total carbon and nitrogen were determined by dry combustion with a Flash EA1112 elemental analyser (Thermo Scientific, Rodana, Italy). The ambient air temperature regime in the study plots was measured by temperature loggers (iButtons 1-wire Thermochron temperature logger, Dallas Semiconductor Corporation, USA).

Each response variable for a given sampling period was analysed in a linear mixed model as implemented in R 2.11.0, using the 'nlme' and 'lme4' packages (R Development Core Team, 2011). 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 -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 -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 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 3.

Results

Overall, 67.5 % of the seedlings survived until the end of the experiment in the Pyrenees. Seedling performance was affected significantly by the experimental manipulations. *Rhododendron* played a key role in determining seedling performance and both positive and negative interactions co-occurred during the experimental period. Environmental manipulations also caused significant effects on seedling development. Table 3 shows all the statistically significant results obtained from the mixed model analyses and compares the main effects of the experimental factors between the Pyrenees and the Scandes. See Grau et al. (2012) to check the detailed results of the experiment in the Scandes.

Effects of shrub and site on Pinus seedling performance

Rhododendron reduced the winter damage to *Pinus* seedlings at the treeline site during periods of little snow cover in winter (Fig. 2, Table 3). About 55% of the seedlings growing at the treeline without the protective effect of a shrub showed some damage to their needles. We only observed this phenomenon during the first winter season after transplantation (2006-2007), when snow cover at the treeline site was very thin until mid-January (less than 15 cm) and there were some severe episodes of strong northerly winds. Although the snow layer was also very thin in the forest, the effect of wind was not as noticeable as in the treeline site and snow was more homogeneously distributed; at the treeline, microtopography and vegetation caused substantial influences on snow distribution after windy episodes and snow cover showed a rather irregular pattern (O. Grau, personal observation). Thus, winter damage caused by needle abrasion was observed exclusively at the treeline site. Approximately one third of the seedlings had more than 25% of the needles damaged and about 7 % had all needles injured (Fig. 2). 57.6 % of the seedlings which did not survive at the treeline had been affected by winter damage. Two-thirds of those seedlings with more than 50 % of their needles damaged died.

Rhododendron presence increased proportional tree seedlings stem growth for the periods 2007-2008 and 2007-2009 (Fig. 3; Table 3, Table S1a), especially in microplots without OTCs. Also, seedlings growing close to the shrub developed thinner stems and

had lower C percentage, in both the treeline and the forest, and higher N percentage in the forest. The C/N ratio was lower in seedlings with shrubs in the forest (Table 3).

Rhododendron offered a clear protection against capercaillies. Two years after transplantation, 41 % of the seedlings growing without shrubs in the forest had been partly browsed by this bird species during winter, whereas only 12 % of the seedlings growing under the canopy of the shrub had been affected (Table 3). No herbivory was observed at the treeline site. At the end of the experiment the highest mortality was observed in the forest site (Table 3, Table S1b); 49.2 % of the seedlings that did not survive in the forest had previously suffered from capercaillie browsing.

The position across the ecotone (i.e. forest *versus* treeline) also had a strong influence on seedling development. During the periods 2007-2008 and 2007-2009 the seedlings in the forest grew proportionally less and developed thinner stems compared to the seedlings at the treeline. The N content was higher, and the C content and the C/N ratio were lower in the needles of seedlings growing in the forest compared to those at the treeline.

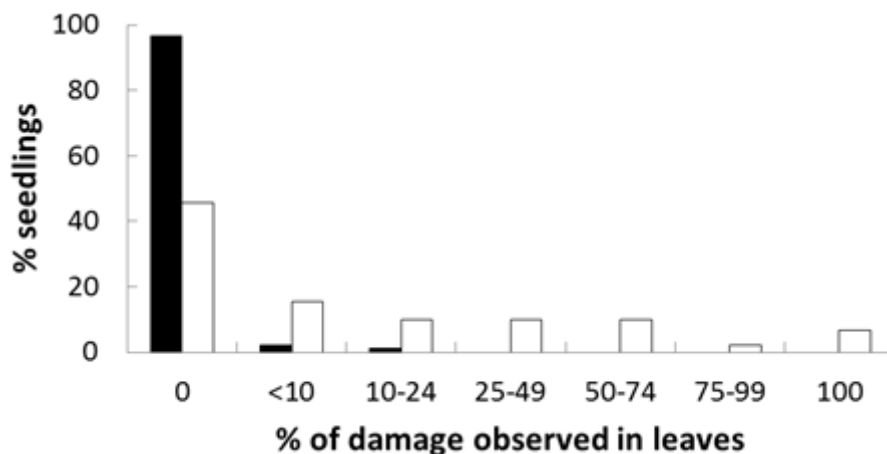


Figure 2. Winter damage observed in *Pinus* needles after winter 2006-07 at the treeline site. Filled columns indicate seedlings protected by *Rhododendron* and empty columns unprotected seedlings.

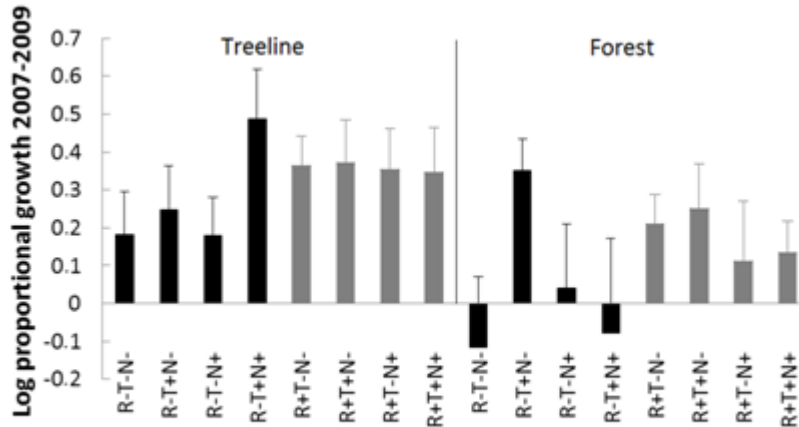


Figure 3. Proportional stem growth (log proportional growth [cm]) during the period 2007-2009 for each treatment. Standard error bars are shown one-sided. Black and grey columns represent the growth of *Pinus* seedlings growing without and with *Rhododendron*, respectively. Negative values in the proportional growth are due to capercaillie herbivory on some of the seedlings. The statistical significance of each factor excluding the effect of herbivory is shown in Table 3. The abbreviations of the treatments correspond to: R-: without *Rhododendron*; R+: with *Rhododendron*; N-: without fertiliser; N+: with fertiliser; T-: without OTC; T+: with OTC. See Table S1a for the mean values of stem length in each treatment without the effect of herbivory.

Table 3 (See next page). Summary table of all the statistically significant results (p -value < 0.05) found in each sampling period for all the variables measured in the experiment in the Pyrenees. The symbols > and < indicate a statistically significant increase or 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. Note that more variables were recorded in August 2009; branching was only measured the first two years and stem diameter and leaf nutrients only in 2009. In the analyses of proportional stem growth we excluded the seedlings which had been affected by herbivory in order to better identify the effect of the original experimental factors. The last column summarises the main effects of the experimental factors considered in this comparative study. See Table 1 in Grau et al. (2012) for further information about the results in the Scandes. *indicates that only second-order interactions were considered to simplify the interpretation; ^rsite a and site b have been analysed separately because of multi-order interactions which confounded the interpretation of the results (in such cases the significance threshold has been set at $p < 0.025$).

Table 3. See table caption on previous page.

	Main effects observed in the Pyrenees			Summarised comparison of the main effects observed in the Pyrenees and in the Scandes
	2007	2008	2009	
FOREST SITE		< proportional stem growth 2007/08 < branching	< proportional stem growth 2007/09 < stem diameter > % N in leaves ^{fr} < % C in leaves ^{fr} < C/N ratio in leaves < survival	Better seedling growth at the treeline compared to the forest in both study regions
WITH SHRUB	< capercaillie herbivory (only in the forest) < winter damage (only in the treeline)	> proportional stem growth 2007/08 < branching < capercaillie herbivory 2007/08 (only in the forest)	> proportional stem growth 2007/09 < stem diameter > % N ^{fr} in leaves (only in the forest) < % C ^{fr} in leaves < C/N ^{fr} ratio in leaves (only in the forest)	Pyrenees: shrub presence decreased seedling damage Scandes: shrub presence increased initial seedling survival, decreased seedling damage, but impaired seedling performance at later stages of the experiment
WITH OTC		> proportional stem growth 2007-08 (especially without <i>Rhododendron</i> ; if without OTC, <i>Rhododendron</i> shows stronger effect)	> proportional stem growth 2007-09 (especially without <i>Rhododendron</i> ; if without OTC, <i>Rhododendron</i> shows stronger effect) > stem diameter	Temperature increase enhanced seedling performance from the second growing season in the Pyrenees and already from the first growing season in the Scandes
WITH NPK		> branching (only in the treeline)	> stem diameter > % N ^{fr} in leaves (only in the forest) < C/N ^{fr} ratio in leaves (only in the forest)	Nutrient addition enhanced seedling performance from the second growing season in the Pyrenees and already from the first growing season in the Scandes

Effects of abiotic manipulations on Pinus seedling performance

The seedlings growing in OTCs responded to passive warming with increased proportional stem growth during the second and third years and thicker stems after three growing seasons, in both the treeline and the forest sites (Fig. 3, Table 3). Such increase was especially important in seedlings growing without *Rhododendron*. Nutrient addition exerted a noticeable effect on the seedlings, which developed thicker stems at the end of the experiment, and at the treeline site they also had more branches. Nutrient addition also increased the N content and decreased the C/N ratio in the needles of *Pinus* seedlings growing in the forest site (Table 3). Neither temperature nor nutrient increase affected seedling survival.

Discussion*Experiment in the treeline ecotone in the Pyrenees*

The protective effect of *Rhododendron* patches on *P. uncinata* seedlings against winter damage observed at the treeline site confirmed our hypothesis 1b. This positive effect was similar to that described for krummholz trees at Pyrenean treelines (Batllori et al. 2009). In winter, strong winds carry small snow crystals which cause cuticular wax abrasion on protruding plant parts (Hadley and Smith 1989), resulting in damaged needles and branches (Neuner 2007). Seedlings growing in sheltered sites such as leeward of shrub-like stunted trees or of *Rhododendron* patches are likely to grow safely as long as the *Pinus* seedlings are shorter than these protective elements.

In addition, the protection of *Rhododendron* against capercaillie browsing indicates that if this bird species is present in the forest, *Pinus* seedlings growing in an exposed position may be seriously damaged and thus have much higher mortality. Capercaillies feed on *Pinus* needles especially in winter and therefore *Pinus* seedlings are particularly affected if there is little snow cover and they remain visible close to the ground. The study area is located within the region with the highest population of capercaillie in the Iberian Peninsula (Canut 1991). Although it is an endangered species and its population has decreased during the last decades (Canut 2001), this bird species might be considered as a potential control on *Pinus* recruitment in those subalpine forests which still host a large population of capercaillies.

However, despite the positive effects of *Rhododendron*, seedlings growing under the shrub had longer and thinner stems, showing a clear phenotypic response to shaded conditions. During the experimental period we did not find evidence for low-temperature photoinhibition in seedlings affected by high sky exposure. This inhibition has been shown to cause multiple stresses that limit the establishment and growth of subalpine trees by impairing carbon accumulation in unprotected tree seedlings (Germino and Smith 1999; Smith et al. 2003). Actually, the fact that *Pinus* is a shade intolerant species (Cantegrel 1983) explains why the small seedlings transplanted into the forest, and thus with low sky exposure, had slower growth and developed thinner stems, compared to those at the treeline, contrary to hypothesis 1a. The lower C/N ratio in *Pinus* needles growing in the forest also suggests that these seedlings are carbon rather than nitrogen limited in their growth, presumably through low photosynthetic rates in the shade. This would correspond also with the lack of stimulation of stem length growth by fertilisation in the forest (Fig. 3, Table 3) even though the stems were thicker in fertilised forest plots (Table 3). At the treeline, though, fertilised seedlings showed a higher number of branches, and those which also had increased temperature without shrub protection (i.e. unshaded) also showed somewhat stronger growth (Fig. 3).

As predicted in hypothesis 1c, both increased temperatures and higher nutrient availability enhanced seedling development. The positive effect of temperature increase on the growth of *Pinus* seedlings confirms that the subalpine Pyrenean forests are indeed sensitive to heat deficiency, not only at the treeline (Körner and Paulsen 2004) but also at the forest immediately below the treeline, and that such sensitivity can be detected already at the seedling stage. However, trees growing at the treeline do not always exhibit a linear response to climate (Daniels and Veblen 2004), due to the marked local spatial variability of biotic and abiotic influences (Stueve et al. 2011). For instance, the scarcity of safe sites (i.e. protective elements such as krummholz trees, *Rhododendron* patches or rocks) may distort the response of treeline ecotones to climate warming (Kullman and Öberg 2009) at local and regional scales in the Pyrenees because of high mortality during winter (Batllori et al. 2009). In our experiment, we observed that the positive effects of temperature on the seedlings

were more evident without the presence of *Rhododendron* in both study sites, suggesting that positive feedbacks from the surrounding vegetation may buffer the effects of climate on tree seedlings (Bader et al. 2008; Elliott and Kipfmüller 2010; Elliott 2011).

Comparison between the Pyrenean and the subarctic treeline ecotone responses to experimental treatments

Betula is well adapted to a short growing season in the subarctic Scandes (Wielgolaski 2002) owing to its high growth rate and marked phenological plasticity (Ovaska et al. 2005), and is very responsive to changes in nutrient availability and temperature (Karlsson and Nordell 1996; Sveinbjörnsson 2001). In contrast, *Pinus*, which shows little phenological plasticity (Ameztegui and Coll 2011) and has a much slower growth than *Betula*, showed less stimulation by the experimental amelioration of the environment. Moreover, N availability does not seem to strongly constrain seedling growth in the Pyrenees, neither in the treeline nor in the forest site (see previous section).

