Arctic fungal communities associated with roots of *Bistorta vivipara* do not respond to the same fine-scale edaphic gradients as the aboveground vegetation

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**Summary**

- Soil conditions and microclimate are important determinants of the fine-scale distribution of plant species in the Arctic, creating locally heterogeneous vegetation. We hypothesize that root-associated fungal (RAF) communities respond to the same fine-scale environmental gradients as the aboveground vegetation, creating a coherent pattern between aboveground vegetation and RAF.
- We explored how RAF communities of the ectomycorrhizal (ECM) plant *Bistorta vivipara* and aboveground vegetation structure of arctic plants were affected by biotic and abiotic variables at 0.3–3.0-m scales. RAF communities were determined using pyrosequencing. Composition and spatial structure of RAF and aboveground vegetation in relation to collected biotic and abiotic variables were analysed by ordination and semi-variance analyses.
- The vegetation was spatially structured along soil C and N gradients, whereas RAF lacked significant spatial structure. A weak relationship between RAF community composition and the cover of two ECM plants, *B. vivipara* and *S. polaris*, was found, and RAF richness increased with host root length and root weight.
- Results suggest that the fine-scale spatial structure of RAF communities of *B. vivipara* and the aboveground vegetation are driven by different factors. At fine spatial scales, neighbouring ECM plants may affect RAF community composition, whereas soil nutrients gradients structure the vegetation.

**Introduction**

Low temperatures, nutrient and water availability are the main factors limiting plant growth in the Arctic (Shaver & Chapin, 1980; Billings, 1987). Accordingly, in the Arctic many plant species are restricted to sites with microclimates favourable for survival (Billings & Mooney, 1968). In arctic–alpine areas, fine-scale topographic variations influence microclimatic conditions such as local temperature and soil moisture (Dahl, 1956; Odland & Munkejord, 2008; Stewart *et al.*, 2014). In addition to biotic interactions and topography, substrate characteristics such as bedrock, quaternary deposits and soil particle size are important determinants of vegetation zonation and plant community composition in the Arctic (Billings & Mooney, 1968; Billings, 1987; Stewart *et al.*, 2014). Fine-scale variation in these factors, as well as environmental stress and disturbance caused by herbivores, wind, snow abrasion and freeze–thaw cycles (van der Wal, 2006; Kumar *et al.*, 2013), create heterogeneity in local arctic plant communities (Billings, 1987).

Fungal activity, which influences vegetation, is an important process in all ecosystems, including those with significant snow cover (Olofsson *et al.*, 2011; Kuhnert *et al.*, 2012). In the Arctic, fungal decomposition of organic material directly affects soil quality (Ludley & Robinson, 2008). Furthermore, fungi play an important role as symbionts for both algae and cyanobacteria (i.e. in lichens) and vascular plants (mycorrhiza, endophytes) (Gardes & Dahlberg, 1996).

Compared to the small size of the arctic plant species pool, the diversity of root-associated fungi (RAF) is relatively high (Väre *et al.*, 1992; Botnen *et al.*, 2014). Ectomycorrhizal (ECM) fungi are important mycorrhizal partners in arctic and alpine ecosystems, and account for a large fraction of the RAF species pool (Gardes & Dahlberg, 1996; Blaalid *et al.*, 2014). ECM are essential for the survival of plant species in nutrient-limited arctic environments by providing nitrogen and phosphorus (Gardes & Dahlberg, 1996; Tedersoo *et al.*, 2010a). Several plant species that are widespread in the Arctic associate with ECM fungi, including shrubs and woody herbs like *Betula*, *Salix*, *Dryas* and *Arctostaphylos*, and the frequent arctic–alpine herb *Bistorta vivipara* (Gardes & Dahlberg, 1996).

Spatial ecology is a field of research that aims to identify the underlying causes of spatial structure of species or communities
(Wolfe et al., 2009). The spatial structure of ECM-forming RAF communities in forests and other environments has been demonstrated from global scales down to a few centimetres (Lilleskov et al., 2004; Burke et al., 2009; Wolfe et al., 2009; Pickles et al., 2012; Bahram et al., 2013; Anderson et al., 2014). For example, fine-scale spatial patterns have been shown around single host plants, where neighbouring plants, soil chemistry and moisture are found to be the major drivers of spatial structure (Bahram et al., 2011; Branco et al., 2013). Which drivers that are important within the heterogeneous arctic tundra are, however, less clear. It has been shown that host plant identity is not alone the driver of fine-scale RAF community structure in the Arctic tundra. In a recent study from the Arctic Archipelago, Svalbard, Botnen et al. (2014) found no evidence for host specificity or any spatial structure of RAF in three arctic plant species (B. vivipara, S. polaris and Dryas octopetala) at scales from 5 cm to 3 m. However, only 10 plants per species were analysed per 3 × 3 m plot, and no abiotic or biotic factors were taken into account in this study. Using a denser sampling scheme within a 2 × 2 m plot, (Blaalid, 2012) detected spatial structure of RAF associated with B. vivipara up to a distance of c. 30 cm in an arctic ecosystem. The structural drivers were, however, not identified.

