



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 (*Cladina* 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, *Cladina* 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 Bertson 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

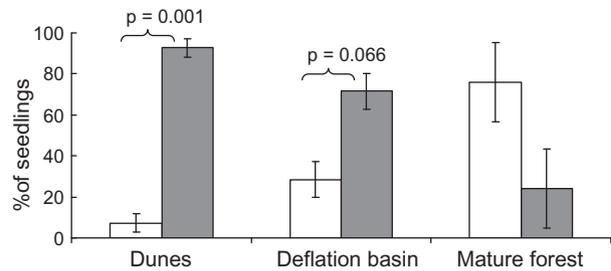


Figure 1. Proportion (%) of total population of Scots pine seedlings in either *Empetrum* (filled bars) and in empty patches (empty bars) along the successional stages. The bars represent the mean (\pm SE) of five locations. p-values above the bars indicate statistical difference according to paired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

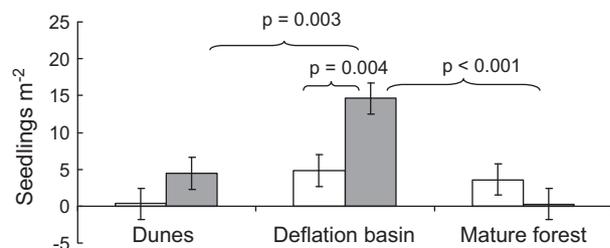


Figure 2. Number of Scots pine seedlings m⁻² in *Empetrum* patches (filled bars) and in empty patches (empty bars) along the successional stages. The bars represent the estimated marginal means (\pm SE), and p-values above the bars indicate statistical difference according to comparison of estimated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

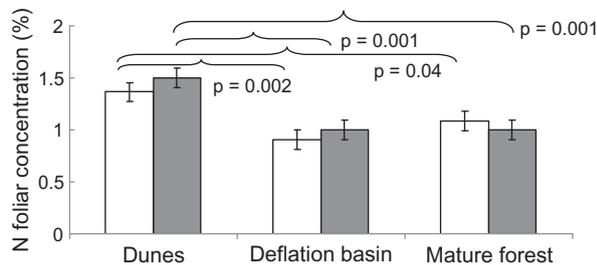


Figure 3. Foliar nitrogen concentration (% of needle dry weight) of Scots pine in *Empetrum* patches (filled bars) and in empty patches (empty bars) along the successional stages. For other details see Fig. 2.

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

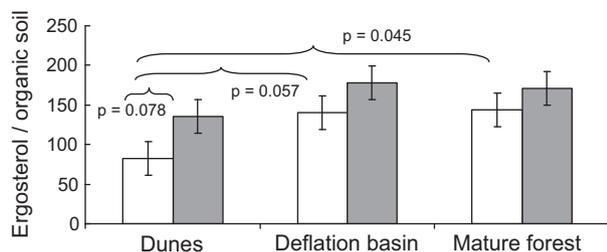


Figure 4. Soil fungal biomass (expressed as μg ergosterol g^{-1} organic matter) in *Empetrum* patches (filled bars) and in empty patches (empty bars) along the successional stages. For other details see Fig. 2.

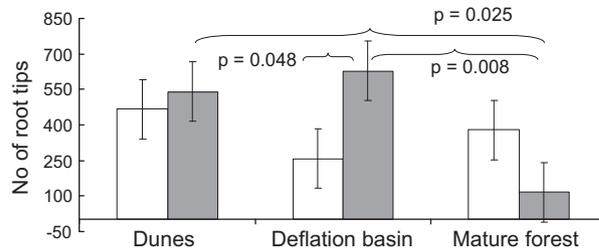


Figure 5. Number of Scots pine root tips colonised by morphotypes with abundant external mycelia (high C) morphotypes / root weight in *Empetrum* patches (filled bars) and in empty patches (empty bars) along the successional stages. For other details see Fig. 2.

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

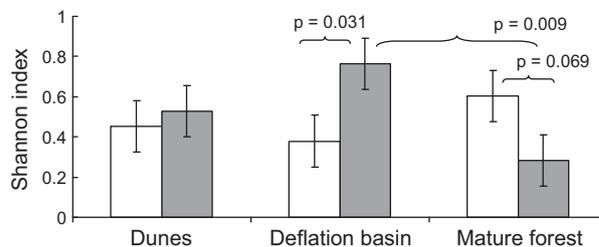


Figure 6. Mycorrhizal morphotype diversity (Shannon index) in Scots pine roots in *Empetrum* patches (filled bars) and in empty patches (empty bars) along the successional stages. For other details see Fig. 2.