Notwithstanding these differences in species characteristics and soil nutrient status, the factors and the underlying processes that drive treeline dynamics in the Scandes and in the Pyrenees share many similarities (Table 3); this could be a reason for the similar pattern during recent decades, i.e. some treelines in these regions have advanced and some have become denser (Batllori and Gutiérrez 2008; Van Bogaert et al. 2011). In both study regions the seedlings developed comparatively better at the treeline than in the forest, contrary to our expectations. This implies that the current growth conditions at the treeline in both study cases are favourable for tree seedling recruitment. Probably, this is partly due to the similar climatic trends occurring in both regions, with increasing mean annual temperatures during the last decades coupled with the responsiveness of treelines to increased temperatures. Moreover, the rather variable livestock pressure at the regional scale during the last decades may have favoured this pattern in both regions (Table 2). During the last decades, shrub cover has expanded into the tundra in the northern Scandes (Hallinger et al. 2010; Rundqvist et al. 2011; Hedenås et al. 2011) and into the subalpine belt in some areas in the

Central Pyrenees (Alados et al. 2011). The projected invasion of shrubs into the tundra (Wolf et al. 2008) during further increases in temperature expected this century (IPCC 2007) are likely to cause a continuing upward shift of the shrub line which could in turn increase the number of potential safe sites where tree seedlings are protected from external damage during the seedling stage.

However, there are many particularities exclusive to each region not formally tested in these two experiments. These peculiarities may vary through time and could also have an important effect on treeline dynamics (see Table 2). For instance, changes in livestock grazing and wild herbivore pressure, frequency of extreme climatic events, climatic trends, pollution and logging pressure will together determine the fate of the recently recruited seedlings at the treeline ecotone and hence, future treeline dynamics in both regions.

The stress gradient hypothesis applied to Pyrenean and subarctic treeline ecotones

Our results partly confirm our hypothesis 2, based on the refined stress gradient hypothesis (Maestre et al. 2009 refined from Brooker and Callaghan 1998; see Table 4). In the Pyrenees we predicted a medium and low resource stress level in the forest and at the treeline respectively when *Rhododendron* was present. However, while the shrubs generally promoted seedling growth, we also observed their competitive effect on tree seedlings (denoted by stem etiolation), indicating resource stress, probably due to low availability of light under the shrub canopy, independently of the position across the ecotone. In the Scandes, we observed positive (i.e. enhanced survival) and negative responses (i.e. poor performance probably because of low availability of nutrients and light), both in the forest and at the treeline. Therefore, in both study regions we found indications of relatively high resource stress across the whole treeline ecotone, contrarily to what we expected.

When we explored non-resource stress, we observed positive interactions at the treeline in both regions as predicted (Table 4). However, we found no indications of competition in the forest, suggesting that non-resource stress is not as low as expected, particularly in the Scandes. Here, symptoms of strong solar radiation (red pigmentation) were noticeable in the forest if *Vaccinium* was not present. Facilitation by shrubs

mediated by protection from herbivores, was evident in both regions. The shrubs decreased damage whenever a dominant vertebrate or invertebrate herbivore was present, independently of the position across the ecotone.

Future changes in the intensity of resource, non-resource or herbivore pressure occurring in the Pyrenees and in the Scandes are likely to induce changes in shrub-tree interactions. For instance, under the predicted scenario of increasing temperatures (IPCC 2007), higher nutrient availability (Rustad et al. 2001) and increasing shrub cover (Wolf et al. 2008; Alados et al. 2011), we expect changes in the stress level, which will affect tree seedling performance both directly and indirectly through the shifting balance between facilitative and competitive interactions between plants within each study region. Our results confirm that higher temperatures favour tree seedling performance in both regions, because the treeline ecotones are limited by temperature (Körner and Paulsen 2004). However, if temperatures continue to increase, water stress in summer and thus, resource stress could develop (Wilmking and Juday 2005; Zhang and Wilmking 2010). This effect may be especially noticeable in the Pyrenees, where there has been a slight trend of decreasing rainfall in summer (Esteban et al. 2009); moreover, snow accumulation in winter as a possible source of water in summer is not expected to increase. In contrast, precipitation in the northern Scandes has increased (Callaghan et al. 2010) and the drought effect is likely to be less noticeable, despite decreases in snow depth and duration (Andrews et al. 2011). An increase in nutrient availability from warming soils in both regions would decrease resource stress, which could in turn favour neutral or positive interactions between shrubs and tree seedlings (Maestre et al. 2009). Moreover, if the frequency of extreme climatic events increases in both study regions (Bokhorst et al. 2010; SMC 2012), there will be an increase of non-resource stress, which will in turn favour positive interactions of shrubs on trees.

Conclusions

We show that treeline dynamics in the Central Pyrenees and in the subarctic Scandes are strongly influenced by shrub-tree interactions, temperature and nutrient increase, as these factors control tree seedling performance across the treeline ecotone. The

presence and distribution of shrubs in this ecosystem clearly determine the amount and spatial distribution of safe sites and thus the recruitment of new saplings. The gradients of stress occurring from closed forest to open areas have been found to be less marked than usually anticipated as different resource and non-resource stresses co-occur along this gradient; growth conditions for the transplanted seedlings at the treeline have been found to be better than in the forest in both experiments. Any future changes in the intensity of resource and/or non-resource stress and/or herbivore pressure occurring in the Pyrenees and in the Scandes will alter the balance between positive and negative shrub-tree interactions and hence, will affect the fate of treeline dynamics in these regions.

Although we focused on only one stage of the life cycle of a long-lived tree species, our detailed results present mechanisms for understanding the recently observed variability of local responses of treeline to currently changing climate while identifying some local commonalities that can be used to generalise on large scale response of treelines to climate warming. We identified the ways in which important local drivers of treeline dynamics, such as facilitation and herbivory, interact with general drivers such as climate change and soil nutrient supply. The mechanisms identified that underpin tree seedling establishment could lead to an improved projection of future treeline location if included in dynamic vegetation models.

Table 4. Predicted *versus* observed responses and interactions between shrubs and tree seedlings in relation to stress type in both study regions.

Type of stress	Predicted stress level and predicted interactions* between shrubs (benefactors) and tree seedlings (beneficiary) in Pyrenean and subarctic regions		Observed responses and observed interactions* in relation to stress on tree seedlings caused by shrub presence			
	Forest	Treeline	Pyrenees		Scandes	
			Forest	Treeline	Forest	Treeline
Resource stress (water, light, nutrients)	Medium stress (FACILITATION**)	Low stress (NEUTRALISM**)	Increased stem growth but thinner stems (etiolation) (COMPETITION)	Increased stem growth but thinner stems (etiolation) (COMPETITION)	Enhanced survival; poor development at later stages (FACILITATION AND COMPETITION)	Enhanced survival; poor development at later stages (FACILITATION AND COMPETITION)
Non-resource stress (temperature, wind, salinity, soil structure, solar radiation)	Low stress (COMPETITION**)	Medium/High stress (FACILITATION**)	Not observed (NONE)	Decreased winter damage (FACILITATION)	Enhanced survival; decreased effects of solar radiation (FACILITATION)	Enhanced survival; decreased effects of solar radiation (FACILITATION)
Herbivore pressure	Highly dependent on the varying spatio-temporal herbivore pressure (FACILITATION**)		Decreased capercaillie herbivory (FACILITATION)	Not observed (NONE)	Decreased caterpillar herbivory (FACILITATION)	Decreased caterpillar herbivory (FACILITATION)

*The latter are shown in capital letters in the table.

**based on the predicted relationship between abiotic stress and the outcome of a given plant-plant interaction, as described in Maestre et al. (2009). The shrubs were considered stress-tolerant benefactors and the tree seedlings were considered competitive beneficiary species based on the classification by Grime (2001).

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Appendix

Table S1. Mean value \pm standard deviation of **a)** Stem length; **b)** Survival. The abbreviations of the treatments correspond to: a: treeline; b: forest; R-: without *Rhododendron*; R+: with *Rhododendron*; N-: without fertiliser; N+: with fertiliser; T-: without OTC; T+: with OTC.

Upper table: Mean stem length (cm) per group of *Pinus* seedlings growing in a given treatment, excluding the seedlings which had been browsed by capercaillies

Lower table: Mean percentage of surviving *Pinus* seedlings growing in a given treatment

Stem length

Treatment	2007	2008	2009
aR-N-T-	8.36 \pm 1.85	8.49 \pm 1.79	9.73 \pm 2.35
aR-N-T+	8.70 \pm 1.28	9.51 \pm 1.69	11.29 \pm 2.32
aR-N+T-	8.86 \pm 1.76	9.22 \pm 2.10	10.56 \pm 2.38
aR-N+T+	8.63 \pm 1.44	11.18 \pm 2.26	14.04 \pm 3.38
aR+N-T-	8.55 \pm 1.70	10.0 \pm 1.53	12.33 \pm 1.84
aR+N-T+	8.76 \pm 1.40	10.36 \pm 2.03	12.99 \pm 2.86
aR+N+T-	8.40 \pm 1.93	9.54 \pm 2.57	11.98 \pm 3.32
aR+N+T+	8.84 \pm 2.29	11.01 \pm 3.38	12.74 \pm 3.61
bR-N-T-	9.71 \pm 2.05	8.34 \pm 2.75	9.17 \pm 3.69
bR-N-T+	8.39 \pm 2.04	9.34 \pm 2.60	11.77 \pm 3.20
bR-N+T-	8.96 \pm 2.56	9.01 \pm 4.47	10.69 \pm 4.97
bR-N+T+	9.36 \pm 1.68	6.95 \pm 2.91	9.68 \pm 4.28
bR+N-T-	10.92 \pm 1.54	12.02 \pm 1.72	13.76 \pm 2.19
bR+N-T+	9.60 \pm 2.24	10.77 \pm 2.5	12.28 \pm 3.32
bR+N+T-	9.27 \pm 2.46	9.46 \pm 3.43	11.83 \pm 3.32
bR+N+T+	9.49 \pm 1.81	10.39 \pm 2.24	12.19 \pm 3.54

Survival

	2007	2008	2009
aR-N-T-	100 \pm 0.00	79.17 \pm 15.96	79.17 \pm 15.96
aR-N-T+	100 \pm 0.00	70.83 \pm 39.38	70.83 \pm 39.38
aR-N+T-	100 \pm 0.00	83.33 \pm 23.57	83.33 \pm 23.57
aR-N+T+	95.83 \pm 8.33	79.17 \pm 31.55	79.17 \pm 31.55
aR+N-T-	100 \pm 0.00	100 \pm 0.00	100 \pm 0.00
aR+N-T+	100 \pm 0.00	79.17 \pm 20.97	79.17 \pm 20.97
aR+N+T-	75.00 \pm 50.00	70.83 \pm 47.87	70.83 \pm 47.87
aR+N+T+	100 \pm 0.00	75.00 \pm 21.52	75.00 \pm 21.52
bR-N-T-	95.83 \pm 8.33	62.50 \pm 20.97	62.50 \pm 20.97
bR-N-T+	95.83 \pm 8.33	75.00 \pm 21.52	75.00 \pm 21.52
bR-N+T-	100 \pm 0.00	37.50 \pm 31.55	33.33 \pm 36.00
bR-N+T+	100 \pm 0.00	54.17 \pm 39.38	45.83 \pm 41.67
bR+N-T-	100 \pm 0.00	75.00 \pm 28.87	75.00 \pm 28.87
bR+N-T+	100 \pm 0.00	66.67 \pm 27.22	54.17 \pm 36.96
bR+N+T-	95.83 \pm 8.33	45.83 \pm 36.96	37.50 \pm 43.83
bR+N+T+	100 \pm 0.00	60.42 \pm 35.60	60.42 \pm 35.60

Capítol III

Un arbust ericoide exerceix la doble funció de reclutar pins i els seus simbiotes fúngics al llarg d'un gradient de successió primària

An ericoid shrub plays a dual role in recruiting both pines and their fungal symbionts along primary succession gradients

Oriol Grau, Pasi Rautio, Juliana Heikkinen, Karita Saravesi, Mikhail V. Kozlov & Annamari Markkola

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Resum

La importància relativa de les interaccions positives i negatives entre les espècies vegetals poden variar al llarg de gradients de pertorbació i de disponibilitat de recursos. Segons prediu la hipòtesi del gradient d'estrès, les interaccions positives predominaran en ambients sotmesos a estrès marcat, amb poca disponibilitat de recursos i poca productivitat, mentre que les interaccions negatives prevaldran en ambients on no hi hagi aquestes limitacions. Se sap que el subarbust *Empetrum nigrum* ssp. *hermaphroditum* té capacitat al·lelopàtica que impedeix tant el desenvolupament de *Pinus sylvestris* com dels seus simbionts ectomicorrízics. Aprofitant aquest coneixement, es va voler veure si els impactes d'aquest arbust en plançons de pi roig variava al llarg del gradient de successió primària en una illa emergent de la badia de Bòtnia, a Finlàndia, on l'estrès abiòtic canvia gradualment cap a estrès d'origen biòtic al llarg de la successió.

Es va veure que *Empetrum* exercia un efecte facilitador malgrat els seus efectes al·lelopàtics, ja que la proporció de plançons de pi roig establerts en claps on hi dominava l'arbust era superior que en claps on no hi dominava, tant en estadis joves com en estadis intermedis de la successió primària, mentre que en estadis més avançats els plançons preferien claps sense *Empetrum*. La quantitat de biomassa fúngica micèl·lica (estimada pel contingut en ergosterol) en el sòl a les proximitats de les arrels dels plançons era més gran en claps d'*Empetrum* que en absència de l'arbust, i augmentava al llarg del gradient de successió. La proporció d'arrels colonitzades per morfotipus ectomicorrízics suil·loids amb miceli abundant a l'exterior i la diversitat de morfotipus ectomicorrízics era més gran en estadis de successió intermedis en claps amb *Empetrum* respecte els claps sense arbust. Aquests resultats suggereixen que en les dures condicions físiques de la zona costanera dominada per dunes, *Empetrum* facilita l'establiment de plançons de pi en estadis joves i intermedis de la successió, afavorint una protecció mecànica i física, mentre que en estadis més avançats les interaccions negatives entre l'arbust i els pins hi prevalien. Segons ens consta, aquest estudi és el primer en indicar que un arbust ectomicorrízic ericoide afavoreix tant el desenvolupament de l'arbre hoste ectomicorrízic com els simbionts fúngics de l'arbre.



