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Substantial compositional turnover of fungal communities in an alpine ridge-to-snowbed gradient

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Abstract

The main gradient in vascular plant, bryophyte and lichen species composition in alpine areas, structured by the topographic gradient from wind-exposed ridges to snowbeds, has been extensively studied. Tolerance to environmental stress, resulting from wind abrasion and desiccation towards windswept ridges or reduced growing season due to prolonged snow cover towards snowbeds, is an important ecological mechanism in this gradient. The extent to which belowground fungal communities are structured by the same topographic gradient and the eventual mechanisms involved are less well known. In this study, we analysed variation in fungal diversity and community composition associated with roots of the ectomycorrhizal plant *Bistorta vivipara* along the ridge-to-snowbed gradient. We collected root samples from fifty *B. vivipara* plants in ten plots in an alpine area in central Norway. The fungal communities were analysed using 454 pyrosequencing analyses of tag-encoded ITS1 amplicons. A distinct gradient in the fungal community composition was found that coincided with variation from ridge to snowbeds. This gradient was paralleled by change in soil content of carbon, nitrogen and phosphorus. A large proportion (66%) of the detected 801 nonsingleton operational taxonomic units (OTUs) were ascomycetes, while basidiomycetes dominated quantitatively (i.e. with respect to number of reads). Numerous fungal OTUs, many with taxonomic affinity to Sebacinales, *Cortinarius* and *Meliniomyces*, showed distinct affinities either to ridge or to snowbed plots, indicating habitat specialization. The compositional turnover of fungal communities along the gradient was not paralleled by a gradient in species richness.

Keywords: *Bistorta vivipara*, ectomycorrhizae, high-throughput sequencing, microbial ecology, ridge-to-snowbed gradient, root-associated fungi

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Introduction

There is a strong relationship between the local topographic gradient from ridge-to-snowbed and the species composition of vascular plants, bryophytes and lichens in alpine areas (Gjærevoll 1956; Dahl 1957; Økland & Bendiksen 1985; Odland & Munkejord 2008). The vegetation gradient is shaped by uneven distribution of snow cover in winter over years, brought about by strong winds (Vestergren 1902; Dahl 1957). Species are distributed along the ridge-to-snowbed gradient by their tolerance to environmental stress. The risk of frost, wind abrasion and desiccation increases towards the windswept ridges, where as the snow cover in the snowbeds provides more stable conditions during winters. Biological activity, such as growth of fungal hyphae has been detected under snow-covered sites in alpine areas (Kuhnert *et al.* 2012). Between these

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extreme habitats, the lee sides provide optimal habitats for many species with snow protection in winter, which melts out early in the summer (Wijk 1986). Important environmental complex gradients such as soil moisture supply, soil organic content, soil reaction, soil stability and concentrations of important elements may covary with parts of the ridge-to-snowbed gradient, depending on local conditions (Gjærevoll 1956; Dahl 1957; Baadsvik 1974; Økland & Bendiksen 1985; Odland & Munkejord 2008).

The mycorrhizal symbiosis between fungi and plant roots is among the most ancient and prevalent eukaryotic symbioses on Earth, crucial for the composition and functioning of terrestrial ecosystems. Ectomycorrhizae (ECM) is most common in woody plants and is highly important for plant productivity in boreal and temperate forests. Nutrient availability in these ecosystems is usually low, and most of the nutrients are present in organic form in litter and humus (Vestergren 1902; Dahl 1957; Smith & Read 2008). Several recent studies have demonstrated high diversity of ECM fungi in alpine and arctic ecosystems (Bjorbækmo *et al.* 2010; Geml *et al.* 2011; Blaalid *et al.* 2012). Most ECM-forming plants are trees and shrubs, and due to the size of their root systems, it is difficult to explore their entire root-associated fungal assemblages. However, a few herbs, including the circumpolar perennial plant *Bistorta vivipara* (L.) Delarbre (syn. *Polygonum viviparum* L.) in family Polygonaceae, form ECM (e.g. Resvoll 1917; Nordhagen 1943; Dahl 1957; Read & Haselwandter 1981). The small and condensed root system of *B. vivipara* allows the entire fungal community associated with each plant to be sampled and analysed (Blaalid *et al.* 2012; Kauserud *et al.* 2012). *B. vivipara* produces an underground rhizome that grows with age (Diggle 1997), from where the finer root threads are attached. *B. vivipara* has a wide ecological amplitude and often occurs as a pioneer species in arctic and alpine environments (Dormann *et al.* 2002).

The structure and composition of fungal communities are influenced by numerous factors and complex interactions. Toljander *et al.* (2006) found that pronounced changes in ECM fungal communities along an environmental gradient in a boreal forest in Sweden were strongly correlated with various soil properties. For example, extractable NH_4 was a strong determinant of the ECM community, the composition of which was also related to moisture availability (Toljander *et al.* 2006). Similarly, variation in composition of soil fungal communities in an alpine habitat was structured according to vegetation types and degree of soil organic matter (Zinger *et al.* 2009, 2010). Several studies (e.g. (Zinger *et al.* 2010; Newbound *et al.* 2012) have reported a strong structuring effect of pH on fungal community composition. How the below ground fungal communities are influenced and possibly structured by processes that give rise to the strong ridge-to-snowbed vegetation gradient for plants and lichens is not yet known.

Studying ecological factors that underlie the dynamics of natural microbial communities remain a challenge because of the high taxonomic diversity in such communities (Hawksworth 2001). The availability of high-throughput sequencing (HTS) technologies has initiated a new era of research on fungal ecology and enabled extensive studies of complex fungal communities (Wallander *et al.* 2010; Kauserud *et al.* 2012; Clemmensen *et al.* 2013). HTS analyses of the nuclear ribosomal internal transcribed spacer (ITS) region, which has been adopted as a validated DNA barcode marker for fungal species identification (Seifert 2009; Schoch *et al.* 2012), provide a powerful tool for studying fungal diversity in environmental samples.