Spatial structure can typically be related to abiotic factors, biotic factors, or a combination of these. Bedrock and local soil environmental factors such as nutrient status, quality and quantity of organic matter, moisture, pH, soil C : N ratio, and temperature have been suggested as likely abiotic causes of (spatial) structure in RAF communities in the Arctic (Deslippe et al., 2011; Timling & Taylor, 2012; Blaalid et al., 2014). Biotic factors, which may include the distribution of host plants and the aboveground vegetation encompassing the host plants (Burke et al., 2009; Bahram et al., 2012; Peay et al., 2013), can affect belowground communities both directly and indirectly through their interaction with abiotic factors (Wardle, 2002, 2006).

We aimed to assess the structural relationship between the aboveground vegetation encompassing the host plant B. vivipara and the RAF community of this host plant, and to determine the possible biotic and abiotic drivers of fine-scale spatial patterns in richness and composition of arctic RAF. Congruent compositional shifts of RAF communities and the associated vegetation along important complex environmental gradients, as observed by (Yao et al., 2013), may be due to both being affected by similar biotic and/or abiotic factors, or arise because one component influences the other directly. In the Arctic, where vegetation patterns show high heterogeneity due to fine-scale variation in soil conditions and microclimate (Billings & Mooney, 1968; Billings, 1987), we predict that the B. vivipara RAF community responds to the same or similar fine-scale spatial gradients as those affecting the aboveground vegetation encompassing the host species.

Materials and Methods

Study plant

Bistorta vivipara (L.) Delarbre (Polygonaceae; syn.: Polygonum viviparum) is an ectomycorrhizal, perennial herbaceous plant species with a wide distribution in circumpolar arctic and alpine habitats (Aiken et al., 2007). The small and compact root system of B. vivipara allows the entire fungal community associated with each plant root system to be studied (Kaiserud et al., 2012). Morphological evidence for ECM colonization of B. vivipara in the Arctic was provided by Hesselmann (1900), and was later confirmed by various studies (Blaalid et al., 2014; Botnen et al., 2014).

Site description and sampling

The study site is located in stabilized vegetation on a flat ridge built up of with fine-grained fluvial and glacial and marine deposits close to the sea in front of the Midtre Lovénbreen glacier in the Svalbard archipelago (78.91° N; 12.06° E). This area was de-glaciated 8000–9000 yr ago (Forman, 1990). The study site is situated in bioclimatic zone B (Northern Arctic Tundra Zone) (Elvebakk, 1999), with mean annual temperature and precipitation of −6.3°C and 385 mm, respectively, and mean July temperature of +4.9°C (1961–1990; The Norwegian Meteorological Institute, www.met.no). The studied 3 × 3 m plot was divided into 81 equal-sized subplots, each measuring 0.33 × 0.33 m. A single B. vivipara individual was randomly selected from each subplot, and excavated with the root system fully intact, except from two subplots in which B. vivipara was absent. Soil samples collected from each sampling spot, brought to the laboratory and stored at −20°C. Plants were stored at +4°C and treated within 36 h after sampling. The root systems were rinsed in tap water to remove visible soil, plant debris and roots not attached to the rhizome. Subsequently, the roots were rinsed thoroughly in milliQ water for 5 min and their weight was measured. All roots were then placed in 2% Cetyl Trimethyl Ammonium Bromide (CTAB) buffer and stored at −20°C until DNA extraction.

Recording of soil, root and vegetation characteristics

Intact soil samples were used for gravimetric determination of water content. Soil samples were further sieved (2 mm) and visible roots removed. The soil fraction was divided into two subsamples; one was used for determination of pH and conductivity, the other for analysis of total C and N content. Soil pH and conductivity were measured by shaking the dried soil in distilled water (1 : 2 v/v) and using a pH-meter (Portable lab™, Lab 482 pH Sensor). Total C and N contents of soil fractions were determined using a CHNS-O Elemental Analyzer 1110 (CE Instruments Ltd, Wigan, Lancashire, UK).

For each sampled B. vivipara plant, morphometric data (rhizome length, height, width and area, inflorescence number, length of the longest leaf and its stipe, root length and weight; Supporting Information Table S1) were recorded. Vegetation structure (i.e. plant and lichen species composition) was determined through recording the total percentage covers of all species of vascular plants, bryophytes and macrolichens. Covers of stones, open soil and biological crusts were also recorded for each of the 81 subplots. A full table of recorded species and other properties of plots is provided (Table S2).
DNA extraction and pyrosequencing