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An ericoid shrub plays a dual role in recruiting both pines and their fungal symbionts along primary succession gradients

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Relative importance of positive and negative interactions between plant species may change along disturbance and resource gradients. Positive interactions are suggested to prevail in low resource, low productivity (high stress) conditions and negative interactions in high resource availability. A dwarf shrub, mountain crowberry *Empetrum nigrum* ssp. *hermaphroditum*, is known to have allelopathic impacts on both Scots pine *Pinus sylvestris* and its ectomycorrhizal symbionts. We aimed to study if the outcome of *Empetrum* impacts on Scots pine changes along primary succession gradients on the dune shores of Bothnian Bay, in Finland, where abiotic stress gradually changes to biotic stress along the succession. We found that *Empetrum* may act as a facilitator despite its allelopathic effects, since the proportion of Scots pine seedlings established in *Empetrum* patches was higher than in patches without the shrub in early and mid succession stages, whereas patches without *Empetrum* were preferred in late succession. The amount of mycelial fungal biomass (ergosterol) in the soil in the vicinity of the seedling roots was higher in *Empetrum* patches than in patches without *Empetrum* and it increased along the succession gradient. Proportion of pine root tips colonised by suilloid morphotypes with abundant external mycelia and the diversity of ectomycorrhizal morphotypes were higher in mid successional stage in *Empetrum* patches compared to patches without *Empetrum*. Our results suggest that in the harsh physical conditions of the dune shore *Empetrum* facilitates pine seedling establishment in the early and mid stages of succession by providing mechanical and physical shelter whereas in late succession negative interactions (competition and allelopathy) between the shrub and the pine are dominating. To our knowledge we present the first finding that an ericoid mycorrhizal shrub could enhance both the performance of the ectomycorrhizal host tree and the tree's fungal symbionts.

Traditional ecological theories have emphasised the role of competition in plant–plant interactions. Numerous studies have shown that plant species interact by competing for nutrients, light and space, and that allelopathic substances may cause a negative impact on neighbours. However, support for positive relations between plant species has also been reported in many ecosystems especially during the last decade. In fact, it has been suggested that positive interactions (i.e. facilitation) might be as important as negative interactions (i.e. competition) (Brooker and Callaghan 1998, Bruno et al. 2003, Zvereva and Kozlov 2004, Maestre et al. 2005, Goldenheim et al. 2008, le Roux and McGeoch 2008). On the other hand, the balance of facilitative and competitive interactions seems to be dependent on the harshness of the abiotic environment (Callaway and Walker 1997, Eränen and Kozlov 2008). In a continuum of species interactions and intensity of abiotic stress, the importance of facilitation in plant communities has often been found to increase with increasing abiotic stress (Bertness and Callaway 1994, Brooker and Callaghan 1998), although there are some

other models which hypothesise that facilitation could be especially important in intermediate levels of stress (Maestre et al. 2005, Gilad et al. 2007).

Primary succession of plant and fungal communities in land uplift areas provides a unique possibility to study positive and negative interspecific interactions along a gradient, as there is a shift from high abiotic stress in early stages (because of low availability of water and high mechanical stress due to aeolian forces) to low abiotic stress but increasing biotic stress in late stages. In order to investigate if such stresses are mirrored in the balance between positive and negative interactions between a dwarf shrub, mountain crowberry *Empetrum nigrum* ssp. *hermaphroditum*, referred to as *Empetrum* hereafter, and Scots pine *Pinus sylvestris* seedlings, we examined pine seedling recruitment, performance and ectomycorrhizal colonisation along a successional gradient in a coastal land uplift area on sandy soil in a boreal region.

We expected that *Empetrum* would act either as a facilitator or as a competitor depending on the position where the interaction took place along the gradient. First, we assumed

to find differences in soil fungal biomass, ectomycorrhizal colonisation of the roots as well as recruitment and growth of pine seedlings in *Empetrum* patches when compared with patches without *Empetrum*. Further, we expected these differences to change along the successional gradient along with the increase in soil organic matter and development of vegetation. Based on the assumptions that facilitation increases with abiotic stress, and that competition increases with biotic stress, we predicted that:

- 1) Scots pine seedling recruitment would be easier in *Empetrum* patches in early successional stages, whereas in older stages (in the closed canopy forest) *Empetrum* free patches would have more seedlings;
- 2) ectomycorrhizal symbiosis of pine seedlings (measured as ectomycorrhizal colonisation and fungal diversity in the roots and soil fungal biomass) would be enhanced in *Empetrum* patches in early successional stages, whereas in older stages negative impacts of *Empetrum* would override its positive effects.

Material and methods

Study species

Empetrum is a dwarf evergreen shrub, which grows in subarctic and circumpolar areas (Hämet-Ahti et al. 1998). It often forms dense monospecific mats dominating in heathland ecosystems on acidic and nutrient poor soils when disturbances are infrequent and the climate is cool (Tybirk et al. 2000). *Empetrum* produces allelopathic phenolic compounds which leach to the soil and impair seed germination of neighbouring species especially in late successional stages (Nilsson et al. 1993, Tybirk et al. 2000). It has also been suggested to impair ectomycorrhizal fungal partners of Scots pine (see Nilsson et al. 1993 reporting adverse effects on *Paxillus involutus*). Scots pine *Pinus sylvestris* is the dominating conifer in dry and nutrient poor boreal soils, and in Finland it is a dominant tree species in ca 70 % of the forest and scrubland areas (Peltola and Ihalainen 2006). In our study sites it is the only species forming mature forests.

Study sites

We did the fieldwork in 2003, on the relatively young island of Hailuoto (less than 2000 years), on the Finnish coast of the Bothnian Bay, which has been subjected to primary succession due to post-glacial isostatic land uplift (Alestalo 1979, Hellemaa 1998). The sandy soil of the island is dry and very nutrient-poor (Kuikka et al. 2003).

We chose five locations along the western coast of Hailuoto within five km distance from the Marjaniemi biological station (65°02'N, 24°34'E). In each location, we explored three stages of a successional gradient: 1) the inland side of the dune ridge at a distance of ca 50–80 m from the shoreline (early stage), 2) the deflation basin, ca 150–250 m from the shoreline (mid stage) and 3) the mature inland pine

forest, ca 400–600 m from the shoreline (late stage). In the early stage of succession near the dunes, soil organic layer is almost absent and vegetation is very sparse, consisting mainly of *Empetrum*, *Salix repens*, pine seedlings and a few herbs (*Hieracium umbellatum*) and grasses (*Festuca ovina* and *Deschampsia flexuosa*). In the deflation basin (mid stage), where young Scots pine trees locate sparsely, *Polytrichum* mosses and lichens (*Cladonia* spp., *Cladonia* spp.) dominate in the ground layer and *Empetrum*, *Vaccinium uliginosum* and *Salix repens* occur patchily in the field layer. The mineral soil of the deflation basin is affected by wind-erosion before the development of vegetation, and this soil contains more coarse particles than the soil of either the dunes or the late stage forest. The late stage is composed by mature pine forest with closed canopy and an even layer of organic soil of 1–2 cm thick, *Cladonia* lichens and mosses (e.g. *Pleurozium schreberi*) in the ground layer, *Empetrum*, *Vaccinium vitis-idaea* and other dwarf shrubs dominating in the field layer. Soil pH declines along succession gradient from early stages to the forest (Table 1, $F_{2,24} = 24.84$, $p < 0.001$). Soil electric conductivity shows the opposite pattern ($F_{2,24} = 6.37$, $p = 0.006$) but it is also clearly higher in *Empetrum* patches ($F_{1,24} = 9.36$, $p = 0.005$). Soil organic matter content is generally low, but increases towards later stages of succession (Table 1, $F_{2,20} = 2.66$, $p = 0.095$) (see also Pennanen et al. 2001).

Sampling method and seedling processing

We investigated the co-occurrence of *Empetrum* and pine seedlings on three 50 m transects parallel to the shoreline in each location and successional stage. In these transects, we systematically placed a 25 × 25 cm frame every two meters. This resulted in 1170 frames studied (26 frames in each of the three lines in each of the three successional stages in each of the five locations) of which four observations were disregarded due to large stones or ant (*Formica cinerea*) colonies making the total number of observations 1166. We classified each frame as *Empetrum* or empty (*Empetrum* free), and counted the number of pine seedlings in it. Original (seedlings/frame) values were used to compute seedlings m⁻², and mean values were then computed for *Empetrum* and empty patches in each successional stage within each five locations to be used in further analysis.

To study N foliar content and ectomycorrhizal symbiosis, in each successional stage in each location we sampled three pine seedlings from both patch types (a total of 90 seedlings) with a steel corer (ø 7 cm) to the depth of 10 cm for mycorrhizal and ergosterol analysis. The seedlings were selected randomly, avoiding those growing close to older Scots pines. In this sampling, we used a stronger contrast between *Empetrum* and empty (*Empetrum* free) patches than in the previous survey. We contrasted seedlings growing either within 15 cm from the main root of the *Empetrum* shrub, or at least 50 cm from it.

We separated the roots of the seedlings from the above ground parts by cutting the stem just above the point where the first lateral root was growing. Needles were separated from stems, dried at 50°C for 48 h, weighed and pulverised for total N analysis using an automatic CN-analyser. Roots

Table 1. Soil organic matter (SOM, % of soil dry weight), pH and conductivity ($\mu\text{S cm}^{-1}$) along the successional gradient (dunes, deflation basin and mature forest) in samples collected in empty and in *Empetrum* patches. Values are mean \pm SE of five locations.

	Dunes		Deflation basin		Mature forest	
	empty	<i>Empetrum</i>	empty	<i>Empetrum</i>	empty	<i>Empetrum</i>
SOM	0.3 \pm 0.03	0.5 \pm 0.08	0.3 \pm 0.02	0.5 \pm 0.06	1.2 \pm 0.8	0.9 \pm 0.3
pH	4.8 \pm 0.03	4.7 \pm 0.08	4.5 \pm 0.04	4.6 \pm 0.06	4.3 \pm 0.05	4.1 \pm 0.1
conductivity	7.9 \pm 0.8	11.1 \pm 1.7	7.4 \pm 0.7	10.9 \pm 1.6	11.2 \pm 0.8	21.3 \pm 4.8

were pre-cleaned by removing the biggest particles of sand attached to them, and pH and conductivity were measured in a fresh soil subsample (1:1.5 vol. sand:water). The rest of the sand was frozen and kept at -20°C for analyses of fungal biomass and soil organic matter. Scots pine roots were frozen (-20°C) in plastic bags with water. For the inspection of mycorrhizal colonisation roots were defrosted and cleaned carefully under dissection microscope. In case we did not manage to properly remove the sand particles from the mycelia and roots, roots were cut into pieces of 1–2 cm and centrifuged in water (10 min, 10 000 rpm) to remove sand. After this, we recorded root fresh weight and preserved them in 50% ethanol.

Mycorrhizae morphotyping and classification

We counted the number of non-mycorrhizal and mycorrhizal root tips in each sample. Then we classified the mycorrhizal root tips according to their morphotypes under dissection microscope. Classification of ectomycorrhizal types was based on Agerer (1987–1998) using the colour and morphology of the mycorrhizal mantle as the main criteria (Table 2). Molecular identification would have naturally given more detailed information about the fungal symbionts, but since we have previously conducted morphotyping and molecular identification of the dominating Scots pine morphotypes in the study area (Kuikka et al. 2003) and have a long-term experience on the composition of ECM communities there, we chose to use only morphotyping here.

We calculated the proportion of each morphotype of all mycorrhizal root tips (except young and non-mycorrhizal) in the samples as well as the proportion of root tips colonised

by different morphotypes. In order to compare the diversity of morphotypes, we calculated the Shannon diversity index (H) by using the equation:

$$H = -\sum p_i \times \ln p_i,$$

where p_i is the proportion of i th mycorrhizal morphotype, calculated after excluding the non-mycorrhizal and young rootlets. Shannon index increases when the morphotype diversity of the sample increases.

The mycorrhizal morphotypes were divided into two groups according to the amount of fungal structures associated with the roots: morphotypes that presumably require high amounts of carbon for the fungal structures, and morphotypes with low requirement of carbon (Godbold and Berntson 1997, Saikkonen et al. 1999, Gorissen and Kuyper 2000, Kuikka et al. 2003, Saravesi et al. 2008, Ruotsalainen et al. 2009). Morphotypes with abundant external mycelia, with fluffy appearance due to abundant attached mycelia, or forming tubercles (type 1, 2, 6, 7; Table 2) were classified in the first group (high carbon demand). Morphotypes with smooth, thin mantles (type 3, 4, 5) were classified in the second group (low carbon demand). Of the 90 samples we excluded one extreme outlier because of anomaly high number of mycorrhizal tips.

Soil fungal biomass and soil organic matter

Soil samples were freeze dried and sieved (mesh size 1.5 mm) to remove litter. We determined soil fungal biomass as ergosterol using a modified assay (Nylund and Wallander 1992, modified by Ola Kären, pers. comm.). Ergosterol was quantified with HPLC using a reverse-phase-C18

Table 2. Description and assumed carbon demand of the mycorrhizal morphotypes into which the mycorrhizal tips were classified.

Morphotypes	Description and comments	Assumed carbon demand
1) Dark and woolly	Loose cover of abundant dark hyphae around the tip, with fluffy appearance	High
2) Light and woolly	Loose cover of abundant light hyphae around the tip, with fluffy appearance	High
3) Dark and smooth	No visible loose hyphae around the tip, a compact mantle with dark brown-blackish appearance	Low
4) Light brown and smooth	No visible loose hyphae around the tip, a compact mantle with light brown appearance	Low
5) Black-yellow and smooth	No visible loose hyphae around the tip, a compact mantle with black and yellow areas	Low
6) Tubercle/suilloid	Thick mantle covers several densely ramified woolly mycorrhizae, enclosing them in a tuberculate formation; often rhizomorphs	High
7) Tubercle-like	Similar to 6), but it lacks a thick mantle, with only a partial or loosely arranged cover	High
8) Young rootlet	No root hairs and too young for determination of morphotype	–
9) Old and non mycorrhizal	No visible mantle, dark, with no root hairs	–

column and methanol as the eluant. Ergosterol (5, 7, 22-ergostatrien-3B-ol) was used as a standard. Ergosterol provides a conservative estimate of the living fungal biomass including ericoid, ectomycorrhizal as well as saprophytic fungi in soil. The amount of ergosterol in fungal biomass may depend on the species and age of mycelium (Antibus and Sinsabaugh 1993). Soil organic matter was determined by ignition loss after heating the soil samples at 500°C for 4 h.