Our aim was to explore fungal communities of ectomycorrhizal root systems of *B. vivipara* in a ridge-to-snowbed gradient using 454 high-throughput sequencing of the ITS 1 region. We wanted to test (i) if there are differences in the composition and diversity of fungi associated with *B. vivipara* between ridge and snowbed, (ii) if the fungal communities are spatially structured across sampling sites, independent of vegetation type and (iii) if soil nutrient concentrations affect the diversity and composition of fungi associated with *B. vivipara* roots.

Materials and methods

Sampling

The study area (60°35′N, 007°30′E, 1229 -1244 m above sea level) was located in the mid-alpine region at Finse, Hordaland, Norway. All samples were collected during a two-day period in July 2011. Five 1.5 m × 1.5 m plots were placed within each of ridge and snowbed. A grid with mesh width of 15 cm was superimposed on each plot. The 81 (9 × 9) grid-line intercepts were used as a point frame for cover estimation by the point intercept method (Bråthen & Hagberg 2009): at each grid-line intercept, the species (or, alternatively, stone or bare soil) first touched by a pin passed vertically through the vegetation were given a score of one. Species present in the plot, but not registered by the point intercept method were assigned a score of 0.5. Within each plot, the *B. vivipara* plant situated closest to each of five predefined positions was sampled: one at the intersection between the two diagonals and four halfway between the intersection of the diagonals and each corner. Soil samples were collected under each plant. A total of 50 plants and 50 soil samples were collected. The soil samples were stored at –18 °C within three hours after

collection. The root system of each plant was carefully washed to remove all soil and plant debris and placed in 50-mL BD Falcon™ tubes (BD Bioscience, San Jose) before fresh weight of the root system was recorded. To each tube, 1200 μL CTAB-lysis buffer (AppliChem GmbH, Ottoweg, Germany) was added before storage at –18 °C. Lengths, widths and heights of the plant rhizomes were measured.

DNA extraction and 454 sequencing

After addition of 10 6.2 mm Ceramic beads (M.P. Biomedicals, CA, USA) and additional 5 mL 2% CTAB buffer with 2-mercaptoethanol (Sigma Chemical Co, Steinheim, Germany), all 50 root systems were crushed for 60 s at 4.0 m/s on a Fast Prep-24 beadbeater (M.P. Biomedicals) and centrifuged at 1300 rpm for 30 s. Two samples (R1-73 and R2-63) were crushed for additional 60 s (to be properly crushed) and centrifuged again. From each tube, 2 mL of the aquatic phase was frozen at –18 °C. The samples were randomized before DNA extraction to reduce methodological biases. Five randomly selected samples were run in parallel as replicates to test for methodological biases (see Kauserud *et al.* 2012). DNA extraction using 600 μL of the crushed material was performed with the Soil DNA isolation Kit (OMEGA Bio-tek, Norcross, GA, USA) according to the manufacturer's directions.

The ITS1 region was amplified by subjecting 2 μL DNA of each sample to a nested PCR approach, as outlined in Blaalid *et al.* (2012). A nested approach was chosen in order to generate highly replicable results (see Kauserud *et al.* 2012) and to avoid biases known to occur in barcode ligation approaches (Gillevet *et al.* 2010). However, both rounds of PCR might exclude certain fungal groups, such as Tulasnellaceae due to primer biases (Taylor & McCormick 2008; Bellemain *et al.* 2010). In the first PCR, the fungal-specific primers ITS1F and ITS4 were used to amplify the entire ITS region (White *et al.* 1990; Gardes & Bruns 1993) using the following PCR protocol: denaturation for 30 s at 98 °C, followed by 30 cycles of denaturation for 10 s at 98 °C, annealing for 20 s at 50 °C and extension for 20 s at 70 °C, and then followed by a step of final extension for 7 min at 70 °C and cool-down at 10 °C. Subsequently, the ITS1 region was amplified using the primers ITS2 and ITS5 (White *et al.* 1990) with 4 μL 20× diluted template from the first PCR. In the second PCR, samples were tagged in both ends by different pyrotags with a length of 10 bp. The same PCR protocol was used. We ran all PCR reactions in triplicates and pooled them to level out tentative stochastic PCR biases. The resulting PCR products were cleaned up by using the Wizard® SV Gel and PCR Clean-Up System kit (Promega, Madison, WI, USA) and normalized using the SequalPrep™ Normalization Plate (96) kit (Invitrogen Inc., CA, USA). The PCR products were pyrosequenced in four lanes on a half 454 plate (Roche GS FLX Titanium Series) at the Norwegian Sequencing Center (University of Oslo, Norway).

Soil sample analyses

We thawed the soil samples before sieving them using sterile milliQ water to remove plant roots and debris. The remaining soil was then dried at 60 °C. The concentration of phosphorus (P) was measured by the potassium persulfate ($K_2S_2O_8$) extraction method. Samples were placed in 10 ml 1% $K_2S_2O_8$ at 121 °C for 30 min, and the extract then analysed using a Bran Luebbe autoanalyzer (Bran Luebbe, Norderstedt Germany) with the Multitest MT method (no. G-297-03). The concentration of soil carbon (C) and nitrogen (N) was measured by a Thermo Finnigan EA 1112 Series Flash Elemental Analyzer (Thermo Scientific, Italy).

Bioinformatics analyses

We analysed the read data using QIIME v. 1.5.0 (Caporaso *et al.* 2010). Reads with length <250 bp or >500 bp, an average phred quality score <50 and/or any mismatch against the tags or ITS1 primers, were removed. Denoiser v. 1.5.0 (Reeder & Knight 2010), as implemented in QIIME v. 1.5.0, was used to denoise the remaining 151,642 reads. The denoised reads were clustered into operational taxonomic units (OTUs) using a 97% similarity threshold and the uclust algorithm as implemented in QIIME v. 1.5. The most abundant read in each cluster will be referred to as the representative sequence. Putative chimeras were identified and removed based on: (i) being identified as chimeric by the perseus algorithm as implemented in mothur v. 1.26.0 (Schloss *et al.* 2009), (ii) having a top BLAST match with <90% coverage and <90% identity to a known fungal sequence and (iii) not occurring independently in more than one sample. OTUs represented by a single read (singletons) and OTUs detected in the negative control were also removed from the data set. The representative sequence from each of the remaining OTUs were submitted to BLASTn (Altschul *et al.* 1997) for comparison against the GenBank nonredundant (NCBI-nr) database. OTUs with best matches to nonfungal accessions were removed. Moreover, only one randomly chosen sample from each of the replicated pairs (S2.78.2, R1.55, R3.68, R4.45 and R5.32.2) was included in the further analyses. The raw data have been accessioned in Dryad, accession doi:10.5061/dryad.216tp.