DNA was extracted from the entire plant roots using a modified CTAB extraction protocol (Murray & Thompson, 1980). The extracted DNA was further purified using the E.Z.N.A. soil DNA kit (Omega Biotek, Norcross, GA, USA) following the manufacturer’s protocol. Samples were prepared for 454 pyrosequencing using a nested PCR amplification approach (Berry et al., 2011), which has provided consistent results (Kauzerud et al., 2012). The fungal specific primers ITS1F and ITS4 (White et al., 1990; Gardes & Bruns, 1993) were used in the first step, whereas the ITS5 and ITS2 fusion primers (White et al., 1990) were used in the nested step that targeted the ITS1 region. Fusion primers were constructed by adding 29 unique 10-bp tags and the 454 pyrosequencing adapters A or B to the ITS5 and ITS2 primers, respectively. PCR amplifications were performed in 20-μl reaction volumes following the protocol of Blaalid et al. (2012). PCR products from the second PCR were cleaned with a Wizard® SV Gel and PCR Clean-Up System (Promega), quantified using a Sequalprep™ normalization kit (Invitrogen), and pooled into equimolar amplicon libraries. The 454 Titanium sequencing of the tagged amplicons was performed at the Norwegian High Throughput Sequencing (HTS) Centre (http://www.sequencing.uio.no) using three lanes of a 454 plate. Sequence data are available in Dryad (doi:10.5061/dryad.2343k).

Bioinformatics analyses

A total of 272 595 sequence reads were processed using QIIME v1.5.0 (Caporaso et al., 2010) on the Abel cluster at the University of Oslo. In order to account for tag-switching, which can lead to false positives in HTS amplicon datasets (Carlsten et al., 2012), 3786 reads with noncompatible tag combinations were removed using a Python script available upon request. Reads with length < 200 bp and > 550 bp, average phred quality score of < 25, mismatches in the tags, homopolymers exceeding 6 bp, ambiguous base calls of > 1, and more than one mismatch in the forward primer sequence, were removed from the dataset. In addition, a 50-bp sliding window was used to identify regions of low sequence quality (average quality score < 25), which were truncated at the beginning of the low-quality window. Truncated sequences that still met the minimum length requirement were retained in the dataset.

After quality control, the resulting sequences (268 809) were denoised using Denuiser v091 (Reeder & Knight, 2010) implemented in QIIME v1.5.0. By using UCLUST algorithm (Edgar, 2010) sequences were clustered into operational taxonomic units (OTUs) at 97% similarity threshold (Tedersoo et al., 2010b). Although a 97% threshold is not sufficient to define all fungi at the species level (Köjalg et al., 2013), owing to high (up to 24.2%) variability in the ITS region (Nilsson et al., 2008), we used a 97% threshold to avoid over- and underestimation of species richness. We deleted all global singleton OTUs from our dataset before downstream analyses (Tedersoo et al., 2010b).

OTUs were considered chimeric when identified as such by both the uchiime and perseus algorithms implemented in MOTHUR v1.25.0 (Schloss et al., 2009) and having a top BLAST match with < 90% coverage and < 90% identity to a known fungal sequence (Blaalid et al., 2014). Those tentative chimeric OTUs were removed from the dataset. OTUs that occurred in the negative control and three samples containing < 300 sequences were discarded from the dataset. The 76 plant root samples finally used in downstream analysis are presented in Fig. S1. One representative sequence from each OTU was subjected to a BLAST search against the GenBank-nr/nt (nonredundant nucleotide) database and a customized database combining the UNITE and INSDC databases (downloaded April 2013). All OTUs with BLAST matches to nonfungal organisms and those that could not reliably be identified as fungi (best BLAST match < 80% sequence similarity or < 50% coverage) were also removed. Taxonomic assignments were made following Davey et al. (2013).

Statistical analyses

A total of 192 354 sequences that clustered into 676 OTUs for the 76 plant-root samples were used for further analyses. Six samples were sequenced twice in order to assess the validity of within-sample comparisons. A global nonmetric multidimensional scaling (GNMDS) ordination demonstrated that intra-sample variation was much lower than inter-sample variation, indicating that sequencing-induced variation was much smaller than biological variation in the samples (Fig. S2). To downweigh abundant OTUs, abundance read data were Hellinger transformed (Ramette, 2007). In addition, the read abundance OTU matrix was normalized using 50 random rarefactions to an even sampling depth of 598 reads per sample. The normalized abundance and presence–absence matrices were analysed in parallel with the Hellinger transformed data matrix. Percentage cover data for plants were transformed to a 1–10 abundance scale before further ordination analysis to better balance qualitative and quantitative information in the data (van Son & Halvorsen, 2014). Power-function weighing of each matrix element was used (Maabel, 1979; Ökland, 1986). All soil and plant morphological variables were transformed to zero skewness using the formula of Ökland et al. (2001). Downstream statistical analyses were carried out in R v2.0–7 (R Core Development Team, 2013). Multivariate analyses were performed using the vegan package (Oksanen et al., 2013) in R. Species accumulation curves and extrapolated species richness estimators were obtained following Ugland et al. (2003).