Data analysis

For the statistical data analyses we computed mean values for both patch types in each of the three successional stages in all locations. We analysed the data by mixed model ANOVA with the location (five levels, considered as blocking factor) as random factor and successional stage (three levels) and patch type (*Empetrum* versus empty patches) as fixed factors. Because most of the parameters indicated a statistically significant (or near significant) interaction between successional stage and patch type (indicating that patch type had different roles in different successional stages), we designed contrasts to compare 1) patch types within each successional stage and 2) between different successional stages given a patch type (*Empetrum* or empty patch) (Fig. 2–6). Because the proportions of seedlings in different patches were not independent (percentages of the two patch types sum up to 100% in each successional stage), and further because percentages sum up to 100% within location (block) (i.e. there is no variation between successional stages or blocks), we could not perform ANOVA for these data. Instead, we used paired-sample t-test to compare proportion of *Empetrum* versus empty patches within each successional stage (total of three comparisons of which those having p-value < 0.1 are presented in Fig. 1). Data analyses, including the computation of the estimated marginal means (\pm SE) displayed in Fig. 2–6, were performed using SPSS ver. 16.0.

Results

Seedling recruitment and performance

Even though the proportion of *Empetrum* patches was lower in the earliest successional stage (60% of the studied patches were *Empetrum* free) over 90% of the pine seedlings were found in *Empetrum* patches (Fig. 1). In the older successional stages the proportion of the two patch types became equal (*Empetrum* vs empty patches was 49:51% in the deflation basin and 50:50% in mature forest). However, in the deflation basin the proportion of seedlings in *Empetrum* patches was over 70% whereas in the mature forest it was less than 25% (Fig. 1).

The number of seedlings within the 25 × 25 cm frames varied from 0 to 11, approaching negative binomial distribution; the most common numbers were 0 and 1 (mean = 0.3 seedlings frame⁻¹). In terms of absolute density (no. of seedlings m⁻²) the amount of seedlings was higher in *Empetrum* patches especially in the deflation basin (Table 3, Fig. 2), where the highest number of seedlings was recorded. Total

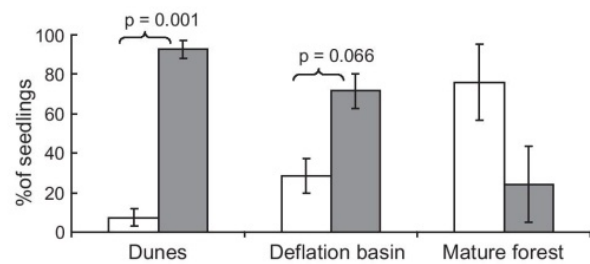


Figure 1. Proportion (%) of total population of Scots pine seedlings in either *Empetrum* (filled bars) and in empty patches (empty bars) along the successional stages. The bars represent the mean (\pm SE) of five locations. p-values above the bars indicate statistical difference according to paired t-tests.

N concentration of Scots pine's needles was highest at the youngest succession stage (Table 3, Fig. 3).

Fungal biomass and mycorrhizal colonisation

Soil fungal biomass (expressed as μg ergosterol g⁻¹ soil organic matter) was higher in samples collected from *Empetrum* patches than in those collected from patches without *Empetrum*. The amount of soil fungal biomass increased towards mid and late succession stages (Table 3, Fig. 4) mainly due to differences between empty patches.

The number of root tips colonised with mycorrhizal morphotypes with abundant external mycelia (high C morphotypes) calculated per root weight was higher in pine root samples collected from *Empetrum* patches in the mid successional stage compared to empty patches (Table 3, Fig. 5). Among *Empetrum* patches this ratio was significantly lower in the late stage compared to the mid and early stages.

Similarly, the diversity of ectomycorrhizal morphotypes was also higher in *Empetrum* patches compared to empty patches in mid succession, whereas in late succession seedlings in *Empetrum* patches had lower ectomycorrhizal diversity than seedlings in empty patches (Table 3, Fig. 6).

Discussion

Role of *Empetrum* on Scots pine seedling recruitment

The proportion of pine seedlings found in *Empetrum* patches compared to empty patches clearly shows that this ericoid

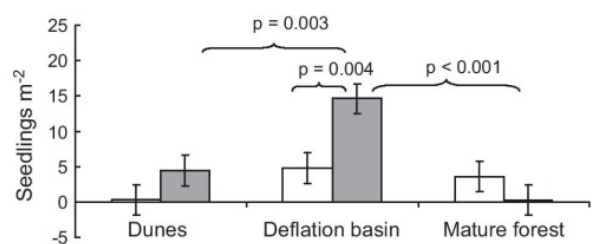


Figure 2. Number of Scots pine seedlings m⁻² in *Empetrum* patches (filled bars) and in empty patches (empty bars) along the successional stages. The bars represent the estimated marginal means (\pm SE), and p-values above the bars indicate statistical difference according to comparison of estimated marginal means.

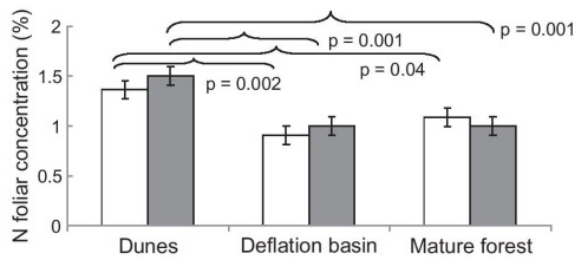


Figure 3. Foliar nitrogen concentration (% of needle dry weight) of Scots pine in *Empetrum* patches (filled bars) and in empty patches (empty bars) along the successional stages. For other details see Fig. 2.

shrub facilitates to a greater extent the recruitment of pine seedlings in early and mid successional stages in relative terms (Fig. 1). Contrarily, in the mature forest pine regeneration is somewhat easier in places without the influence of the shrub. These results give strong support to the hypothesis that the importance of facilitation in plant communities increases with increasing abiotic stress and disturbance (Bertness and Callaway 1994, Brooker and Callaghan 1998, Callaway et al. 2002, Franks and Peterson 2003, Klanderud and Totland 2004, Sthultz et al. 2007, le Roux and McGeoch 2008) and that competition may play a more important role under more benign physical conditions (Bertness and Leonard 1997, Sthultz et al. 2007). Present results also further emphasise the previously understated role of positive interactions in shaping and maintaining populations and communities (Goldenheim et al. 2008).

In early stage of succession, the harsh aeolian forces and the lack of suitable places for germination represent a marked bottleneck for seedling success, as only 40% of studied patches were occupied by *Empetrum*. However, once Scots pine seedlings manage to germinate – mainly in *Empetrum* patches (Fig. 1) – they seem to grow well due to a competition free environment, as can be deduced from their high foliar nitrogen content (Fig. 3). This may be also due to the higher soil pH (Table 1) improving the availability of nutrients for plants. Mechanic sheltering effects have been found in primary successional environments (Jumpponen et al. 1998), in high-alpine environments (Dona and Galen 2007) and in stressed seashore and high elevation conditions with strong winds and extreme temperatures (Eränen and Kozlov 2008). In the mature forest seedling recruitment is as difficult as in the dunes but, instead, competition is severe: seedlings

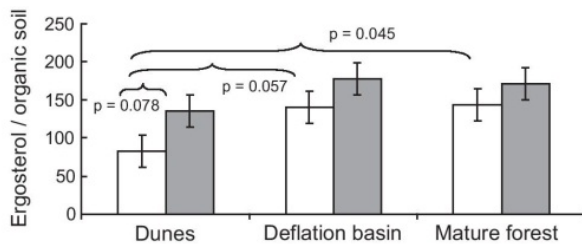


Figure 4. Soil fungal biomass (expressed as μg ergosterol g^{-1} organic matter) in *Empetrum* patches (filled bars) and in empty patches (empty bars) along the successional stages. For other details see Fig. 2.

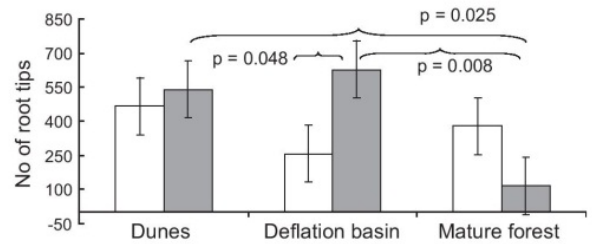


Figure 5. Number of Scots pine root tips colonised by morphotypes with abundant external mycelia (high C) morphotypes / root weight in *Empetrum* patches (filled bars) and in empty patches (empty bars) along the successional stages. For other details see Fig. 2.

are forced to compete with *Empetrum*, but also with other shrubs, forest mosses and lichens in *Empetrum* free patches. Furthermore, shading by tree canopy causes additional stress and competition for species on forest floor.

However, in terms of absolute density of seedlings, the highest density was recorded in the intermediate stage of succession in *Empetrum* patches (Fig. 2), suggesting that the combined effects of abiotic and biotic stress gradients which constrain the establishment of seedlings, is at the lowest under such conditions.

Facilitation mechanisms by *Empetrum*

On the rapidly emerging coast of subarctic Canada, shrub-like willows are reported to control forest margin expansion towards the shoreline. Dense shrub mats act as snow traps providing protection for conifer seedlings (Gregoire and Begin 1993). Similarly, in the early stage of Hailuoto island, the *Empetrum* patches growing on windy and dry dunes may trap airborne seeds and provide a shelter for newly established Scots pine seedlings. Along with physical protection from the wind, *Empetrum* can enhance soil organic matter content, assure soil development and stability, moderate temperature fluctuations, keep moisture in the soil (Choler et al. 2001), and/or provide protection of seeds against granivores.

The fact that soil under *Empetrum* contained more fungal biomass (Fig. 4), and that in the two younger succession stages there was a tendency for higher diversity of ectomycorrhizal species in pine roots collected in *Empetrum* patches (Fig. 6), suggest that *Empetrum* may not only enhance the establishment of Scots pine seedlings through a complex combination of facilitative mechanisms as discussed above, but

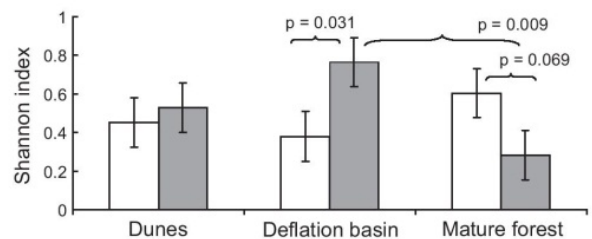


Figure 6. Mycorrhizal morphotype diversity (Shannon index) in Scots pine roots in *Empetrum* patches (filled bars) and in empty patches (empty bars) along the successional stages. For other details see Fig. 2.

Table 3. Results for mixed model analysis of variance for number of seedlings m^{-2} , foliar N concentration, soil fungal biomass (ergosterol concentration), number of root tips colonised by morphotypes with high carbon demand, and mycorrhizal morphotype diversity (Shannon index). Error DF = 20, except in * where the block-term was redundant and error DF = 24.

Source	DF	Seedlings m^{-2}		N concentration		Ergosterol	
		F	p	F	p	F	p
Successional stage	2	8.54	0.00	16.00	<0.001	3.90	0.04
Patch type	1	4.17	0.05	0.44	0.51	5.60	0.03
Succession \times Patch	2	4.79	0.02	0.86	0.44	0.21	0.81

Source	DF	High C morphotypes*		Shannon index	
		F	p	F	p
Successional stage	2	2.25	0.13	0.61	0.55
Patch type	1	0.35	0.56	0.24	0.63
Succession \times Patch	2	3.18	0.06	4.52	0.02

also benefit their fungal symbionts. *Empetrum* may improve the environment for mycorrhizal and other root-associated fungi and may possibly provide fungal inocula for the seedlings as suggested by Vrålstad et al. (2000). These findings are supported by Nara (2006) who found that ectomycorrhizal mycelial networks are important for seedling establishment and plant community structure during early primary succession, which suggests that the observed ameliorated seedling establishment and ectomycorrhizal performance are not independent from each other. However, part of these facilitative impacts may also be indirect, as *Empetrum*, by keeping soil moist underneath the dense shoots, enhances the occurrence of *Polytrichum* mosses (unpubl.), which stabilise the sandy soil by their dense rhizoids and also increase soil organic matter content.

Our data therefore suggest that *Empetrum* can facilitate Scots pine seedlings by changing both abiotic environment and root fungal colonisation. However, the physiological processes that control and direct these interactions remain to be solved, by e.g. in situ experiments where seedlings and soil are transposed between successional stages.

Effects of *Empetrum* on ectomycorrhizal fungi

Even though the soil ergosterol results suggest that *Empetrum* patches maintain higher mycelial biomass than the empty patches (Fig. 4), on the basis of ergosterol it is not possible to distinguish between mycelia of ectomycorrhizal or ericoid or saprophytic fungal origin. However, the higher frequency of mycorrhizal morphotypes with abundant external mycelia found in pine roots of *Empetrum* patches in the mid succession (Fig. 5) suggests that *Empetrum* actually ameliorates the mycorrhizal symbiosis by enhancing the growth, quality and diversity of ectomycorrhizal fungi in harsh physical conditions. This finding contrasts with the results of Nilsson et al. (1993) reporting that *Empetrum* extracts impair ectomycorrhizal fungal partners of Scots pine, which in turn reduces nitrogen acquisition from soil (Wardle et al. 1998). Allelopathic effects of *Empetrum* appear to be linked to a hydroxylated batatasin III, which occurs in high concentrations in *Empetrum* leaves and aqueous leaf extracts (Wallstedt et al. 1997). Apparently, in the harsh environment characterised by high mechanical disturbance and drought of the early dune succession, the positive sheltering impacts of *Empetrum* patches overcome the negative allelopathic influ-

ences. High light availability of the early and mid succession possibly enables the pine seedlings to maintain fungal symbionts with abundant external mycelia, whenever *Empetrum* is present. In spite of the high carbon cost, this is beneficial for the seedlings as the external ectomycorrhizal mycelia are efficient in nutrient harvesting from soil (Read 1992).