Statistical analyses

All statistical analyses were made after transformation to binary (presence/absence) data. EstimateS v. 7.5.2 (Colwell 2009) was used to calculate the shared number of OTUs in each pair of samples. We tested whether the OTUs were significantly associated with either ridge or snowbed using the *G*-test as implemented in QIIME v. 1.5.0, with the null hypothesis that each OTU was evenly distributed among groups. Patterns of OTU richness and community composition were analysed using the R software v. 2.15.2 (R Core Team 2012). Accumulation curves for OTU richness versus sampling effort and estimates of total OTU richness were calculated according to (Ugland *et al.* 2003), as implemented in the R package vegan (Oksanen *et al.* 2012). GLM was used to relate OTU richness to environmental variables and rhizome characteristics. In order to test if larger rhizomes and root systems inhabit more fungal OTUs and number of fungal reads, correlation tests were performed in R. Tests were run using the cor.test() function with Pearson method between rhizome size (rhizome length × rhizome height × rhizome width), rhizome length, rhizome height, rhizome width as well as root system weight against number of OTUs and reads.

Two-dimensional global nonmetric multidimensional scaling (GNMDS; (Kruskal 1964; Minchin 1987) and detrended correspondence analysis (DCA; (Hill 1979; Hill & Gauch 1980) ordinations were performed in R, using the R packages vegan and MASS (Venables & Ripley 2002). The GNMDS was run with the following options and settings: number of axes = 2, number of random starts = 100; Bray–Curtis dissimilarities [(Bray & Curtis 1957), which for qualitative data reduces to Sørensen's index (Sørensen 1948)], maximum number of iterations = 1000, stress convergence criterion = 10^{-7}. The minimum stress solution, found from more than one random start, was used after postprocessing by which axes were rotated to principal components and rescaled to half-change (H.C.) units of compositional turnover. DCA was run with default settings. Correspondence between DCA and GNMDS axes was assessed using Kendall's rank correlation coefficients, calculated between axis pairs. Environmental variables and size and weight of plant rhizomes were fit into the GNMDS ordination diagram by use of the envfit() command of vegan. An ANOVA was run to test if the GNMDS scores obtained for each vegetation type along both GNMDS axes showed any sign of spatial structure within the ordination.

An OTU/sample matrix containing only known ECM fungi (Tedersoo *et al.* 2010) was extracted from the full data matrix and analysed separately. A GNMDS analysis was run, using the above settings, along with a *t*-test where the number of ECM OTUs per root system was compared across ridge and snowbeds.

The original vegetation scores obtained from the pin intercept method, measured on a scale of 0.5–56, were transformed to a new scale of 1–8 by use of the following formula $y = 1.33(x^{0.411})$, as recommended by Eilertsen *et al.* (1990) and successfully implemented by Birkeland (2012). y gives the new weighted value, 1.33 is the abundance scale and 0.411 the weighting parameter. The weighted vegetation scores were used to run a GNMDS with three axes, using the same conditions as for the GNMDS of fungal OTUs, as well as a DCA with default settings. Correspondence of the GNMDS and DCA axes was assessed using Kendall's rank correlation coefficients, calculated between axis pairs. The correspondence of GNMDS axes of vegetation data and fungal communities was tested using a Procrustes test in R with the package vegan. To test whether the concentrations of N, C and P in soil were significantly different between the sample types, a split-plot GLM (Crawley 2007) was run.

Results

Vegetation and soil characteristics of ridges and snowbeds

Altogether, 47 plant species were recorded in the ten plots, of which six were bryophytes, 32 vascular plants and nine lichen species. Thirty-two species were recorded in the ridge plots and 38 species in the snowbed plots. The weighted data of the vegetation cover showed a clear dominance of lichens in the ridge plots (28.4%), compared to the snowbeds (6.6%). *Salix herbacea* dominated the vegetation in the snowbeds (16.0%), whereas it was less numerous in the ridge plots (5.4%; Fig. S1, Supporting information). The GNMDS analysis of the vegetation data separated the snowbed and ridge plots along axis one (Fig. 1a). The average concentration of C in soil was 9.9% (by weight) in the ridge plots, whereas it was 32.6% in the snowbed plots. Correspondingly, the average concentration of N was 0.6% in ridge plots and 2.2% in snowbed plots. The average concentration of P was 1.4% in ridge plots and 1.1% in snowbed plots. The concentrations of C and N were significantly different in the ridge and the snowbed plots ($P < 0.05$).

Data set properties

We obtained 191,099 reads of which 151,642 were retained after filtering. Using a 97% read similarity cut-off, the reads clustered into 1,172 OTUs. Fifty-six of the OTUs were identified as chimeras and removed from