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 × 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 × 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 hydrotilbene (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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References

- Agerer, R. 1987–1998. Colour Atlas of Ectomycorrhizae. – Einhorn Verlag, Schwäbisch Gmünd.
- Alestalo, R. 1979. Land uplift and development of the littoral and aeolian morphology on Hailuoto, Finland. – Acta Univ. Ouluensis Geol. A 82: 109–120.
- Antibus, R. and Sinsabaugh, R. 1993. The extraction and quantification of ergosterol from ectomycorrhizal fungi and roots. – Mycorrhiza 3: 137–144.
- Bertness, M. and Callaway, R. 1994. Positive interactions in communities. – Trends Ecol. Evol. 9: 191–193.
- Bertness, M. and Leonard, G. 1997. The role of positive interactions in communities: lessons from intertidal habitats. – Ecology 78: 1976–1989.
- Brooker, R. and Callaghan, T. 1998. The balance between positive and negative plant interactions and its relationship to environmental gradients: a model. – Oikos 81: 196–207.
- Bruno, J. et al. 2003. Inclusion of facilitation into ecological theory. – Trends Ecol. Evol. 18: 119–125.
- Callaway, R. and Walker, L. 1997. Competition and facilitation: a synthetic approach to interactions in plant communities. – Ecology 78: 1958–1965.
- Callaway, R. et al. 2002. Positive interactions among alpine plants increase with stress. – Nature 417: 844–848.
- Choler, P. et al. 2001. Facilitation and competition on gradients in alpine plant communities. – Ecology 82: 3295–3308.
- Connell, J. 1978. Diversity in tropical rain forests and coral reefs – high diversity of trees and corals is maintained only in a non-equilibrium state. – Science 199: 1302–1310.
- Dona, A. and Galen, C. 2007. Nurse effects of alpine willows (*Salix*) enhance over-winter survival at the upper range limit of fireweed, *Chamerion angustifolium*. – Arct. Antarct. Alp. Res. 39: 57–64.
- Eränen, J. and Kozlov, M. 2008. Increasing intraspecific facilitation in exposed environments: consistent results from mountain birch populations in two subarctic stress gradients. – Oikos 117: 1569–1577.
- Franks, S. and Peterson, C. 2003. Burial disturbance leads to facilitation among coastal dune plants. – Plant Ecol. 168: 13–21.
- Gilad, E. et al. 2007. Dynamics and spatial organization of plant communities in water-limited systems. – Theor. Popul. Biol. 72: 214–230.
- Godbold, D. and Berntson, G. 1997. Elevated atmospheric CO₂ concentration changes ectomycorrhizal morphotype assemblages in *Betula papyrifera*. – Tree Physiol. 17: 347–350.
- Goldenheim, W. et al. 2008. Switching from negative to positive density-dependence among populations of a cobble beach plant. – Oecologia 158: 473–483.
- Gorissen, A. and Kuyper, T. 2000. Fungal species-specific responses of ectomycorrhizal Scots pine (*Pinus sylvestris*) to elevated [CO₂]. – New Phytol. 146: 163–168.
- Gregoire, M. and Begin, Y. 1993. The recent development of a mixed shrub and conifer community on a rapidly emerging coast (eastern Hudson Bay, subarctic Quebec, Canada). – J. Coast. Res. 9: 924–933.
- Hämäläinen, L. et al. 1998. Retkeilykasvio (Field Flora of Finland). – Ed. Finn. Mus. Nat. Hist., Bot. Mus., Helsinki.
- Hellemaa, P. 1998. The development of coastal dunes and their vegetation in Finland. – Fennia 176.
- Jumpponen, A. et al. 1998. Effects of established willows on primary succession on Lyman Glacier forefront, North Cascade Range, Washington, USA: evidence for simultaneous canopy inhibition and soil facilitation. – Arct. Alp. Res. 30: 31–39.
- Klanderud, K. and Totland, O. 2004. Habitat dependent nurse effects of the dwarf-shrub *Dryas octopetala* on alpine and arctic plant community structure. – Écoscience 11: 410–420.
- Kuikka, K. et al. 2003. Severe defoliation of Scots pine reduces reproductive investment by ectomycorrhizal symbionts. – Ecology 84: 2051–2061.
- le Roux, P. and McGeoch, M. 2008. Changes in climate extremes, variability and signature on subantarctic Marion Island. – Climatic Change 86: 309–329.
- Mackey, R. and Currie, D. 2001. The diversity–disturbance relationship: is it generally strong and peaked? – Ecology 82: 3479–3492.
- Maestre, F. et al. 2005. Is the change of plant–plant interactions with abiotic stress predictable? A meta-analysis of field results in arid environments. – J. Ecol. 93: 748–757.
- Nara, K. 2006. Ectomycorrhizal networks and seedling establishment during early primary succession. – New Phytol. 169: 169–178.
- Nilsson, M. et al. 1993. Allelopathic effects by *Empetrum hermaphroditum* on development and nitrogen uptake by roots and mycorrhizae of *Pinus sylvestris*. – Can. J. Bot. 71: 620–628.
- Northup, R. et al. 1995. Polyphenol control of nitrogen release from pine litter. – Nature 377: 227–229.
- Nylund, J. and Wallander, H. 1992. Ergosterol analysis as a means of quantifying mycorrhizal biomass. – Meth. Microbiol. 24: 77–88.
- Peltola, A. and Ihalainen, A. 2006. Forest resources. – In: Peltola, A. (ed.), Finnish statistical yearbook of forestry. SVT, Agriculture, forestry and fishery. ISSN 1456-8268. pp. 33–74.
- Pennanen, T. et al. 2001. Microbial and plant community structure across a primary succession gradient. – Scand. J. For. Res. 16: 37–43.
- Read, D. 1992. The mycorrhizal mycelium. – In: Allen, M. F. (ed.), Mycorrhizal functioning. Chapman and Hall. pp. 102–133.
- Read, D. et al. 1989. The nitrogen nutrition of mycorrhizal fungi and their host plants. – In: Boddy, L. et al. (eds) Nitrogen, phosphorus and sulphur utilization by fungi. Cambridge Univ. Press. pp. 181–204.
- Ruotsalainen, A. et al. 2009. Mycorrhizal colonisation of mountain birch (*Betula pubescens* ssp. *czerepanovii*) along three