Detrended correspondence analysis (DCA) (Hill & Gauch, 1980) and GNMDS (Kruskal, 1964; Kruskal et al., 1973), were applied in parallel to describe patterns of variation in fungal OTU composition and plant species composition. The GNMDS ordinations were run with the following specifications, following to Liu et al. (2008): distance measure = Bray-Curtis Distance; dimensions = 2; initial configurations = 100; maximum iterations = 1000; minimum stress improvement in each iteration = 10−5. A minimum-stress GNMDS solution had to be obtained from more than one starting configuration to be accepted. Assessment of similarity between GNMDS ordinations
was made by Procrustes analysis (Oksanen et al., 2013). Accepted GNMDs solutions were rotated to principal components and the rotated axes were rescaled to half-change (HC) units by the postMDS() procedure in the vegan package. DCA ordinations were run with default options. All ordinations were inspected for known artefacts such as arch effects, tongue effects and strong outliers (Ökland, 1990). A reliable gradient structure was inferred if similar results were obtained by the use of the two methods (GNMDS and DCA) and no obvious artefacts were observed (Ökland, 1996). Similarity of ordinations was evaluated by calculating Kendall’s rank correlation coefficient τ (Kendall, 1938) between pairs of DCA and GNMDS axes. Axes were considered similar if |τ| > 0.4 (Liu et al., 2008). Explanatory variables such as soil physico-chemical variables, plant morphological variables, percentage cover of individual plant species, as well as subplot scores along axes in the ordination of vegetation data, were used for the interpretation of axes obtained by GNMDS ordination of fungal OTUs. Interpretation was performed by calculation of τ between GNMDs axes and each explanatory variable, and by the use of the envfit function in vegan, in which each explanatory variable is separately regressed on GNMDs axes 1 and 2 by linear regression analysis. The strength of the relationship between each variable and the ordination axes was assessed by the multiple coefficient of determination (R²) of this regression. The vegetation dataset was subjected to GNMDs ordination by the same settings and options as used for the ordination of the fungal OTUs, and the same means were used for interpretation of ordination axes.

A generalized linear model (Venables & Ripley, 2002), with forward selection of variables and quasipoisson errors, was used to model the relationships between the response variables (soil variables, plant morphological variables) and OTU richness. Bonferroni correction was used at each step in the forward selection procedure to prevent bias due to multiple testing (Legendre & Legendre, 2012). The F-ratio test was used to compare nested models in the model selection procedure.

Empirical semi-variance analyses, as implemented in the R package geoR (Ribeiro & Diggle, 2001) was used to describe the spatial structure of ordination axes for fungal OTUs, vegetation, and environmental explanatory variables. Standardised isotrophic semi-variance, that is, half the standardised variance of differences between sample pairs (Rossi et al., 1992), was calculated for each variable for each of the six lag classes (0–0.50, 0.51–1.00, 1.01–1.50, 1.51–2.00, 2.01–2.50 and 2.51–3.00 m), into which the range of distances between the studied samples was divided. Envelopes for each standardised semi-variance value were obtained by permutation, using the variog.mc.env function of geoR by which data values were randomly allocated to spatial locations 999 times and new variograms calculated on each permutation. The envelopes represent, for each lag class, the minimum and maximum of the standardised semi-variance values for the permuted data. A spatial structure was considered to be present in distance intervals at which the standardised semi-variance was below the lower bounds of the envelope. The vegan function ordisurf, which fits a smooth surface to observed values for a variable that is, defined for points in a grid using thinplate splines in generalized additive models (GAM), was used to visualise the spatial structure of selected variables in the 3 × 3 m study plot.

Results

Soil, root and vegetation characteristics

The average soil moisture was 32.1% (range: 10–50%), and the average pH was 6.99 (range: 6.7–7.3). Electrical conductivity varied strongly among samples (range: 0–277 Sm⁻¹), with an average value of 63.6 Sm⁻¹. The average C and N contents were 8.70% and 0.56% (ranges: 3.2–20.8%, and 0.2–1.2%, respectively). Soil C as well as soil N content were strongly correlated with each other (Kendall’s τ = 0.853 and P < 0.001). We found considerable heterogeneity in soil physico-chemical variables. Even samples that were collected as close to each other as 30 cm differed considerably in values for many of the variables (Table S1).

The average B. vivipara root length and fresh root weight were 53.5 ± 17.1 mm (mean ± SD) and 0.12 ± 0.07 g, respectively. These two variables were strongly correlated (Kendall’s τ = 0.310 and P < 0.001). Root variables (length and weight) were not significantly correlated with rhizome variables (height, length, width and area) (Kendall’s τ < 0.20 and P > 0.05).

A total of twelve vascular plant species, five moss taxa and four lichen species were recorded in the plot. The ECM plant Dryas octopetala and biological crusts dominated the study plot (average cover per subplot of 44.22% and 18.20%, respectively). B. vivipara was found in all subplots except two, with an average cover of 1.69 ± 1.20% per subplot. The other ECM plant present in the plot was S. polaris (1.05% cover).

Sequence data characteristics

A total of 232 120 reads passed the quality control criteria and clustered into 933 OTUs. Of these, 128 OTUs were singletons, which were removed. We retained a total of 676 OTUs after the removal of 88 chimeras, five OTUs present in the negative control and duplicated samples, 29 OTUs with < 80% sequence similarity or < 50% coverage in the BLAST analysis and 7 OTUs representing nonfungal organisms, out of 805 OTUs. Finally, 676 non-singleton OTUs (consisting of 192 354 reads), were used for further analysis (Table S3). The average number of OTUs detected per sample was 70 (range: 13–113) and the mean number of reads per sample was 2531 (range: 598–7383). The number of reads per sample was not correlated with root weight (τ = −0.036, P = 0.638) or root length (τ = −0.095, P = 0.229). On average, each OTU occurred in 7.9 samples (range: 1–74) with 284 reads (range: 1–27097). The numbers of OTUs and reads per root system were strongly correlated (τ = 0.285, P < 0.001).