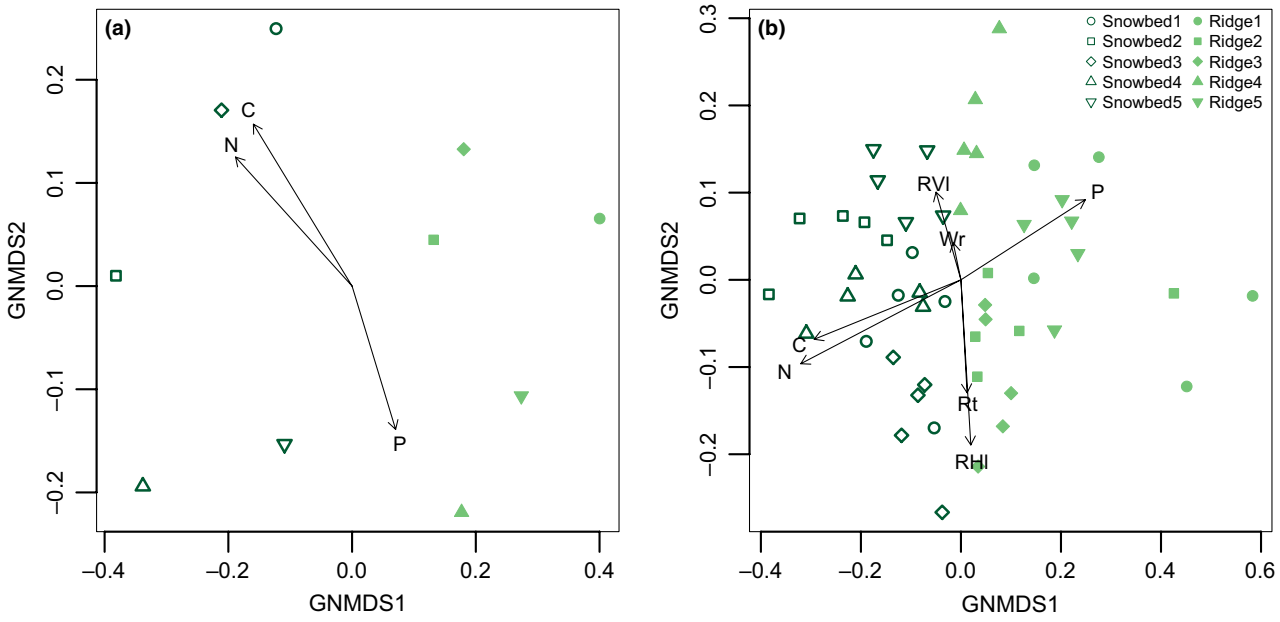

Fig. 1 (a) GNMDS ordination based on weighted data of vegetation cover within five sampled 1.5 m × 1.5 m plots of snowbed (dark green open symbols) and ridge (light green closed symbols). C, N and P give the concentration of carbon, nitrogen and phosphor contents in the soil. (b) GNMDS ordination of the fungal communities associated with *Bistorta vivipara* roots systems of the ridge samples (light green solid symbols) and snowbed samples (dark green open symbols) based on presence/absence data of fungal OTUs. C, N and P give the concentration of carbon, nitrogen and phosphor contents in the soil. RHl, RVl and Rt give the horizontal length, vertical length and thickness of the rhizome; while Wr gives the weight of the root system.

the data set. After removal of 298 singleton OTUs, six OTUs appearing in the negative control, and 11 OTUs that had best BLAST match against nonfungal reads, 801 nonsingleton OTUs remained in the data set that was subjected to further analyses. Samples run in parallel as methodological replicates were more similar with respect to OTU composition than between-sample comparisons (Fig. S2, Supporting information).

Fungal richness

The accumulation curves of fungal OTU richness (Fig. 2) levelled off, indicating that a large part of the pool of fungal species associated with *B. vivipara* root systems within the study area was included in our sample. No significant difference in observed or estimated fungal OTU richness was found between ridge and snowbed (Table 1). In samples from ridge, on average, 83 OTUs were found per sample (range: 13–132), while in snowbeds, the average number of OTUs per sample was 93 (range: 59–137; Fig. S3, Supporting information). OTU richness per root system was significantly correlated with the concentration of P in soil (Table S1, Supporting information). No significant relationship was found between OTU richness per root system and C and N soil contents, plot type, rhizome size or root weight. A significant negative correlation was found

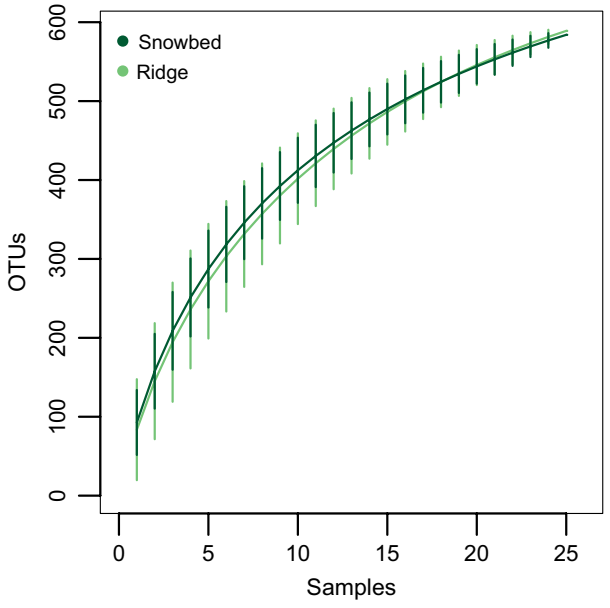

Fig. 2 Accumulation curves of fungal OTU richness associated with *Bistorta vivipara* root systems for the ridge (light green) and snowbed (dark green) samples.

between number of OTUs and rhizome size in snowbeds, but not in ridge plots (Table 2). No significant correlations were found between number of OTUs and

Table 1 Observed and estimated fungal species (OTU) richness associated with *Bistorta vivipara* root systems in snowbed and ridge. Richness estimates are given in (1) order jackknife (and corresponding confidence intervals), (2) order jackkinfe, as well as bootstrap (with corresponding confidence intervals)

	Observed	Chao	(1) Order jackknife	(2) Order jackknife	Bootstrap
Snowbed	584	722.1 ± 27.0	755.84 ± 41.7	818.25	666.90 ± 23.4
Ridge	589	718.4 ± 24.5	722.40 ± 43.5	823.77	679.43 ± 26.4

Table 2 Correlation tests (with corresponding *P*-values) between number of OTUs or number of reads of the fungal communities and rhizome measurements and root weight of the associated *Bistorta vivipara* plant. Rhizome size is calculated as: rhizome length × rhizome height × rhizome width. The variability gives the minimum and maximum sizes as well as the average (in brackets)