In the mature forest, however, mycorrhizal symbiosis and seedling recruitment seem to be aggravated by *Empetrum*, possibly due to its allelopathic and competitive effects (Nilsson et al. 1993). The organic soil layer becomes thicker and better developed towards older stages of the succession, as organic matter increases due to litter accumulation and decomposition (Pennanen et al. 2001). Because thicker organic layer retains the allelochemicals more effectively, allelopathic effects are most likely stronger in older stages of succession. *Empetrum* is a strong competitor also because it contributes to building up an organic soil horizon where nutrients are retained as organic compounds mainly available only to shrubs with ericoid mycorrhizae (Read et al. 1989) and those ectomycorrhizal fungi that are able to use complex N sources (Northup et al. 1995).

We found the highest ectomycorrhizal morphotype diversity in the mid stage of succession (Fig. 6). This pattern fits the prediction of the intermediate disturbance hypothesis proposed by Connell (1978), according to which an intermediate incidence of disturbance is often translated into higher diversity of organisms (Sousa 1979, Valiela 1995, but see also Mackey and Currey 2001). In our case the strong competition in the mature forest, and especially the allelopathic effects of *Empetrum*, most likely hampers root colonisation capacity and survival of some mycorrhizal fungi. In the youngest stage of succession, hyphal growth in dry sand is limited, and also some of the potential fungal symbionts may not find hosts in patchy vegetation and may become carbon limited. However, in the mid stage, *Empetrum* patches seem to favour the diversity of fungal morphotypes, which is most likely increased due to simultaneous occurrence of both ruderal fungi (*Rhizopogon* sp., *Suillus bovinus*, *Inocybe* sp.) typically present in the early stages of succession, and some of the competitive fungal species (Kuikka et al. 2003) usually occurring later in the succession.

In summary, we found support for the assumption that there is a change from facilitation to competition in species interactions along a gradient from abiotic to biotic stress. Scots pine, ectomycorrhizal fungi and *Empetrum* interact

with each other and their interactions vary along the successional gradient: *Empetrum* facilitates Scots pine seedlings at the early stages of successions, and competes with them at late successional stages; however, suilloid ectomycorrhizal morphotypes are facilitated by *Empetrum* at intermediate levels of disturbance. These processes have remarkable effects on Scots pine seedling density, amount of fungal biomass in soil, and quality and diversity of fungal morphotypes along the primary succession gradient. Despite the limited understanding of the mechanisms underlying these interactions, our results are especially interesting as, to our knowledge, they are the first indication that an ericoid mycorrhizal shrub can act as a facilitator for both the ectomycorrhizal host and the host's fungal symbionts.

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Capítol IV

**Interaccions vegetals i composició de la vegetació àrtica al llarg d'un
gradient de nivositat al nord-est de Grenlàndia**

***Plant interactions and high-arctic vegetation composition along a snow-
depth gradient in NE Greenland***

Oriol Grau, Josep M. Ninot, Aaron Pérez-Haase & Terry Callaghan

Manuscrit enviat / *Submitted manuscript*

Resum

L'escalfament del clima i els canvis en els règims de precipitació de neu que tenen lloc en zones àrtiques en l'actualitat, i que s'espera que persisteixin en el futur, estan causant alteracions notables en la vegetació, com ara la expansió de les comunitats arbustives. D'acord amb la hipòtesi del gradient d'estrès (SGH), variacions en el règim d'estrès ambiental poden determinar la naturalesa i la direcció de les interaccions planta-planta, és a dir, el balanç entre facilitació i competència, que són factors causals rellevants en l'establiment de les plantes i en la diversitat vegetal dels ecosistemes. En aquest estudi s'ha investigat si alguns tipus de vegetació dominant en zones àrtiques (les moltes i els subarbusts *Salix arctica* i *Dryas octopetala x intermedia*) tenen alguna funció moduladora de l'establiment i de la diversitat vegetal en tres comunitats vegetals comunes al nord-est de Groenlàndia. Aquestes comunitats eren la congestera amb *Salix*, la formació subarbustiva de *Dryas* i els erms amb vegetació esparsa, que estan associades a un gradient altitudinal creixent i a un gruix de neu alt, mig i baix durant el període hivernal, i per tant a un règim d'estrès creixent.

Vam observar que la SGH no s'adequava com s'havia previst al llarg del gradient de gruix de neu. Per exemple, *Dryas* no va mostrar cap indicatiu que originés cap efecte facilitador creixent cap a la zona amb més severitat ambiental; les moltes i *Salix* van esdevenir punts calents de biodiversitat en l'extrem més sever del gradient, però el seu efecte en la diversitat i en el reclutament de plantes va ser semblant en les zones de menys severitat, on s'esperava una prevalença de les interaccions competitives. Aquests resultats no neguen la validesa de la SGH, però remarquen la necessitat de fer més recerca sobre aquest tema per tal de preveure millor els efectes de la competència i la facilitació al llarg de gradients d'estrès, i de millorar el coneixement dels factors específics implicats en aquestes interaccions. Aquest estudi ajudarà a preveure millor els futurs canvis que hi podria haver en la riquesa d'espècies vegetals, en l'establiment i en la composició de la vegetació que viu a l'alt Àrtic, si els règims de precipitació de neu canvien en el futur, com s'ha pronosticat.

Plant interactions and high-arctic vegetation composition along a snow-depth gradient in NE Greenland

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Keywords

Arctic; competition; facilitation; plant establishment; snow; stress gradient.

Abstract

Current climate warming and changes in snow precipitation regime are causing, and are expected to continue to cause major changes in vegetation, such as shrub expansion in arctic ecosystems. Changes in snow cover lead to varying regimes of abiotic and resource stress on plants. According to the stress-gradient hypothesis (SGH), variations in stress regime determine the nature and direction of plant-plant interactions, namely the balance between facilitation and competition, which are expected to drive plant establishment and diversity. We investigated whether distinct dominant arctic plant patch types, i.e. the mosses and the dwarf shrubs *Salix arctica* and *Dryas octopetala* × *intermedia* drive the establishment of vascular plants and species composition in three common plant communities in NE Greenland, i.e. the *Salix* snowbed, the *Dryas* heath and the fell-field, respectively associated with an increasing altitudinal gradient and high, medium and low snow-depth during winter, and thus with varying regimes of stress.

We found that the SGH did not apply as expected over the snow-depth gradient. *Dryas* showed no evidence of increasing facilitation at the harshest site as predicted; moss

and *Salix* patches acted as biodiversity hotspots at the harshest site, but their roles were similar in the two less harsh habitats in which competitive exclusion was expected. These findings do not negate the SGH but emphasise the need to understand the mechanisms involved in plant-plant interactions along stress gradients. Our results will help to forecast future changes on plant species richness, establishment and composition occurring in the high Arctic if snow precipitation regime changes in the future as predicted.

Introduction

Arctic regions are expected to continue to experience substantial changes in climate in the coming decades according to most recent models and assessments (ACIA 2005; IPCC 2007; AMAP 2011). There is growing confidence that there will be a marked increase of mean annual temperatures and changes in precipitation regimes, particularly snow (Brown & Mote 2009). The regional models project much larger local temperature increases in winter, compared with other seasons, particularly along Greenland's east coast. This is related to retreat of sea ice, and increased precipitation in northern Greenland (Stendel et al. 2008). The marked effect of climate change on organisms (e.g. Post et al. 2009; Callaghan et al. 2011a) and on ecosystem functioning (e.g. Euskirchen et al. 2006; Myers-Smith et al. 2011; Lara et al. 2012) in the Arctic and the consequences of these effects at a larger scale has led to an increasing importance of understanding climate-ecosystem interactions in northern latitudes, particularly as not all ecosystems have responded as expected to climate change (Van Bogaert et al. 2011; Callaghan et al. 2011a).

Snow cover plays a major role in climate, hydrology and ecological systems through its influence on the surface energy balance, water balance, thermal regimes and trace gas fluxes. Also, it is a source of soil moisture in spring and summer and greatly determines the damaging effects of abiotic stress on vegetation, since snow provides protection for plants from extreme low temperatures, large temperature fluctuations, ice crystal blast, solar radiation, desiccation and herbivory (Callaghan et al. 2011b). Thus, snow cover and length of the growing season are key factors determining the nature and functioning of plant communities occurring in deglaciated areas in the Arctic (Evans et

al. 1989; Callaghan et al. 2005; Elberling et al. 2008). For instance, substantial phenological changes have already been detected recently in plants in response to advanced snowmelt in north-eastern Greenland (Høye et al. 2007) and to manipulated and natural extreme thaw events in the sub-Arctic in winter (Bokhorst et al. 2009). Furthermore, manipulations of snow cover that affect growing season length have important effects on vegetation (Wahren et al. 2005)

Snow acts both as a resource for vegetation (e.g. moisture store) and as a stressor (e.g. constraining the length of the growing season). Variations in the importance of the resource and abiotic stresses on plants along stress gradients associated with varying snow cover and length of the growing season are important because the dynamic balance between them is expected to determine the nature of plant-plant interactions (i.e. facilitative or competitive) (Lortie et al. 2004; Maestre et al. 2009). According to the stress-gradient hypothesis (Brooker & Callaghan 1998), positive interactions will prevail under harsh abiotic conditions, whereas negative interactions will be more frequent under more favourable abiotic conditions. The potential facilitation mechanisms include amelioration of disturbance (e.g. Altieri et al. 2007), accumulation of nutrients and water (e.g. Brooker et al. 2008), or protection from wind (e.g. Carlsson & Callaghan 1991) and herbivores (e.g. Rao et al. 2003; Grau et al. 2012), which may lead to enhanced survival of plants, higher species richness or higher productivity (Callaway 2010).

Plant-plant interactions in the high Arctic in relation to stress gradients have been poorly examined, with the exception of studies in primary succession gradients in glacier forelands in the high Arctic. For instance, Jones & Henry (2003) found that facilitation by bryophytes may have been one of the processes initiating the succession following glacier retreat. Chapin et al. (1994) and Breen and Lévesque (2006) found that biotic interactions favoured plant recruitment in biological soil crusts in proglacial succession. On the other hand, Sohlberg & Bliss (1984) demonstrated that bryophytes and lichens facilitate vascular plant recruitment in high-arctic plant communities. Thus, these few studies suggest that plant-plant interactions play a key role in plant species establishment in several high-arctic ecosystems. However, the distinct role of life

forms of plants and individual species in relation to plant–plant interactions in arctic environments, and particularly in relation to varying snow cover, are still relatively unexplored (Pajunen et al. 2011).

Current climate warming and changes in snow precipitation regime are causing, and are expected to continue to cause major changes in vegetation in arctic ecosystems in many areas (Van Bogaert et al. 2007; Callaghan et al. 2011a; Myers-Smith et al. 2011; Elmendorf et al. 2012). An early impact of climate warming is a change in plant dominance (Press et al. 1998) and it is therefore important to explore the role of indirect effects of environmental changes through variations in dominance of plants (Wookey et al. 2009) through both their effects on the environment and interactions with co-occurring species. For instance, the current expansion of shrub cover in northern latitudes (Hallinger & Wilming 2011; Myers-Smith et al. 2011; Rundqvist et al. 2011) may in turn alter snow distribution on the ground and vary shrub-snow interactions (Sturm et al. 2001). Further, shrubs have been described as drivers of plant seedling establishment in cold environments, such as in alpine (Cavieres et al. 2007; Klanderud 2010), boreal (Grau et al. 2010) and subarctic (Graae et al. 2011; Grau et al. 2012) ecosystems. On the other hand, moss cover is expected to decrease under a warmer climate (Wijk et al. 2003; Walker et al. 2006) or with increasing snow cover (Wahren et al. 2005) and mosses have been also described as key drivers of vascular plant establishment in the Subarctic (Soudzilovskaia et al. 2011). Nevertheless, it remains unknown if and how shrubs or mosses affect plant establishment and plant community composition along stress gradients related to snow-depth in high-arctic ecosystems.

This study investigates if distinct common arctic plant patch types involving presence of dwarf shrubs and mosses drive the establishment of vascular plants and species composition in distinct plant communities associated with varying snow cover. The plant patch types selected were *Salix arctica* Pall., *Dryas octopetala* L. *x intermedia* Vahl. –*Salix* and *Dryas* hereafter, respectively– and patches without any dominating dwarf shrub but covered with a thin layer of mosses. These three patch types show obvious structural differences between them (for instance, the density of the covering

canopy, litter accumulation, biomass dry weight/fresh weight; see patch type descriptions below) which may result in different plant establishment patterns. The selected plant communities were the *Salix* snowbed, the *Dryas* heath and the fell-field, which are associated with decreasing snow-depth from the snowbed to the fell-field. This gradient is thus associated with varying growing conditions derived from changing regimes of abiotic stress, nutrient and water availability and length of the growing season (Elberling et al. 2008).

We make two assumptions. First, we assume that the overall balance determining plant growth along the snow-depth gradient is less favourable at higher altitudes, as indicated by the progressively decreasing vegetation cover towards the fell-fields and above. However, we acknowledge that extreme snowbeds in other areas can be less favourable to plant growth than other habitats at higher altitudes. Secondly, we assume that the presence or absence of individuals in a patch is related directly to competitive or facilitative properties of the species dominating the patch. However, we acknowledge that what we measure in practice is an association among species and there is a small possibility that this might be controlled by environmental features other than those mediated by the dominant species.