Richness of RAF

The estimated species accumulation curve (SAC) approached an asymptote (Fig. S3), which indicated that the majority of the OTUs in the plot were found. Extrapolative estimates of total OTU richness slightly exceeded (observed OTUs-676, chao-748,
jack1-794, jack2-816 and boot 737) the observed OTU richness of the 76 root samples (Fig. S3). A GLM analysis showed a significant relationship between per-plant OTU richness and root variables (length and weight; \( P < 0.05 \)) (Table 1). In separate GLM analyses for individual variables, length explained slightly more of the variation in OTU richness than root weight (data not shown). The number of sequence reads was positively related to OTU richness (\( P < 0.05 \)) (Table 1).

### Taxonomic distribution of RAF

A list of all detected OTUs with their best match against the UNITE and GenBank-nr/nt databases is shown in Table S3. Of the recorded OTUs, 73.1% were assigned to specific taxa using the UNITE database, whereas 87.4% were assigned to specific taxa using the GenBank-nr/nt database. The results from the GenBank-nr/nt database were hence used in the downstream analysis.

Based on identification of the most similar reference sequences in the GenBank-nr/nt database, 27 orders of fungi were represented in the data set (Table S3). A total of 55% of the OTUs (69% of the reads) belonged to the Basidiomycota, 32% (22% of the reads) belonged to Ascomycota and the remaining 13% (9% of the reads) were not assigned to a taxon at the phylum or order levels. Three OTUs (0.4%) remained unclassified at the phylum level. The dominant basidiomycete orders were the Thelephorales, Agaricales and Sebacinales, whereas the Helotiales and Pezizales were the main ascomycete orders (Table 2). ECM genera such as Inocybe, Tomentella, Cortinarius, Sebacina and Heloboma were frequently observed. The 20 most frequently observed OTUs represented 56% of all reads (Table 3). Although most of the identified OTUs were ECM fungi, putative root pathogens, saprobes and endophytes were also observed. For example, one of the dominating OTUs, which was observed in >70 samples, had a 100% match to Leptodontidium orchidicola (Genbank acc. no. JX630667), which is a dark sepatate endophyte (DSE).

### Ordination of vegetation

GNMDS axes were strongly correlated with the corresponding DCA axes (\(|t| > 0.6\); Table S4). GNMDS ordination of vegetation data revealed a distinct community composition in the plot (Fig. 1a). Subplots 19–26, dominated by Sanionia uncinata agg. and situated in the lower right corner of the sampling plot, obtained low scores along the GNMDS axis 1 whereas subplots 54–61, dominated by biological crust and lichens and situated in the upper left corner of the sampling plot, obtained high scores along GNMDS axis 1 (Figs 1a,S1). Percentage cover of several vegetation elements were significantly related to the observed compositional structure of the plot (all with \(|t| > 0.2\) with GNMDS axes 1 and 2, and \( P < 0.05 \); Table S5). The highest \( R^2 \) values were obtained for ground-covering vegetation elements, such as S. uncinata agg. and biological crust (\( R^2 = 0.680, P = 0.001 \) and \( R^2 = 0.593, P = 0.001 \), respectively). Soil carbon concentration and C:N ratio were also significantly related to GNMDS axes (\(|t| > 0.2\) and \( P < 0.05 \); Table S5). The C:N ratio increased with increasing cover of biological crust (Fig. 1a), and the isoline diagrams for GNMDS axis 1 of vegetation cover and C:N ratio reflected similar spatial trends across the sampling plot (Fig. 2).

### Ordination of RAF OTUs

GNMDS ordination axes obtained by use of Hellinger transformed RAF OTU data were correlated with the corresponding DCA axes (\(|t| > 0.4\); Table S4). However, no significant RAF community compositional structure was detected among the 76 root samples (Fig. 1b). The analysis of normalised abundance and presence–absence data showed similar results (data not shown). GNMDS axes of vegetation structure were not significantly related to GNMDS axes obtained for RAF data. The aboveground cover of B. vivipara, S. polaris and the lichen Cetraria delisei were, however, significantly related to fungal GNMDS axis 2 (all \(|t| \geq 0.2\) and \( P \leq 0.05 \); Table S6). In the ordination, vectors for S. polaris and the lichen Cetraria delisei was pointing in the same direction (Fig. 1b). The RAF community composition was not significantly related to any of the recorded soil variables (Table S5).