	Snowbed			Ridge		
	Variability	No. of OTUs	No. of reads	Variability	No. of OTUs	No. of reads
Rhizome size (cm^3)	0.25–4.31 (1.61)	**−0.432 (*P* = 0.035)**	0.128 (*P* = 0.551)	0.49–4.17 (1.40)	0.017 (*P* = 0.938)	0.238 (*P* = 0.252)
Rhizome length (cm)	0.47–2.13 (1.06)	−0.029 (*P* = 0.893)	0.229 (*P* = 0.281)	0.50–2.59 (1.25)	0.264 (*P* = 0.203)	0.303 (*P* = 0.141)
Rhizome width (cm)	0.46–0.86 (0.65)	−0.251 (*P* = 0.237)	0.207 (*P* = 0.331)	0.49–1.62 (0.70)	0.011 (*P* = 0.958)	**0.668 (*P* < 0.001)**
Rhizome height (cm)	0.73–6.81 (2.35)	−0.372 (*P* = 0.074)	0.053 (*P* = 0.806)	0.49–2.66 (1.62)	−0.193 (*P* = 0.355)	−0.050 (*P* = 0.811)
Root weight (g)	0.01–0.17 (0.07)	−0.297 (*P* = 0.159)	0.124 (*P* = 0.564)	0.01–0.15 (0.06)	0.098 (*P* = 0.648)	0.130 (*P* = 0.544)

Bold numbers indicate significant $P < 0.05$.

root weight or between number of reads and rhizome size and root weight in any of the habitat types, except for a significant positive correlation between rhizome width and number of reads in the ridge plots. The number of ECM OTUs was significantly higher in snowbeds (26.3 in average) than in the ridges (14.8 in average, *t*-test *P*-value < 0.001) (Fig. S4, Supporting information).

Taxonomic composition

The taxonomic distribution of detected fungal OTUs is summarized in Table 3, and the 15 most common OTUs are listed in Table 4. Most OTUs (66.5%) belonged to Ascomycota and the order Helotiales (52.7%). Only 25.4% of the OTUs belonged to Basidiomycetes, of which most (23.2%) belonged to Agaricomycetes. Other fungal groups, including Glomeromycota, Zygomycota and Chytridiomycota, only comprised 1.9% of the OTUs. Summarizing the total number of reads across taxonomy (Table 3) showed that Basidomycota (65.7%), class Agaricomycetes (64.7%), was quantitatively most abundant. While Russulales only accounted for 2.6% of the OTUs, as many as 26.9% of the obtained reads were affiliated with this order. Conversely, the ascomycetous groups were far less abundant when recorded as number of reads. Although not significantly different (chi-square tests, $P > 0.05$), the Basidiomycetes and Agaricomycetes were relatively more abundant in snowbed than in ridge plots (Table 3). The *G*-test revealed that some OTUs were affiliated with either ridge or snowbed (Table S2).

Fungal community composition

The most commonly encountered OTU (OTU ID 1089 with the best match to *Articulospora* sp.; GenBank Accession no JN995644) appeared in all root systems, while a high proportion of the OTUs (20.6%) was detected in only one single root system (Fig. S3c). The fungal community composition differed highly between root systems: on average, only 22 of the 801 (2.7%) OTUs were shared in pairwise comparisons across all samples. However, both the GNMDS and DCA ordination analyses based on presence/absence data revealed clear differences in fungal community composition between ridge and snowbeds, both for the data set containing all fungal OTUs (Fig. 1b; DCA not shown) as well as for the data set containing only known ECM-forming fungi (data not shown). The GNMDS ordination axes 1 and 2 were strongly correlated with the corresponding DCA axes 1 and 2 (Kendall's $\tau = 0.88$ and 0.54, respectively). This is a strong argument for the existence of strong compositional structure in the

Table 3 Summary of the distribution of OTUs and reads of fungal lineages found in the root systems of *Bistorta vivipara*. The first two columns show the distribution (%) of OTUs and reads over all samples, while the last four show the distribution in the ridge and snowbed plots, respectively

	Total		Snowbed		Ridge	
Taxonomic group	% OTUs	% reads	% OTUs	% reads	% OTUs	% reads
Ascomycota	66.47	32.47	33.66	16.24	32.8	16.23
Leotiomycetes	54.54	27.75	26.93	12.82	27.61	14.94
Helotiales	52.75	26.63	26.18	12.46	26.57	14.17
Rhytismatales	1.02	0.97	0.52	0.33	0.5	0.64
Eurotiomycetes	1.27	0.13	0.61	0.05	0.66	0.08
Chaetothyriales	1.16	0.11	0.59	0.05	0.57	0.06
Dothideomycetes	1.18	1.97	0.79	1.68	0.39	0.29
Sordariomycetes	1.13	0.37	0.5	0.29	0.64	0.08
Basidiomycota	25.43	65.72	14.95	37.72	10.48	28
Agaricomycetes	23.21	64.67	13.61	36.74	9.6	27.93
Agaricales	9.46	19.61	5.94	12.99	3.52	6.62
Thelephorales	5.13	12.74	3.99	7.06	1.13	5.68
Sebacinales	2.99	3.76	0.79	1.2	2.2	2.56
Russulales	2.59	26.91	1.52	14.85	1.07	12.06
Tremellomycetes	1.13	0.77	0.64	0.71	0.5	0.05
Other fungal divisions	2.75	0.59	1.43	0.3	1.31	0.29
Glomeromycota	0.98	0.35	0.48	0.14	0.5	0.22
Fungi spp.	5.35	1.22	2.54	0.54	2.81	0.68

Table 4 The 15 most common OTUs found in the 50 root samples of *Bistorta vivipara*. Top hit in GenBank gives the best match of the representative sequences to NCBI GenBank, with accession number in bracket. Cov (Query coverage) gives the percentage of sequence match against the top hit in GenBank. Iden (Identity) gives the sequence similarity to the top hit. N_A, N_R and N_S give the number of samples in which the OTU was observed across all samples, ridge samples and snowbed samples, respectively. R_A (%), R_R (%) and R_S (%) give the percentage of reads that clustered as the OTU across all samples, ridge samples and snowbed samples, respectively. Only one OTU was found in all 50 samples, with the best match to *Articulospora*. *Russula* sp. was the most abundant OUT found, constituting 24.6% of all reads