We hypothesise that: (1) on the basis of the stress-gradient hypothesis, the selected patch types (i.e. *Salix*, *Dryas* and mosses) will have more positive effects on seedling establishment and on species richness in plant communities occurring at high altitudes with little snow cover than in plant communities at lower altitudes; (2) the structural differences between the distinct patch types will lead to varying seedling establishment and species richness patterns independently of the community type in which they occur; we expect that the effects described in hypothesis 1 will be more evident in dwarf shrubs, as they offer stronger physical protection and seed-trapping ability than mosses.

Material and Methods

Study area

The study was conducted in July 2011 along the south-western slope of Mount Aucellabjerg, in the Zackenberg valley, near the Zackenberg Research Station (74°30'N, 20°30'W), on the north-eastern coast of Greenland. This area was deglaciated about 10,000 years ago and currently its high-arctic climate is strongly affected by the wide and dense belt of polar pack ice on the coast, which makes the climate more continental, with very cold winters, little precipitation and sunny summers (Meltofte & Rasch 2008). During the polar night, monthly mean air temperatures are below -20°C, with dominating northerly winds. In the snow-free summer period, the mean monthly air temperatures vary between 3°C and 7°C in July and August and daily temperatures rarely get below zero during this warmest part of the summer season (Hansen et al. 2008). The growing season at Zackenberg is from late May in early snow-free areas, while extensive snow-cover may prevail into early summer in snowdrift areas (Meltofte & Rasch 2008). The average annual accumulated precipitation at Zackenberg was 261 mm for the years 1996-2005, of which 10 % was rain and 7 % was mixed precipitation. For the period 1958-2005, there has been an increase of 1.9 mm/year in the annual precipitation and a significant annual warming of 2.25°C for the period 1991-2005 (Hansen et al. 2008).

Plant communities

We selected three main plant communities occurring along the altitudinal gradient (35 m to 450 m above sea level) in Aucellabjerg. These were representative of the vegetation commonly found at low, medium and high elevation, respectively. The communities were the *Salix* snowbed, occurring mainly at the bottom of the valley, with high vegetation cover; the *Dryas* heath, which is found at intermediate altitudes, still with substantial plant cover; and the fell-field, dominated also by *Dryas* but with sparse vegetation (Bay 1998; Table 1). These three plant communities are also associated with decreasing snow thickness from *Salix* snowbeds in the valley bottom to the fell-fields at higher altitude; wind-blown snow accumulates on valley bottoms where snowdrifts persist until early summer (Hinkler et al. 2008). At higher altitudes the effect of wind becomes progressively stronger, and snow is frequently blown away

throughout the winter season. Therefore, there is intermediate snow accumulation in the *Dryas* heath and less accumulation in the fell-fields. Soil water content and storage of organic matter increases from the fell-field to the *Salix* snowbed; the maximum active layer depth (as determined by the 0°C isotherm, Christiansen et al. 2008) is reached by the end of August and decreases from about 80 cm at *Dryas* sites to about 45 cm in *Salix* snowbeds; soil temperature is also lower at the snowbeds (Elberling et al. 2008; Meltofte & Rasch 2008).

Patch types and study species

In each plant community, we searched for three distinct patch types, each dominated by either 1) *Salix*; 2) *Dryas*; 3) mosses:

- 1) *Salix* is a dominating dwarf shrub species in the Zackenberg valley (Bay 1998) which thrives in a wide niche range, from sand and almost barren moraine tills, to snowbeds –where it is dominant– and open fell-fields. It shows wide morphological plasticity, forming diffuse mats with long twigs and large leaves in disturbed spots or under low competitive conditions, and smaller structures in fell-fields or in denser tundra. *Salix*, compared to *Dryas*, makes up a much larger fraction of the diet of musk oxen and lemmings in summer and of the winter diet of the collared lemming (Klein & Bay 1994; Berg et al. 2008).

- 2) *Dryas* is also a dominating dwarf shrub in the study area and is an arctic–alpine species, which has a circumpolar distribution in the northern hemisphere. It has also a wide niche, although it is less opportunistic and also less adapted to long lasting snow cover than *Salix* (Bay 1998). In the Zackenberg region, it forms rather dense cushion-shaped mats with small leaves, which protrude from the bare soil in the fell-fields, or from flat ground or small convexities in moister tundra and snowbeds. *Dryas* mats have this compact, dense structure as they retain a great amount of dead leaves attached to the stem. It shows higher dry weight/area (results not shown) and higher dry weight/fresh weight ratio than *Salix* (Fig. A1 a in Appendix). *Dryas* dominates in the heaths but also occurs sparsely in the snowbed and in the fell-fields.

- 3) Mosses without any dominant co-occurring shrub; the distinct moss species forming this patch type could not be identified, partly because of their early stage of development during the sampling period. The moss patches showed a rather homogeneous structural aspect and stage of development. This patch type was dominated by poorly developed moss carpets or tiny layers of prothalli, although some small spots with cushion-shaped acrocarpic mosses (like *Polytrichum* sp.) and sparse macrolichens (like *Cetraria ricetroum* Opiz, *Peltigera rufescens* (Weiss) Humb. and *Stereocaulon* gr. *alpinum* Laurer ex Funck) were present. In this patch type there was very little biomass production and litter accumulation compared to *Salix* and *Dryas* patches. Thus, the plants growing in the mosses were not generally affected by shade from a covering canopy as occurred in the shrub patches.

Table 1. Description of the communities sampled.

	Fell-field	<i>Dryas</i> Heath	<i>Salix</i> snowbed
Altitude (m a.s.l.)¹	300-600	150-300	< 150 m
Snow accumulation¹	Little	Intermediate	High
Slope angle (~°)	13	11	0
Vegetation cover (%)	28 ± 13.82	85.53 ± 16.61	87.45 ± 10.77
Cover of main patches (%):			
<i>Salix</i>	7.85 ± 3.42	25.17 ± 7.25	50.09 ± 8.76
<i>Dryas</i>	14.07 ± 7.38	40.29 ± 5.03	11.59 ± 9.78
mosses	6.44 ± 4.09	19.27 ± 17.57	24.17 ± 14.13

¹ According to Bay (1998)

Data collection

We selected three study plots at similar altitudes within each plant community (i.e. *Salix* snowbed, 35-43 m a.s.l.; *Dryas* heath, 182-240 m; and fell-field, 415-450 m), separated by a few hundred meters from each other, but with similar orientation (mainly south-west). In each plot we searched for the three distinct patch types described above. We sampled four 25 × 25 cm quadrats of each patch type, which were treated as subsamples of a given patch type within each plot (n=4 subsamples × 3 patch types × 3 plots × 3 plant communities = 108 quadrats in total). We analysed each quadrat with a rigid ITEX-like frame divided into 100 2.5 × 2.5 cm squares. To clearly elucidate the effects of each patch type, mixed patches were rejected, thus only those

where *Dryas* or *Salix* or mosses clearly dominated (>80 % and often >90 %) were considered; for instance, we avoided those *Dryas* or *Salix* patches which contained high cover of mosses. The distinct quadrats of each patch type were separated by only a few meters (2-8 m generally) from each other within each study plot. In each quadrat we identified the species and estimated the number of individuals of each species found. Clonal species growing in adjacent 2.5 cm squares within the frame were considered as one single individual when counting the total number of individuals, unless they could be clearly identified as separate individuals (i.e. if visibly recently germinated). However, it is acknowledged that determining an individual of a clonal plant is difficult in practice (Callaghan et al. 1999).

In order to sample the aboveground biomass in the *Salix* and *Dryas* quadrats, we clipped a surface of 10 × 10 cm for *Salix* and 7 × 7 cm for *Dryas* (the latter showed a more uniform canopy and this amount was considered sufficient). The fresh leaves were kept moist in small, sealed plastic bags and brought quickly to the laboratory, where we sorted the green leaves, weighed them fresh, and then dried them at 70°C for 24 h and measured dry weight. To make sure that the shrubs sampled did not show big structural differences and that they could be used to compare their role in the distinct plant communities, the leaf dry matter content (dry weight/fresh weight) and the ratio 'leaf dry weight/area covered' were calculated (Fig. A1 a-b). To evaluate the cover of each patch type in each plant community, we recorded the presence or absence of the distinct patch types every 5 cm along five parallel lines of 5 m length in each study plot.

Data analyses

The data were analysed using R (R Development Core Team 2012). The number of individuals and the number of species were calculated by obtaining a mean value of each of these variables for each plot in each community and then by averaging the three plots in each community, thereby avoiding pseudoreplication. The trends observed in the data were analysed in a linear mixed model, using the 'nlme' package (Pinheiro & Bates 2000). 'Plant community' and 'patch type' were integrated as fixed

factors. To account for the grouping structure of the data, 'patch type' was nested within 'plot' and was considered a random factor in the model.

Species accumulation curves were obtained by counting the number of species found when increasing the area sampled for each patch type in each community; assuming that our samples provide a proper random representation of the three communities, all the quadrats of a given patch type \times plant community combination were taken as a whole and were added in a random order for 100 times to obtain the final accumulation curves with the 'vegan' package (Oksanen et al. 2009). From these curves we calculated the β -diversity (Whittaker 1972), defined here as the ratio 'final species richness in the accumulation curve' / 'mean initial richness value in the accumulation curve'.

Since species accumulation curves did not reach saturation after adding all the sampled plots, we used rarefaction to estimate species richness for the distinct patch types in each community, using the 'vegan' package (Oksanen et al. 2009). This method accounts for incomplete sampling by estimating the expected species richness in a given subsample of the total number of observations randomly drawn from a large pool of individuals (Gotelli & Colwell 2001). Under the assumption that our samples are a random representation of the community (Gotelli & Colwell 2001), the rarefied species richness provides an unbiased estimation of the true species richness, i.e. an estimation of the relative species richness between the distinct patch type \times plant community combinations. We summed all the individuals of the four quadrats of each patch type in each plot; because the lowest number of individuals in one of these plots was 13, the rarefaction was calculated by randomly drawing 13 individuals from the total pool of each sample. In this way we included all the sampled plots, which generally had a much larger number of individuals. The results were also analysed in the mixed model described above.

We performed a PCA ordination which assessed the floristic differences between patch type \times plant community combinations; such floristic differences can be appropriately estimated by using a distance coefficient, i.e. the Hellinger distance, which reduces

more strongly the highest abundance values compared to low values and avoids the similarities derived from sharing absent species (Legendre & Legendre 2003; Borcard et al. 2011). Since a PCA cannot be calculated from a distance matrix, we transformed the species abundances by emulating the Hellinger distance coefficient with the Ginkgo data analyser (de Cáceres et al. 2012). The abundance values were based on the total number of individuals of each species from all the sampled quadrats of each patch type in each community. The PCA was obtained by using the 'FactoMineR' package (Husson et al. 2011).

Species abundance distribution was firstly analysed by classifying the species from most to least abundant (i.e. rank-abundance plots, not shown) using the 'BiodiversityR' package (Kindt 2004) in each patch type × plant community combination. In this classification we considered all the individuals of each species from all quadrats together. Then, we selected the model which best fitted their species abundance distribution, that is, with the lowest AIC value compared to all the other potential models (Kindt & Coe 2005). Log-Normal and Zipf models were fitted as a generalised linear model with a logarithmic link function. A pre-emption model was fitted as a purely nonlinear model (see Fig. A2 for a graphic description of the fitted models).

Species fidelity was calculated as implemented in Ginkgo (de Cáceres et al. 2012) for all species in each patch type × plant community combination. The fidelity measures the degree to which a species is confined to a given group (Legendre and Legendre 2003). We used the phi fidelity statistic (Φ) as defined by Chytrý et al. (2002),

$$\Phi = \frac{N \cdot np - n \cdot Np}{\sqrt{n \cdot Np \cdot (N - n) \cdot (N - Np)}}$$

which takes into account the number of relevés in the data set (N); the number of relevés in the particular vegetation unit (Np), the number of occurrences of the species in the data set (n); the number of occurrences of the species in the particular vegetation unit (np). The value 1 indicates that the species and the vegetation unit are completely faithful to each other. Fidelity values higher than 0.3 or lower than -0.3 were considered to be representative of high or low fidelity, respectively.

Results

Species richness and establishment

The total number of vascular plant individuals counted was 974 in the snowbed community, 994 in the heath and only 556 in the fell-field. The total number of species found was 48 over all these plant communities. There were no differences in the number of individuals (Fig. 1a) and total species number (Fig. 1b) between the distinct patch types in snowbed and heath communities, but these numbers were significantly lower in *Dryas* patches in the fell-field, at the highest altitude. However, rarefied species richness (Fig. 1c) showed a marked decreasing trend in *Dryas* patches from the snowbeds at the bottom of the altitudinal gradient to the fell-field. It was also significantly lower in *Dryas* patches compared to moss patches in both the heath and the fell-field.