### Spatial structure of vegetation and RAF communities

Standardised semi-variance for vegetation GNMDS axis 1 was below the lower bounds of the confidence envelope, indicating a

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**Table 1** Results from general linear models fitted to the number of operational taxonomic units (OTUs) detected in each sample, as functions of three explanatory variables

|         | Estimate | SE   | \( t \) value | \( Pr(>|t|) \) |
|---------|----------|------|---------------|----------------|
| Intercept        | 3.53     | 0.11 | 31.46         | <0.001         |
| Reads            | 0.76     | 0.15 | 4.99          | <0.001         |
| Root length      | 0.53     | 0.16 | 3.29          | <0.001         |
| Root weight      | 0.33     | 0.14 | 2.41          | 0.018          |

**Table 2** Overview of the taxonomic composition of root-associated fungal (RAF) communities of Bistorta vivipara

<table>
<thead>
<tr>
<th>Taxonomic affinity</th>
<th>% of total OTUs</th>
<th>% of total reads</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascomycota</td>
<td>31.95</td>
<td>21.64</td>
</tr>
<tr>
<td>Helotiales</td>
<td>11.54</td>
<td>6.65</td>
</tr>
<tr>
<td>Pleosporales</td>
<td>5.03</td>
<td>9.20</td>
</tr>
<tr>
<td>Pezizales</td>
<td>1.33</td>
<td>0.83</td>
</tr>
<tr>
<td>Unassigned Ascomycota</td>
<td>8.88</td>
<td>2.58</td>
</tr>
<tr>
<td>Basidiomycota</td>
<td>55.03</td>
<td>69.38</td>
</tr>
<tr>
<td>Thelephorales</td>
<td>21.89</td>
<td>23.09</td>
</tr>
<tr>
<td>Agaricales</td>
<td>17.60</td>
<td>34.57</td>
</tr>
<tr>
<td>Sebacinales</td>
<td>12.13</td>
<td>10.64</td>
</tr>
<tr>
<td>Cantharellales</td>
<td>1.33</td>
<td>0.98</td>
</tr>
<tr>
<td>Unassigned Basidiomycota</td>
<td>0.30</td>
<td>0.05</td>
</tr>
<tr>
<td>Mortierellales</td>
<td>0.44</td>
<td>0.01</td>
</tr>
<tr>
<td>Unassigned</td>
<td>12.57</td>
<td>8.98</td>
</tr>
</tbody>
</table>

Operational taxonomic units (OTUs).
Table 3 The 20 most frequent operational taxonomic units (OTUs) found in the root systems of *Bistorta vivipara* plants, listed with number of reads and best BLASTN hit

<table>
<thead>
<tr>
<th>OTU ID</th>
<th>Best hit in Genbank</th>
<th>Accession no.</th>
<th>Query coverage</th>
<th>% ID</th>
<th>Reads</th>
<th>Freq.</th>
</tr>
</thead>
<tbody>
<tr>
<td>527</td>
<td><em>Cortinarius diospermus</em></td>
<td>JQ724021</td>
<td>96</td>
<td>100</td>
<td>27097</td>
<td>68</td>
</tr>
<tr>
<td>705</td>
<td>Uncultured <em>Tomentella</em></td>
<td>EU668944</td>
<td>96</td>
<td>100</td>
<td>17762</td>
<td>63</td>
</tr>
<tr>
<td>220</td>
<td><em>Helotoma eburneum</em></td>
<td>JN943851</td>
<td>97</td>
<td>99</td>
<td>15914</td>
<td>40</td>
</tr>
<tr>
<td>549</td>
<td><em>Herpetichia juniperi</em></td>
<td>HM853977</td>
<td>95</td>
<td>91</td>
<td>14299</td>
<td>74</td>
</tr>
<tr>
<td>340</td>
<td><em>Tomentella</em> sp.</td>
<td>HQ215812</td>
<td>90</td>
<td>100</td>
<td>7925</td>
<td>49</td>
</tr>
<tr>
<td>535</td>
<td>Uncultured <em>Inocybe</em></td>
<td>JX630671</td>
<td>98</td>
<td>100</td>
<td>6695</td>
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Discussion

Our data showed a strong spatial structure in aboveground vegetation and lack of spatial structure in the belowground RAF community of *B. vivipara* at a fine spatial scale. Soil variables affected the vegetation structure, but not the RAF community structure at this scale. However, cover of certain vegetation elements, such as the ECM plants *B. vivipara* and *S. polaris*, showed a weak structuring effect on the RAF community.