OTU ID	Top hit in GenBank (Accession nos)	Cov	Iden	N_A	N_R	N_S	R_A (%)	R_R (%)	R_S (%)
1089	*Articulospora* sp.(JN995644)	95	99	50	25	25	5.77	3.2	2.6
444	*Helotiales sp.* (AB598104)*	96	98	43	25	18	2.09	1.6	0.49
858	*Meliniomyces* sp. (HQ157926)	95	99	41	20	21	1.02	0.18	0.84
547	*Articulospora* sp. (EU998923)	95	99	38	15	23	1.16	0.38	0.78
383	*Articulospora* sp. (EU998928)	96	98	37	17	20	1.84	0.65	1.19
452	*Articulospora tetracladia* (EU998923)	95	93	37	16	21	0.21	0.1	0.11
918	Helotiales sp. (EU998923)†	95	96	37	19	18	0.98	0.5	0.48
1059	*Gyoerffyella* sp. (EU998923)	95	100	37	13	24	0.34	0.14	0.2
494	*Phialocephala* sp. (JQ272456)	96	92	36	18	18	0.18	0.09	0.09
75	*Russula* sp. (AY061696)	96	99	35	16	19	24.64	11.36	13.28
334	*Cortin. diasemospermus* (AY061696)	96	100	33	11	22	12.4	2.6	9.8
181	*Phialocephala fortinii* (EU882733)	96	99	32	14	18	0.12	0.05	0.07
376	*Meliniomyces* bicolor (HQ157926)	95	96	29	9	20	0.08	0.01	0.07
1057	Helotiales sp. (HQ157926)‡	95	98	29	17	12	0.4	0.21	0.18
1062	Helotiales sp. (AB598104)§	96	90	29	17	12	0.38	0.22	0.16

Best match at species level: **Leptodontium elatius* (acc.no. JF340290, Cov = 95%, Iden = 96%), †*Phialea strobilina* (acc.no. EF596821, Cov = 96%, Iden = 88%), ‡*Leptodontium elatius* (acc.no. JF340290, Cov = 95%, Iden = 96%), §*Leptodontium elatius* (acc.no. JF340290, Cov = 95%, Iden = 90%.

data set. Even though the amount of compositional turnover along the axes was small (gradient lengths of 1.0 and 0.6 H.C. units were obtained for GNMDS axes 1 and 2, respectively), plots from ridge and snowbed were fully separated along the first GNMDS axis (Fig. 1a). Moreover, samples originating from the same

Table 5 Split-plot GLM analyses where GNMDS axes 1 and 2 (Fig. 1) are related to environmental factors (C, N and P), plant rhizome characteristics, root weigth and interaction effects (for example, C*Veg). V gives the vegetation types (snowbed or ridge). N, C and P give the nitrogen, carbon and phosphorus contents of the soil, respectively. RHl gives the horizontal length of the rhizome, RVl the vertical length of the rhizome, Rt the rhizome thickness and Wr the weight of the root system. Numbers in bold give factors that are significant

	Between veg types				Between plots within veg types				Between plots			
	SS = 1.089				SS = 0.3682				SS = 0.389			
	df = 1				df = 8				df = 40			
GNMDS1	Ssexp	Coef	*F*	*P*	Ssexp	Coef	*F*	*P*	Ssexp	Coef	*F*	*P*
N	0.005	0.014	0.291	0.592	0.011	0.028	0.222	0.652	0.000	−0.006	0.037	0.848
C	0.011	0.001	0.660	0.421	0.017	0.002	0.335	0.581	0.000	0.000	0.014	0.905
P	**0.120**	**0.090**	**8.814**	**0.005**	0.120	0.132	3.403	0.108	0.022	0.053	2.373	0.132
C*Veg	**0.125**	**0.009**	**9.267**	**0.004**	0.113	0.013	2.857	0.142	0.024	0.006	2.508	0.122
N*Veg	**0.118**	**0.142**	**8.511**	**0.005**	0.103	0.185	2.445	0.169	0.021	0.089	2.136	0.152
N*C	**0.105**	**0.004**	**7.573**	**0.009**	0.085	0.005	1.669	0.253	0.019	0.003	1.984	0.167
RHl	0.020	−0.045	1.244	0.271	0.049	−0.162	1.087	0.332	0.003	−0.018	0.246	0.623
P*V	0.032	−0.133	2.449	0.124	0.001	−0.044	0.018	0.898	0.024	−0.134	2.669	0.111
P*C	0.021	−0.004	1.584	0.215	0.015	−0.008	0.357	0.576	0.007	−0.003	0.771	0.386
C*N*V	0.027	0.008	1.943	0.171	0.023	0.031	0.236	0.675	0.004	0.004	0.420	0.521
P*N	0.028	−0.075	2.147	0.150	0.023	−0.164	0.542	0.495	0.009	−0.050	0.957	0.334
RVl	0.023	0.019	1.431	0.238	0.021	0.028	0.422	0.537	0.006	0.013	0.570	0.455
Rt	0.009	−0.076	0.564	0.456	0.036	−0.294	0.776	0.407	0.000	0.004	0.001	0.970
Wr	0.002	−0.141	0.099	0.755	0.005	−0.423	0.088	0.776	0.000	−0.016	0.001	0.970
	Between Veg types				Between plots within veg types				Between plots			
	SS = 0.0108				SS = 0.4429				SS = 0.1771			
	df = 1				df = 8				df = 40			
GNMDS2	Ssexp	Coef	*F*	*P*	Ssexp	Coef	*F*	*P*	Ssexp	Coef	*F*	*P*
N	0.000	−0.003	0.023	0.881	0.003	0.014	0.042	0.843	0.008	−0.027	1.734	0.196
C	0.000	0.000	0.006	0.938	0.009	0.002	0.152	0.708	0.008	−0.002	1.844	0.182
P	0.002	0.011	0.126	0.724	0.000	−0.006	0.004	0.950	0.005	0.025	1.133	0.294
C*Veg	**0.067**	**−0.007**	**5.537**	**0.023**	0.205	−0.017	5.371	0.060	0.004	0.002	0.922	0.343
N*Veg	**0.082**	**−0.119**	**7.027**	**0.011**	**0.234**	**−0.278**	**6.818**	**0.040**	0.005	0.044	1.140	0.292
N*C	**0.080**	**−0.004**	**6.794**	**0.012**	**0.249**	**−0.009**	**8.831**	**0.031**	0.005	0.001	1.013	0.321
RHl	**0.070**	**−0.084**	**6.084**	**0.017**	0.041	0.040	0.736	0.419	0.000	0.001	0.003	0.958
P*V	0.001	0.024	0.079	0.780	0.001	−0.036	0.007	0.936	0.001	0.030	0.265	0.610
P*C	0.012	−0.003	0.851	0.361	0.100	−0.020	1.497	0.276	0.000	0.000	0.000	0.997
C*N*V	0.019	−0.006	1.585	0.215	0.005	−0.014	0.310	0.634	0.000	0.000	0.000	0.993
P*N	0.008	−0.040	0.607	0.440	0.109	−0.360	1.638	0.257	0.000	0.008	0.047	0.830
RVl	0.018	0.017	1.428	0.238	0.041	0.040	0.736	0.419	0.000	0.001	0.003	0.958
Rt	0.031	−0.142	2.544	0.118	0.077	−0.430	1.501	0.260	0.001	−0.036	0.356	0.554
Wr	0.003	0.175	0.193	0.663	0.005	−0.423	0.088	0.776	0.000	−0.016	0.001	0.970