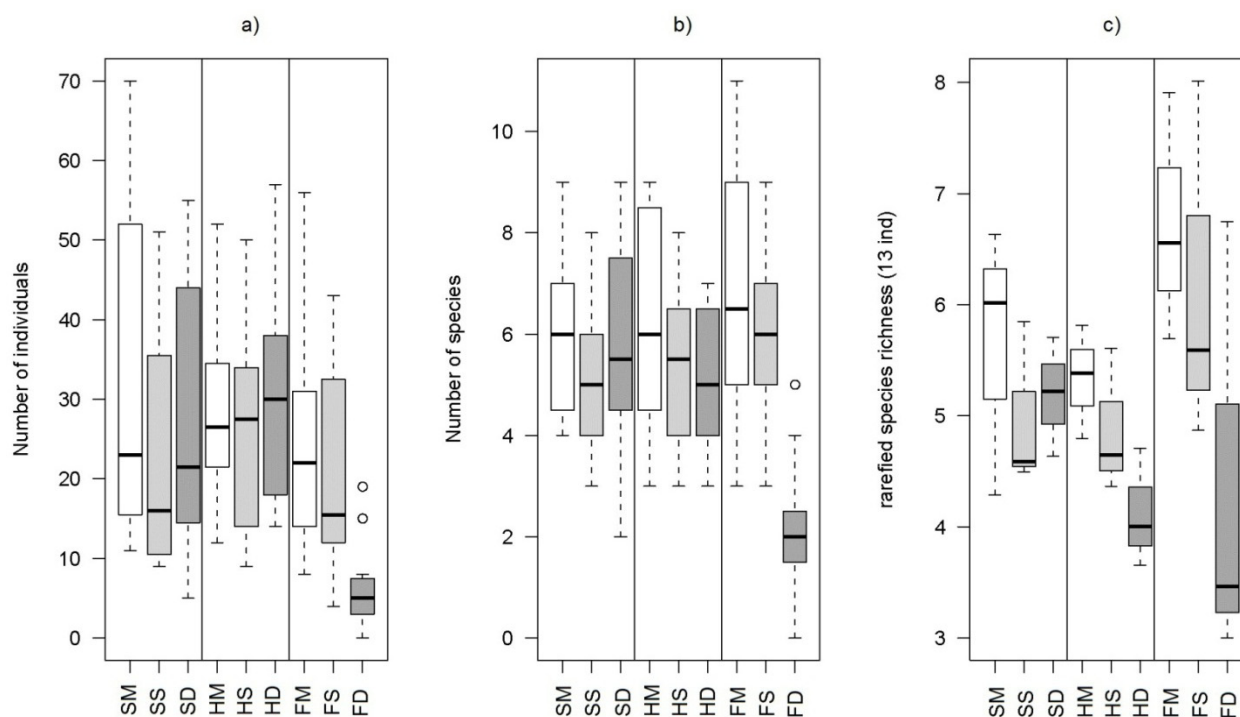


Fig.1 Number of individuals (a), number of species (b) and rarefied species richness (c) measured in the *Salix* snowbed, the *Dryas* heath and the fell-field. The abbreviations correspond to: M: mosses (white columns); S: *Salix* (grey columns); D: *Dryas* (dark grey columns). The abbreviations correspond to: FD: *Dryas* patches in the fell-field; FM: moss patches in the fell-field; FS: *Salix* patches in the fell-field; HD: *Dryas* patches in the *Dryas* heath; HM: moss patches in the *Dryas* heath; HS: *Salix* patches in the *Dryas* heath; SD: *Dryas* patches in the *Salix* snowbed; SM: moss patches in the *Salix* snowbed; SS: *Salix* in the *Salix* snowbed.

Species accumulation curves for the distinct patch type × plant community combinations (Fig. 2) indicated that *Dryas* patches generally accumulate (assuming that associations among species are directly caused by the presence of the dominant species: see Introduction) the lowest absolute number of species when considering all the sampled quadrats, irrespective of the plant community. In the fell-field, *Dryas* patches promoted a clearly lower final accumulation of species with increasing sampling area compared to *Salix* and moss patches in this community. *Dryas* patches also showed lower species accumulation in the heath, but this difference became less evident in the snowbed community. However, the effect of *Dryas* on the increase of the β -diversity was highest in the fell-field and lowest in the other communities (Table A1). The effects of *Salix* and mosses varied less across the distinct communities; species accumulation was especially high in moss patches in all plant communities.

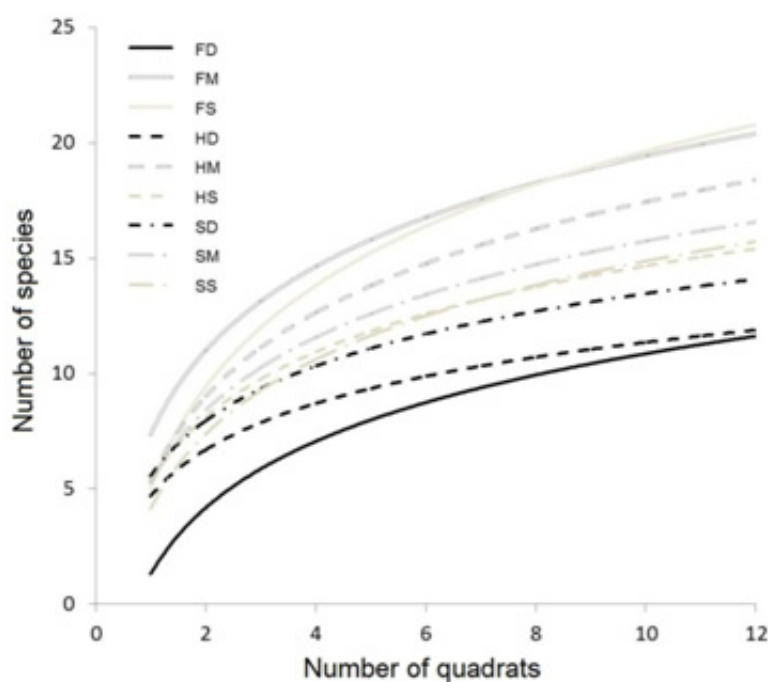


Fig. 2 Species accumulation curves for all the distinct patch type × plant community combinations. See caption in Fig. 1 for details of the abbreviations.

Species distribution

The model which best fitted the species abundance distribution differed between patch types in the fell-field and in the heath but it became similar for all patch types in the snowbed (Table 2; Fig. A2), where species abundances (i.e. number of individuals for each species) in all patch types showed a similar decreasing pattern from the most

to the least abundant species. Moss and *Salix* patches showed a similar pattern in the fell-field and in the heath, whereas *Dryas* patches showed a varying distribution in each plant community. The differences estimated by these fitted models depended basically on how abundant the rest of the species were compared to those most abundant. In nearly all cases the most abundant species was *Polygonum viviparum*, which showed the highest number of individuals in the fell-field and in the heath, and was also very abundant in the snowbed.

Table 2. Models describing the best fit to species abundance distributions and the three most abundant species found in each patch type and plant community.

		<i>Dryas</i>	Mosses	<i>Salix</i>
Fell-field	Best model	Zipf	Log-Normal	Zipf
	Most abundant species	<i>Polygonum viviparum</i> <i>Salix arctica</i> <i>Festuca ovina</i>	<i>Polygonum viviparum</i> <i>Dryas octopetala</i> <i>Saxifraga cernua</i>	<i>Polygonum viviparum</i> <i>Dryas octopetala</i> <i>Festuca ovina</i>
Dryas Heath	Best model	Log-Normal	Log-Normal	Zipf
	Most abundant species	<i>Polygonum viviparum</i> <i>Luzula confusa</i> <i>Salix arctica</i>	<i>Polygonum viviparum</i> <i>Poa arctica</i> <i>Salix arctica</i>	<i>Polygonum viviparum</i> <i>Luzula confusa</i> <i>Dryas octopetala</i>
Salix snowbed	Best model	Pre-emption	Pre-emption	Pre-emption
	Most abundant species	<i>Equisetum arvense</i> <i>Polygonum viviparum</i> <i>Salix arctica</i>	<i>Equisetum arvense</i> <i>Polygonum viviparum</i> <i>Luzula confusa</i>	<i>Equisetum arvense</i> <i>Hierochloe alpine</i> <i>Polygonum viviparum</i>

The PCA in Fig. 3 shows the ecological distances between the distinct patch type × plant community combinations and indicates that the three plant communities studied may be clearly distinguished from each other by their flora. However, there was no clear difference between patch types if plant communities were analysed separately (results not shown), suggesting that the plots sampled within the fell-field, heath and snowbed communities shared similar ecological distances, and that their flora differed less within each plant community, irrespective of patch type, than between communities. When analysing only presence/absence of species in a PCA ordination

(Fig. A3) we observed a very similar pattern and we did not detect a clear segregation related to patch types either.

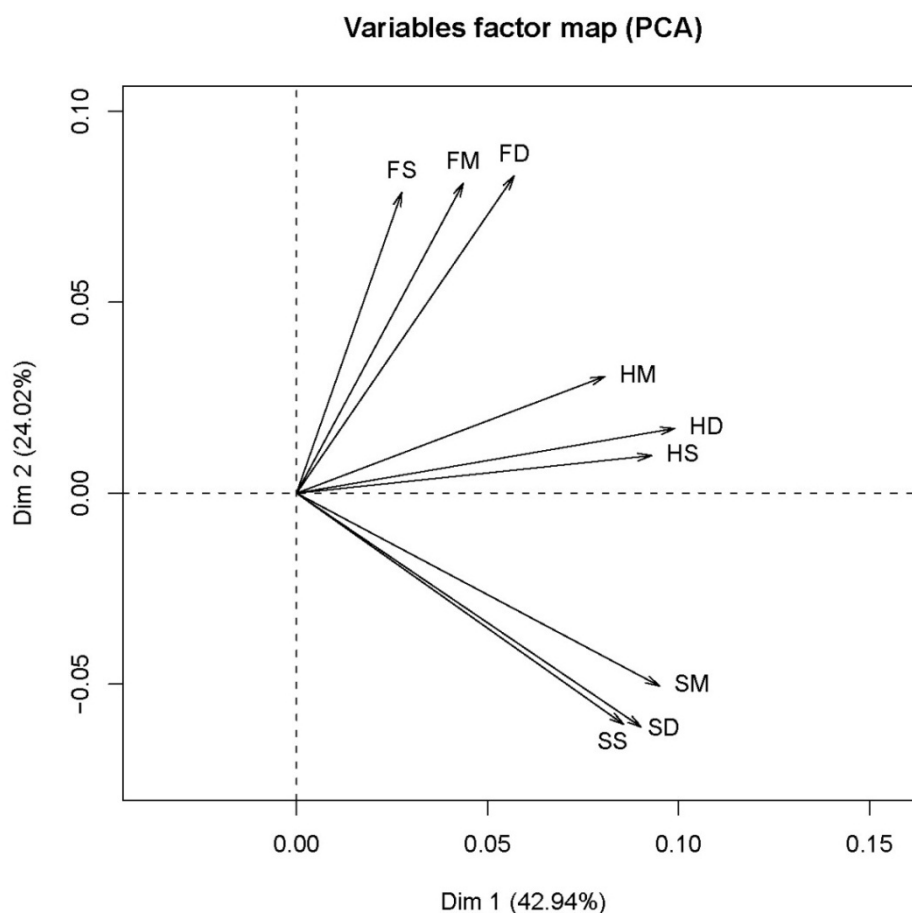


Fig. 3 Principal Component Analysis based on the floristic composition of the distinct patch type × plant community combinations. See caption in Fig. 1 for details of the abbreviations.

Species fidelity

In agreement with the species ordination shown in figure 3, the pool of species showing high or low fidelity to the distinct patch type × plant community combinations (Table 3) varied more between communities than it did between patch types within each plant community. The highest fidelities were reached in moss patches in the fell-field and the lowest in this same community but in *Salix* and *Dryas* patches. No species showed high fidelity to *Dryas* patches in the fell-field. When the fidelity was analysed for the whole species pool, irrespective of the patch type, several species showed high fidelity to each plant community. In the *Salix* snowbeds the species with highest fidelity (Φ value higher than 0.3) were *Hierochloe alpina*, *Arctagrostis latifolia*, *Luzula confusa* and *Alopecurus borealis*; in the heath the species were *Poa arctica*, *Kobresia myosuroides* and *Festuca brachyphylla*; in the fell-field the species showing high fidelity

were mostly of the genus *Saxifraga*: *Saxifraga cernua*, *Saxifraga integrifolia*, *Saxifraga nivalis*, *Saxifraga oppositifolia* and *Saxifraga platysepala*, but also the grass *Poa glauca*.

Table 3. List of species with highest (positive values) and lowest (negative values) fidelity to the distinct patch type × plant community combinations. Only the species with values of fidelity equal to or higher than 0.3 and those with values equal to or lower than -0.3 are shown.

	<i>Dryas</i>		<i>Mosses</i>		<i>Salix</i>
Fell-field	<i>Luzula confusa</i>	-0.41	<i>Saxifraga platysepala</i>	0.62	
			<i>Poa glauca</i>	0.49	<i>Poa glauca</i> 0.41
			<i>Saxifraga cernua</i>	0.45	
			<i>Sagina intermedia</i>	0.43	<i>Sagina intermedia</i> 0.32
			<i>Festuca rubra ssp. richardsonii</i>	0.39	<i>Luzula confusa</i> -0.35
			<i>Saxifraga nivalis</i>	0.39	
Dryas Heath	<i>Kobresia myosuroides</i>	0.52	<i>Poa arctica</i>	0.41	<i>Poa arctica</i> 0.34
	<i>Festuca brachyphylla</i>	0.43	<i>Potentilla hyparctica</i>	0.3	<i>Carex bigelowii</i> 0.3
Salix snowbed	<i>Hierochloe alpina</i>	0.52	<i>Alopecurus borealis</i>	0.44	<i>Hierochloe alpina</i> 0.52
	<i>Arctagrostis latifolia</i>	0.41	<i>Salix arctica</i>	0.34	<i>Arctagrostis latifolia</i> 0.33
	<i>Salix arctica</i>	0.34	<i>Arctagrostis latifolia</i>	0.33	<i>Luzula confusa</i> 0.3
	<i>Stellaria longipes s.l.</i>	0.3			

Discussion

Contrary to our hypothesis 1, the distinct patch types did not show an increasingly positive effect on plant establishment and species richness in the fell-fields compared to the *Dryas* heath and the *Salix* snowbed (Fig. 1) (under the assumption that species associations are related to effects of the dominant species). Actually, *Dryas* patches had an increasingly negative effect on small-scale plant richness and establishment with increasing altitude, which was especially evident in the fell-field, whereas *Salix* and moss patches showed a similar effect in all the plant communities analysed, independently of the general length of the snow-free period associated with each community. These results contrast with those of Klanderud & Totland (2004), who predicted a negative impact of *Dryas* on alpine but not on high-arctic community diversity. We observed that a negative role of *Dryas* on seedling establishment may occur in a high-arctic ecosystem depending on the environmental constraints, even

though it has been commonly described as a facilitator low shrub in northern latitudes (Chapin et al. 1994; Cooper et al. 2004).

Plant establishment within the dominant vegetation has been reported to be potentially governed by many factors. These include factors moderated by the species already present which can affect the abiotic and biotic environment (Pajunen et al. 2011), such as the structure of the canopy of the dominant species, which has important effects on the availability of light to plants growing below (Shevtsova et al. 1995; Totland & Esaete 2002), provision of moisture, shelter and nutrients together with possible allelopathy. They also include external factors such as the availability of air-borne seeds (Klanderud & Totland 2007).