Spatial structure of vegetation vs RAF communities

The strong spatial structure detected in aboveground vegetation within the 3 m plot was mainly due to a shift in species composition from biological soil crust and lichens towards a community dominated by mosses (mainly *S. uncinata* agg.). This suggests presence of an edaphic gradient within the plot, associated with soil moisture and/or nutrients. Moisture is the main limiting factor for moss growth (Skre & Oechel, 1981), whereas biological crusts typically occur in dry habitats (Pointing & Belnap, 2012). However, no clear spatial trend in soil moisture content was recorded, probably owing to a rainfall event during the sampling period confounding eventual existence of a moisture or soil nutrient gradient in the plot. Rather than moisture, the trend in ground-cover vegetation seemingly follows the soil C:N ratio in our study, which decreases with increasing moss cover. This trend in C:N concentrations can be a result of variation in N fixation between these two habitats, in accordance with the fact that the primary source of newly fixed N in nutrient-poor arctic ecosystems is N-fixing cyanobacteria (Alexander et al., 1978; Shaver & Chapin, 1980; Chapin & Körner, 1995; Stewart et al., 2014). Cyanobacterial species form both free-living colonies on the soil surface (i.e. biological crust) and grow epiphytically on bryophytes such as *S. uncinata* (Alexander et al., 1978; Rai et al., 2002; Solheim & Zielke, 2002; Zielke et al., 2005; Stewart et al., 2014). Besides light and temperature, N fixation rates are in particular promoted by increased moisture amounts in the Arctic (Dickson, 2000). Moss mats usually retain more moisture than soil crust, leading to higher net N-fixation in moss cover than in biological crust (Zielke et al., 2005; Stewart et al., 2014). Thus, the observed shift in ground-cover may represent a shift between two different cyanobacterial communities with different N-fixation rates. This may explain the spatial variation in the C:N ratio observed in our study plot.

In the Arctic, bedrock composition and associated edaphic factors are shown to contribute to the fine-scale distribution of both plant (Elvebakk, 1994) and fungal (Blaalid et al., 2012, 2014; Timling & Taylor, 2012) communities. Our results do, however, show that the two communities do not respond in concert. The spatial structure we observed between C:N ratio and vegetation was not reflected in RAF community structure. Similar lack of co-varying spatial structure between structured aboveground vegetation and unstructured arbuscular mycorrhizal (AM) fungal communities of a single host plant has been reported in wetlands by (Wolfe et al., 2007) who do not, however, provide a clear explanation for this discrepancy. Rather, they suggest that either a different, unidentified mechanism was responsible for fungal distributional patterns at fine spatial scales (their plots were 2 m), or the resolution of their analytic methods was too low. Technical constraints are, however, not the most likely explanation for the lack of spatial RAF community structure observed in...
our study, as high-throughput sequencing provides high resolution, and most of the RAF diversity was recovered (Fig. S3). We can, however, not completely rule out the possibility that we sampled the RAF community at a too broad a spatial scale to detect spatial structure. At spatial scales of 1–10 m, arctic communities show high levels of heterogeneity related to periglacial (freeze–thaw) processes (Washburn, 1980). Flat surfaces with fine-grained soils that are strongly subjected to periglacial processes exhibit nonsorted features, especially in the High Arctic bioclimatic zones A and B (Raynolds et al., 2008). Thus, both the scale and the location of our study plot suggest high nonsorted heterogeneity due to periglacial processes. However, the influence of periglacial heterogeneity should be less for distances <1 m (Washburn, 1980), so periglacial processes alone cannot explain the lack of spatial RAF structure.

Periglacial processes combined with extreme climatic conditions also restrict soil development and growth of both roots and fungi, which further affect the extent of mycelial networks (Timling & Taylor, 2012; Botnen et al., 2014; Timling et al., 2014). In addition, freeze–thaw cycles will recurrently lead to fragmentation and destruction of mycelia and, building of extensive external mycelia may therefore be disadvantageous in arctic soils (Lehto et al., 2008; Kumar et al., 2013). By contrast, the formation of larger mycelia networks is facilitated in forest and grassland ecosystems, where soil environments are more stable and growth conditions more favourable. Such habitats are thus more likely to exhibit distinct spatially structured fungal communities (Lilleskov et al., 2004; Bahram et al., 2013; Anderson et al., 2014) at larger spatial scales than high-arctic tundra. In order to assess more accurately existence of spatial structure in RAF of arctic ecosystems, in which soil heterogeneity is high, and root and mycelia extension is restricted (Botnen et al., 2014), we should either narrow down the interplant sampling distance (<30 cm) or collect very small soil samples at the very small distances close to plant-root.

Factors affecting RAF community composition

A weak relationship is found between cover of ECM plants (B. vivipara and S. polaris) around the sampled B. vivipara host and its RAF community composition. Neighbouring plant
species composition is reported as an important factor affecting AM (Mumey et al., 2005; Hausmann & Hawkes, 2009) and ECM (Bahram et al., 2011; Bogar & Kennedy, 2013) fungal communities in forest. The roots of neighbouring plant species may have direct effects on RAF communities by exudation of chemical compounds. Thus Broeckling et al. (2008) show that addition of nonhost root exudate changes the carbon substrates or signalling compounds that bring about increase in the soil fungal load. Furthermore, ECM fungi associated with neighbouring plant roots may serve as reservoirs of ECM inoculation. For example, Nara (2006) observed that pioneer Salix plants serve as ECM fungal reservoirs during primary succession in a volcanic desert landscape, and facilitate the establishment of late colonizer.

In the Arctic, RAF communities associated with B. vivipara, Dryas octopetala and S. polaris have low host specificity (Botnen et al., 2014). Low host specificity may help ECM plants to associate with a range of symbiotic fungi, which may improve nutrient access (Molina et al., 1992). Hence, if a B. vivipara bulbil establishes in a S. polaris community, it may adopt a more developed and mature ECM community than a bulbil establishing outside patches of ECM vegetation.