plot were to some extent clustered in the ordination diagram. The ANOVA of spatial structure within the ordination showed that snowbed plots were not spatially structured along GNMDS axis 1, but were spatially structured along axis 2. The ridge plots were spatially structured along both GNMDS axes ($P < 0.05$). Procrustes analyses showed high correspondence between fungal OTU composition and vegetation in the sampled plots (Fig. S5), $r = 0.9125$, $P = 0.001$. The GNMDS axes 1 and 2 for the vegetation data were highly correlated with the GNMDS axes 1 and 2 for the fungal data (Kendall's $\tau = 0.78$ and 0.65, respectively).

Concentrations of soil P showed a significant correlation with the first GNMDS axis between the vegetation types (Table 5). Moreover, the interaction terms between vegetation type and C or N and the interaction term between C and N were also significantly correlated with the first axis between the vegetation types (Table 5). The second GNMDS axis was significantly correlated with the interaction terms between vegetation

type and C or N, together with the interaction between C and N between the two vegetation types. The horizontal length of rhizome (RHl) was also significantly correlated with the second axis between the two vegetation types (Table 5). The interaction terms between N and vegetation type and interaction between C and N were significant correlated between plots within vegetation types along GNMDS axis 2.

Discussion

Fungal community composition

We found substantial turnover in fungal communities associated with the root system of *Bistorta vivipara* in the ridge-to-snowbed gradient with a higher richness of ECM OTUs in snowbeds. However, several intercorrelated environmental variables vary systematically along the ridge-snowbed gradient, making it difficult to separate their effects and infer causal relationships. These include duration of snow cover, soil moisture, concentrations of C, N and P, and other soil chemical variables. Existence of a gradient in ECM fungal community composition that mirrors variation in soil characteristics and vegetation is reported in several studies. ECM fungal coenoclines gradients in species composition, (Whittaker 1967) have previously been found to relate to soil nutrients in forest ecosystems (Toljander *et al.* 2006; Twieg *et al.* 2009; Reverchon *et al.* 2012). Blaalid *et al.* (2012) reported that the root-associated fungal communities of *B. vivipara* changed along a primary successional gradient at Finse, Norway, and that compositional differences were correlated with changes in pH of the soil.

Several ecto- and ericoid mycorrhizal plant species were present in the ridge and snowbed plots. *B. vivipara* was found in more or less equal amounts in both habitat types. *Salix herbacea* and *Vaccinium uliginosum* were predominantly found in snowbeds, whereas *Empetrum nigrum* and *Vaccinium vitis-idea* were predominantly found at ridges. The presence of ecto- and ericoid mycorrhizal plant species might influence root-associated fungal communities of other species through common mycelial networks, CMNs. CMNs have been suggested to play an important role in facilitating growth of seedlings, as the mycelium of fungi growing in symbiosis with larger plants might serve as an inoculum for the seedlings (Nara 2006). Nara (2006) also observed that pioneering *Salix* plants provided adjacent late colonizers with compatible ECM fungal symbionts during primary succession in a volcanic desert landscape. Moreover, the results indicate that ECM fungal community composition is spatially structured at the fine scales: on average, root systems from the same plot are more similar with respect to community composition than root systems from different plots. Fine-scale spatial structure may arise because neighbouring plants share fungal partners due to belowground vegetative growth between adjacent root systems producing CMNs (Selosse & Duplessis 2006; Bingham & Simard 2012). Such networks can affect the physiology and ecology of plants by facilitating interplant nutrient exchange (Teste *et al.* 2009).

Several OTUs from taxonomically different groups were significantly affiliated with either ridge or snowbed plots. Adaptations to different environmental conditions (Reverchon *et al.* 2012) as well as biotropic interactions (Pickles *et al.* 2012) can influence distribution of species. Several OTUs with affinity to Sebacinales were found to be associated with ridges. This early diverging lineage within Agaricomycotina has been shown to have beneficial influence on plant growth of host plants, as it forms diverse symbiotic associations by ecto-, ericoid- or orchid mycorrhizae as well as endophytic associations (Weiss *et al.* 2004, 2011; Selosse *et al.* 2009; Reverchon *et al.* 2012). In contrast, numerous OTUs with taxonomic affinity to the ECM-forming genera *Tomentella* and *Cortinarius* were strongly associated with the snowbeds. *Tomentella* species have earlier been found to be the dominant ECM partners of alpine plants such as *Kobresia myosuroides* (Mühlmann & Peintner 2008b), *Salix herbacea* (Mühlmann & Peintner 2008a) and *B. vivipara* (Mühlmann *et al.* 2008). *Cortinarius* species are typically associated with well-decomposed organic matter and humus (Lindahl *et al.* 2007), and therefore may thrive better in the snowbeds where the amounts of organic material is higher. Likewise, several OTUs with taxonomic affinity to dark septate root endophytes (DSE), like the *Cadophora finlandica/Meliniomyces* spp. complex and the *Phialocephala fortinii* complex, show distinct preferences for snowbeds. Summerbell (2005) observed that the root endophyte *Meliniomyces variabilis* prefers peat bog sites with more decomposed peat. Two OTUs with taxonomic affinities to the *Rhizoscyphus ericae* complex show contrasting preferences for ridge or snowbed. Further studies are needed to understand the association of fungal OTUs with ridges and snowbeds.