- environmental gradients: does life in harsh environments alter plant-fungal relationships? – *Environ. Monit. Assess.* 148: 215–232.
- Saikkonen, K. et al. 1999. Defoliation and mycorrhizal symbiosis: a functional balance between carbon sources and below-ground sinks. – *Ecol. Lett.* 2: 19–26.
- Saravesi, K. et al. 2008. Defoliation causes parallel temporal responses in a host tree and its fungal symbionts. – *Oecologia* 156: 117–123.
- Sousa, W. 1979. Experimental investigations of disturbance and ecological succession in a rocky inter-tidal algal community. – *Ecol. Monogr.* 49: 227–254.
- Sthultz, C. et al. 2007. Shifts from competition to facilitation between a foundation tree and a pioneer shrub across spatial and temporal scales in a semiarid woodland. – *New Phytol.* 173: 135–145.
- Tyrbirk, K. et al. 2000. Nordic *Empetrum* dominated ecosystems: function and susceptibility to environmental changes. – *Ambio* 29: 90–97.
- Valiela, I. 1995. *Marine ecological processes* (2nd ed.). – Springer.
- Vrålstad, T. et al. 2000. *Piceirhiza bicolorata* - the ectomycorrhizal expression of the *Hymenoscyphus ericae* aggregate? – *New Phytol.* 145: 549–563.
- Wallstedt, A. et al. 1997. A method to quantify the allelopathic compound batatasin-III in extracts from *Empetrum hermaphroditum* using gas chromatography: applied on extracts from leaves of different ages. – *J. Chem. Ecol.* 23: 2345–2355.
- Wardle, D. et al. 1998. An ecosystem-level perspective of allelopathy. – *Biol. Rev.* 73: 305–319.
- Zvereva, E. and Kozlov, M. 2004. Facilitative effects of top-canopy plants on four dwarf shrub species in habitats severely disturbed by pollution. – *J. Ecol.* 92: 288–296.