A weak relationship is also observed between cover of the lichen Cetraria delisei around the sampled B. vivipara host and its RAF community composition, resembling the community structure created by increased S. polaris cover. This lichen is known to co-occur with S. polaris, the two represent a separate, late snow-bed plant community in Svalbard (Hadač, 1989). As this foliose lichen has its thallus almost completely aboveground, the observed relationship with the B. vivipara RAF community is probably an indirect result of its co-variation with S. polaris.

### Fungal richness

The OTU accumulation curve showed that almost the entire fungal diversity associated with the roots of B. vivipara in the studied plot was recovered by our sampling scheme. The various estimates of total OTU richness confirmed this because the estimated total richness is only slightly higher than observed richness (Fig. S3). Furthermore, the size-related characteristics of B. vivipara roots are more important predictors of fungal species richness than soil physico-chemical variables and the composition of the aboveground vegetation. The positive correlation of rhizome length with the age of B. vivipara individuals (Diggle, 1997) suggests that RAF richness increases with plant age in alpine areas (Yao et al., 2013). Although B. vivipara has a restricted root system, the numbers of rootlets and ECM root tips most likely increase with root length, and therefore roots can accumulate more fungal species due to a larger root surface area being available for fungal colonization.

### Taxonomy

Most of the OTUs observed in our study belong to the Dikarya, with Basidiomycota being the dominant phylum. The primers used in this study (ITS1F and ITS4), which preferentially amplify basidiomycetes (Gardes & Bruns, 1993; Bellemain et al., 2010), may partly explain the lower occurrence of ascomycetes in our dataset. However, the retrieved taxonomical distribution, from phylum to genus level, is corresponding to earlier findings in alpine and arctic habitats (Timling et al., 2012; Yao et al., 2013; Botnen et al., 2014). Although our dataset was dominated by basidiomycetes, it is worth noticing that one of the top 20 most frequent OTUs was an ascomycete, with taxonomic affinity to the DSE Leptodontidium orchidicola. L. orchidicola is a ubiquitous root and rhizosphere fungus found in all kinds of habitats (Jumpponen & Trappe, 1998). DSEs may be considered as being analogous to mycorrhizal fungi (Jumpponen, 2001), which under specific conditions are able to enhance plant performance (Newsham, 2011).
Conclusions

Our results suggest that the spatial structure of RAF communities in *B. vivipara* is neither structured by vegetation gradients nor by the soil variables on which vegetation gradients at 0.3–3.0 m scales are conditioned. At this fine spatial scale, the RAF communities seem more strongly influenced by periglacial processes and stochastic processes than by ‘classic’ edaphic gradients and/or the aboveground vegetation cover. Cover of neighbouring ECM plants had some effect on RAF communities of *B. vivipara*, suggesting that plants may serve as reservoirs of RAF for one another. Fungal richness is correlated with root length and weight, suggesting a species-area relationship. Overall, our results show little coherence of spatial patterns between RAF communities in *B. vivipara* and the aboveground vegetation encompassing their host, and suggest that the underlying processes shaping their communities are uncoupled or operating at different spatial scales.

Acknowledgements

We acknowledge Tor Carlsen, Synnøve Botnen and Marie Davey for their help with laboratory work and bioinformatics analyses, and Karin Lagesen for writing the Python script controlling for tag-switching. We thank Mohammad Bahram, Leho Tedersoo, Maarja Õpik and three anonymous reviewers, for helpful comments on an earlier draft of the manuscript. This research was funded by Svalbard Science Forum (SSF) through Arctic Field Grant, and ConocoPhillips and Lundin Petroleum through The Northern Area program, and carried out at University Centre in Svalbard and the University of Oslo.

References


**Supporting Information**

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

**Fig. S1** Map showing the 3 × 3 m study plot and positions from which the 76 *Bistorta vivipara* plants were sampled.

**Fig. S2** GNMDS ordination of presence–absence data for samples that were sequenced twice in different lanes of a 454 pyrosequencing plate.

**Fig. S3** Species accumulation curve (obtained by randomizing 76 samples) for OTUs against sampling effort.

**Table S1** Soil physico-chemical variables and morphological features of *Bistorta vivipara*, as recorded in each subsample

**Table S2** Vascular plant and lichen species, moss groups and ground cover variables for which percentage cover was recorded in subplots of the studied plot

**Table S3** Abundance, frequency and taxonomic affiliation of nonsingleton OTUs identified against a custom database of UNITE+INSDEC sequences and the GenBank-nr/ntdatabase

**Table S4** Relationships between DCA and (two-dimensional) GNMDS ordination axes, assessed by Kendall’s rank correlation coefficients (τ)

**Table S5** Relationships between GNMDS vegetation ordination axes, and each of the soil physico-chemical variables and GNMDS axes of fungal OTUs, assessed by Kendall’s rank correlation coefficients (τ)

**Table S6** Relationships between GNMDS ordination axis of fungal OTUs and percentage vegetation cover of each plant and lichen species, moss group, soil physico-chemical and plant morphometric variable, and vegetation GNMDS ordination axis, assessed by Kendall’s rank correlation coefficients (τ)

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