In terms of external factors, we would expect viable seed rain to decrease with increasing altitude (Thompson 1978). For instance, Klanderud & Totland (2007) showed that seed dispersal in *Dryas* heath limited plant recruitment and diversity in an alpine ecosystem in Norway. However, the equally high number of species observed in *Salix* and in mosses patches in the fell-field compared to the other communities suggests that the differing pattern of plant establishment observed in *Dryas* patches is not primarily limited by seed availability in this case.

In terms of factors internal to the community, our results suggest that structural differences between patch types did not primarily determine their capacity to favour plant establishment (against our hypothesis 2). Our expectation that mosses would show a less visible effect on seedling recruitment and species richness compared to the shrubs was not observed in the field, as mosses showed a very similar pattern to that of *Salix*. This finding is in line with Soudzilovskaia et al. (2011), who found that contrasting structural differences in subarctic bryophytes did not lead to significant changes in vascular plant recruitment.

A possible explanation for the pattern observed in *Dryas* compared to the other patch types, irrespective of the physical structure of the dominant species, could be that *Dryas* lacks energy-demanding N-fixing nodulation at higher elevations, whereas at

lower elevations it becomes nodulated (Kohls et al. 1994). Therefore, we speculate that *Dryas* growing under the severe conditions found in the fell-fields may act as a stronger competitor for soil N and may thus reduce its recruiting capacity for potential neighbour competitors (Tilman et al. 2001). This would contrast with several studies which have shown that species richness is higher in relatively harsh environmental conditions compared to more favourable sites due to the relatively minor role of competition in these systems (e.g. Grime 1979). On the other hand, *Dryas* at the fell-field might actively inhibit recruitment to a higher degree than *Salix* and mosses by possibly producing allelopathic substances, as plants may respond to varying stress factors by changing their content of secondary plant metabolites (Berg et al. 2008). In any case, further research is needed in relation to possible N-fixation and allelopathy to better understand the observed patterns of plant establishment in these poorly studied arctic plant communities.

Salix and mosses in the fell-fields hosted a high number of species in spite of the much less vegetated aspect of this harsh, upper zone. If *Salix* and mosses showed a changing competitive ability along the gradient, it had no varying effect on plant establishment in the studied communities.

The high β -diversity observed in *Dryas* patches in the fell-field (Table A1) seems to be related to the low number of species occurring at the patch scale (Figs. 1 and 2) compared to the higher number of species found at the community scale. This difference favours a high turnover of these few species between the distinct samples as none of the species showed high fidelity (Table 3). The species found in *Dryas* patches in the fell-field differed greatly between samples but they were generally found together with *Polygonum viviparum*, which was highly abundant in all samples (Table 2). Thus, except for *Dryas* patches in the fell-field, all the other patch type \times plant community combinations showed higher species richness and higher fidelity values, but lower β -diversity, as the species composition was more uniform between samples and more similar to the species pool at the community scale.

Species composition varied between plant communities but it did not change very substantially between patch types within each community. Even though species establishment and richness varied at patch scale, species composition in each patch type was determined at community scale, as indicated by the similarity between patch types within each community and by the marked differences between communities (Fig. 3; Fig. A2). The PCAs indicate that the *Dryas* heath always showed an intermediate position between the other two communities, suggesting that the growing conditions which drive species composition in the heath were intermediate between the *Salix* snowbed and the fell-field, consistent with the fact that the heath is found midway along the snow-depth gradient.

Overall, this study found that the stress-gradient hypothesis (Brooker & Callaghan 1998) did not apply as expected over the environmental gradient in the high Arctic for any of the three patch types. Whereas, as would be predicted from the hypothesis, moss and *Salix* patches acted as biodiversity hotspots at the harshest site, their roles were similar in the two less harsh habitats in which competitive exclusion would have been predicted. Furthermore, *Dryas* showed no evidence of increasing facilitation at the harshest site as would have been predicted. These findings do not necessarily negate the stress-gradient hypothesis but emphasise the need to understand the specific mechanisms involved in plant-plant interactions along stress gradients.

Conclusions

Our results suggests that plant composition can be significantly associated with the snow-depth gradient at the community scale but not at the patch scale, because the different patch types shared most of their flora within each community but not between communities. Despite this similarity between patch types, this study indicates that plant species richness and establishment at the patch scale in the fell-field are probably strongly determined by the proportion of each patch type. The high β -diversity and the low species fidelity measured in *Dryas* patches in the fell-field suggest that the reduction in species richness observed at patch scale may be attenuated at community scale.

Any future environmental changes altering the relative proportion of *Salix*, *Dryas* or mosses may have a noticeable influence on plant species richness and establishment in the fell-field, but it may also affect the other communities if these changes alter the competitive capacity of *Dryas*, *Salix* or mosses. The patterns observed in the *Salix* snowbed, the *Dryas* heath and the fell-fields, which correspond to high, medium and low snow-depth, will help to predict future changes on plant species richness, establishment and composition occurring in the high Arctic if snow precipitation regime changes in the future as predicted. However, it is important to do further experimental research on the mechanisms which drive the observed patterns in this study in order to better assess any potential effects of environmental changes on plant interactions and on vegetation composition in arctic ecosystems.

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Appendix 1

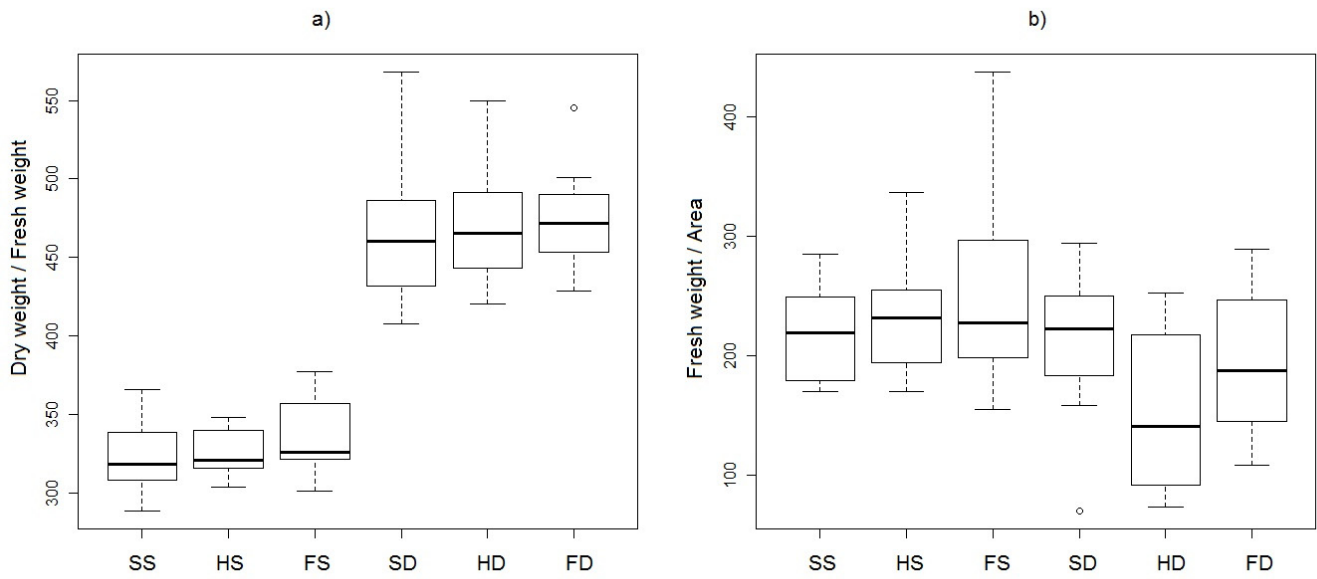


Fig. A1 Dry weight/Fresh weight ratio (a) and Fresh weight/Area (b) in *Salix* and *Dryas* patches in the *Salix* snowbed, the *Dryas* heath and the fell-field. See caption in Fig. 1 for details of the abbreviations.

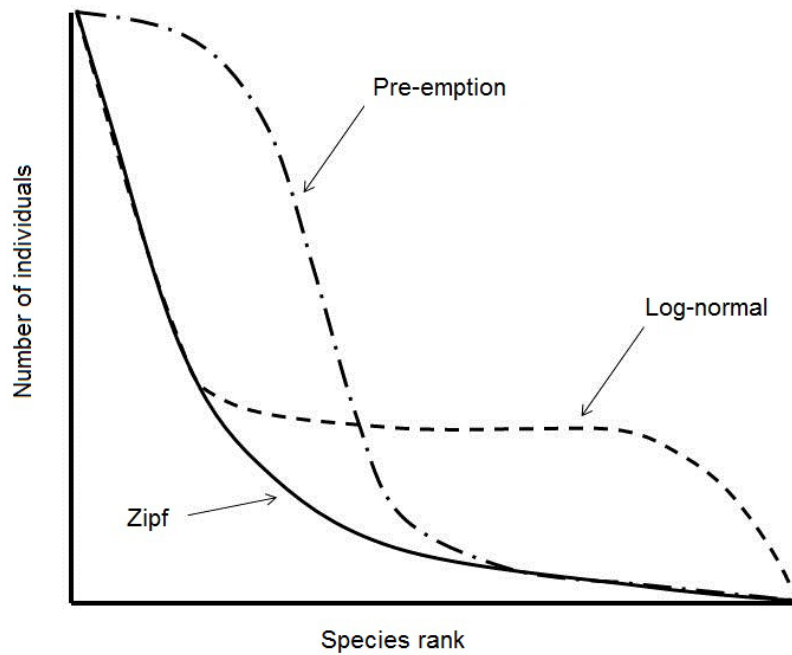


Fig. A2 Pre-emption, Log-normal and Zipf species abundance distribution models.

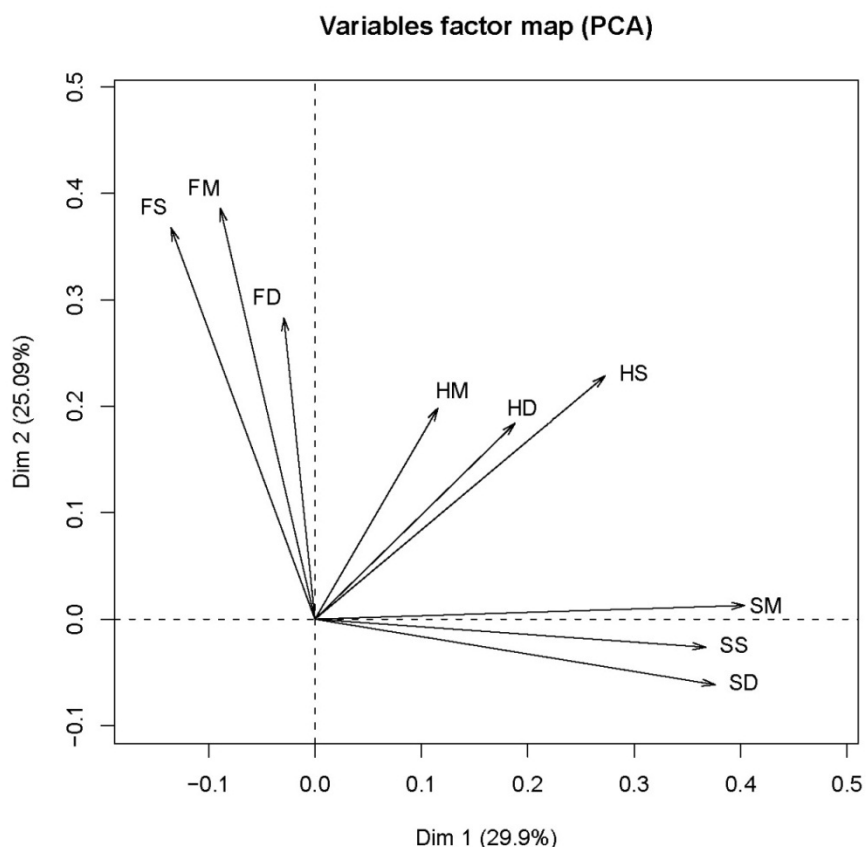


Fig. A3 Principal Component Analysis based on the presence and absence of species in the distinct patch type × plant community combinations. See caption in Fig. 1 for details of the abbreviations.

Table A1. Multiplicative β -diversity: final species richness in the accumulation curve/mean initial number of species from species accumulation curves.

patch type × plant community combination	β-diversity
<i>Dryas</i> patches in the <i>Salix</i> snowbed	2.446
<i>Dryas</i> patches in the <i>Dryas</i> heath	2.478
Moss patches in the fell-field	2.733
Moss patches in the <i>Salix</i> snowbed	2.761
<i>Salix</i> patches in the <i>Dryas</i> heath	2.814
Moss patches in the <i>Dryas</i> heath	2.992
<i>Salix</i> patches in the <i>Salix</i> snowbed	3.267
<i>Salix</i> patches in the fell-field	3.589
<i>Dryas</i> patches in the fell-field	5.678

Apèndix / Appendix

Altres publicacions / Other publications:

Recent changes in vegetation. R. Van Bogaert, D. Walker, G.J. Jia, O. Grau, M. Hallinger, M. De Dapper, C. Jonasson, T.V Callaghan (2007). *Arctic Report Card*, 32-39.

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How do bryophytes govern generative recruitment of vascular plants? (2011). N. A. Soudzilovskaia, B. Graae, J. Douma, O. Grau, A. Milbau, A. Shevtsova , L. Wolters & J.H.C. Cornelissen. *New Phytologist*, 190: 1019-1031.

Altitudinal species richness patterns of vascular plants in the south-eastern Pyrenees and nearby mountains of Catalonia (2012). O. Grau, J.M. Ninot, X. Font, & J.A. Grytnes. *Plant Ecology & Diversity*, DOI:10.1080/17550874.2012.666027.

Functional plant traits and species assemblage in Pyrenean snowbeds (2012). J.M. Ninot, O. Grau, E. Carrillo, R. Guàrdia, A. Lluent & E. Illa. *Folia Geobotanica*, DOI: 10.1007/s12224-012-9138-9