The ordination analyses indicate that not only chemical soil properties of the ridge-to snowbed gradient play important roles in the structuring of the root-associated fungal communities. The significant relationship between the horizontal length of *B. vivipara* rhizomes and the second GNMDS axis indicates that age of the host plant is important for ECM community composition. The size of the rhizome of *B. vivipara* is likely positively correlated with the age of the plant (Diggle 1997). We may speculate that the perennial life history of *B. vivipara*, which allows continuous accumulation of fungal species over several years, is an important reason for the high taxon richness observed. However, the negative correlation between number of OTUs and

rhizome size in snowbeds could be an indication of competition among fungal species within the root systems of larger plants at relatively stable environmental conditions. Perhaps somewhat surprisingly, we do not observe significant differences in fungal species richness between the ridge and snowbed plots, while the species composition changes along the gradient, species richness does not. A similar pattern with no change in species richness but change in community composition was observed in fungal communities in alpine open meadows and willow understory habitat (Becklin *et al.* 2012). It has also been reported for fungal communities along a salinity gradient from fresh- to saltwater marshes (Mohamed & Martiny 2011).

Most of the OTUs observed in our study belong to Dikarya. Ascomycota is most diverse when it comes to OTU richness while basidiomycetes dominate when it comes to proportion of reads. These contrasting patterns probably reflect the different life strategies of the two groups. Many of the observed basidiomycetes are high-biomass ECM fungi that are expected to yield many reads in high-throughput sequencing analyses of bulk samples. Notably, the ECM-forming Russulales have a high proportion of reads (26.9%) but relatively few OTUs (2.6%), which indicates high biomass. In contrast, a higher proportion of the ascomycetes probably represent root endophytes or pathogens of lower biomass. Several OTUs with high sequence similarity to *Articulospora* spp. were among the most common OTUs in our sample. *Articulospora* is mainly known as aquatic hyphomycetes forming characteristic spores that spread through water (Quilliam & Jones 2010; Seena *et al.* 2012). Recent studies indicate that many such 'Ingoldian fungi' (Ingold 1942), including *Articulospora,* spend parts of their life cycle as plant root associates (Carlsen 2002; Selosse *et al.* 2008; Bjorbækmo *et al.* 2010; Blaalid *et al.* 2012).

Although a systematic shift in fungal community composition is observed from ridge to snowbed, we found high heterogeneity and low overlap in the number of shared OTUs across the 50 root systems. This accords with results of several other studies (Izzo *et al.* 2005; Lekberg *et al.* 2011; Blaalid *et al.* 2012; Pickles *et al.* 2012), which report a high degree of stochasticity in the assembly of fungal communities. Stochastic spore dispersal processes may be partly responsible for this pattern. Other explanations for high heterogeneity may be niche partitioning in response to fine-scale environmental gradients (Tedersoo *et al.* 2003), for example, brought about by different enzymatic capabilities (Abuzinadah & Read 1986; Bruns 1995), as well as competitive interactions between different fungal species. Even though the species accumulation curve for the ridge and snowbed plots do level off, the accumulation curves are still not saturated. This indicates that a part of the heterogeneity in the detected fungal communities might be due to the sampling intensity of this study.

Conclusion

This study demonstrates that the fungal communities associated with roots of the ECM-forming plant *B. vivipara* change systematically along the ridge-to-snowbed gradient. Different fungal groups are associated with different environmental conditions. OTUs with taxonomic affinity to Sebacinales are significantly affiliated with the exposed ridges, where the vegetation is dominated by lichens, and the amount of phosphorus is higher than in the snowbeds. In the snowbeds, numerous OTUs with taxonomic affinity to *Tomentella* and *Cortinarius* are over-represented.

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F.Y., U.V., A.K.B., T.C. and H.K. contributed in planning the project. F.Y., U.V., A.K.B. and H.K. conducted the field work. F.Y. and T.C. conducted the lab work. F.Y. and U.V. participated in the bioinformatic analyses. F.Y., U.V. and R.H. participated in the statistical analyses. F.Y. and U.V. drafted the manuscript.

Data accessibility

Raw .sff files from 454 sequencing with corresponding mapping files have been uploaded to Dryad (doi:10.5061/dryad.216tp) along with OTU tables, vegetation cover estimates, environmental factors measured and R-scripts used in this study.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 GLM analyses of environmental variables (C, N and P) and plant root characteristics against number of fungal OTUs in each *Bistorta vivipara* root sample.

Table S2 OTUs that were significantly over- or underrepresented in either ridge or snowbed, according to the *G*-test.

Fig. S1 The weighted distribution (in percentage) of bryophyte, vascular plant and lichen species found within the five sampled 1.5 m × 1.5 m plots of snowbed (dark green bars) and ridge (light green bars).

Fig. S2 GNMDS ordination of the fungal communities associated with *Bistorta vivipara* root systems based on the replicated samples.

Fig. S3 Histograms showing the distribution of a) number of fungal OTUs and b) number of fungal reads found per *Bistorta vivipara* root sample, as well as c) the number of root samples in which an OTU occurred.

Fig. S4 Boxplot showing the difference in number of ectomycorrhizal fungal OTUs in the ridge and snowbed plots.

Fig. S5 Plot showing Procrustes errors between the two GNMDS ordinations of fungal communities associated with *Bistorta vivipara* root samples (light gray squares) and vegetation data (black squares) in ten sampled plots.