Cryptogams signify key transition of bacteria and fungi in Arctic sand dune succession

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Running title: Cryptogams and microbes in Arctic dune succession

Summary

Microbes are the first colonizers of barren soils and an important factor in vegetation establishment. Primary succession models focus on vascular plants, although most soil successions include mosses and lichens. Here, we aim to clarify the role of these cryptogam-dominated stages in plant-microbe interactions by covering the successional trajectory of bacterial and fungal communities in eroded Arctic inland dunes. The earliest successional stages with sparse or no plant cover (bare sand, grass) had low microbial biomass per organic matter. In the cryptogam stages with the moss *Polytrichum piliferum* and lichens, microbial biomass and community structure shifted towards the vascular vegetated stages (heath, mountain birch forest). Soil chemistry explained microbial community variation better than vegetation, but the transitions in chemistry and in plant and microbial community structure did not align. We detected potential autotrophic and trace gas oxidizing bacteria, such as WD272 (WPS-2), Chloroflexi and Cyanobacteria, which could form the basis of biomass accumulation from bare sand to cryptogam stages. The results indicate that when considering also belowground microbes, the ecosystem transition from vegetation loss to vegetation recovery starts at the moss stage. *Polytrichum piliferum* and the associated microbes may help stabilize the Arctic dune sand and facilitate vascular vegetation development.

Keywords: Arctic soil, bacterial community, cryptogam, fungal community, *Polytrichum*, primary succession, sand dune

Introduction

Development of vegetation on bare land and the sequence of vegetative stages that predictably follow each other have been described in several theories of succession (Egler, 1954, Wilson *et al.*, 1992). According to widely accepted views, primary succession is influenced by site abiotic conditions (climate, soil parent material, topography) and biotic factors (species pool present, order of arrival, species interactions). Despite the fact that microbes are the first colonizers of any barren surface and shape soil chemistry, models predicting successional trajectories of bacteria and fungi are few (Jackson, 2003, Fierer *et al.*, 2010, Dini-Andreote *et al.*, 2015, Tripathi *et al.*, 2018, Ortiz-Álvarez *et al.*, 2018). Most models of terrestrial primary succession overlook bryophyte communities (e.g. Connell and Slatyer, 1977, Baker and Walford, 1995), although most reports of primary succession include moss and lichen dominated stages (Chapin *et al.*, 1994, Lichter, 1998, Hodkinson *et al.*, 2003, Jones and Henry, 2003, Sparrius *et al.*, 2012).

Cryptogams, here mosses and lichens, may have diverse but unexplored effects on the development of vascular plant and belowground microbial communities. Firstly, dispersal by spores minimizes dispersal limitation – a feature classically included in models explaining succession of vascular plant communities (Makoto and Wilson, 2019). The rapid arrival of mosses warrants them a priority effect in establishing plant communities. Secondly, although mosses and lichens lack roots and root-mediated effects on microbial communities, they affect soil temperature, moisture, and nitrogen availability, which may promote or inhibit vascular vegetation (Van der Wal and Brooker, 2004, Gornall *et al.*, 2007, Cornelissen *et al.*, 2007, Gornall *et al.*, 2011). Many lichens characteristic of primary succession fix nitrogen in specific structures called cephalodia (Vitousek, 1994), whereas moss leaves contain diazotrophs (DeLuca *et al.*, 2002, Arróniz-Crespo *et al.*, 2014). In addition to nitrogen, rhizoids of mosses provide carbon to the belowground soil (Bowden, 1991). Thirdly, mosses and lichens produce unique secondary metabolites that may have adverse effects on vascular plants and beneficial or adverse effects on microbes (Cornelissen *et al.*, 2007, Xie and Lou, 2009).

Microbial community shifts in early succession are considered to be driven by soil chemistry, external resources and dispersal of microbes, and in later stages by biotic factors, such as establishment and changes in plant community (Jiang *et al.*, 2018, Brown and Jumpponen, 2014). During succession microbes modify soil chemistry, and biotic interactions including plant-microbe interactions can aid plant establishment. Bacterial versus fungal communities can be expected to show distinct trajectories in primary succession: Bacteria are smaller and believed to disperse easier than fungi, which may lead to more deterministic community assembly for bacteria (Schmidt *et al.*, 2014, Powell *et al.*, 2015) and higher influence of priority effects and stochastic effects for fungi (Brown, S. P. and Jumpponen, 2014, Schmidt *et al.*, 2014, Jiang *et al.*, 2018). Further, the physiological diversity in bacteria enables some of them to survive as autotrophs in bare oligotrophic soils of early successional stages in the absence of plants fixing carbon (Nemergut *et al.*, 2007, Schmidt *et al.*, 2008, Duc *et al.*, 2009). Fungi in these habitats depend on already fixed carbon and nitrogen from wind-blown, ancient, or microbially fixed sources (Schmidt *et al.*, 2014). The critical shift to ecosystem based on production by resident plants is associated with changes in microbial community (Bardgett and Walker, 2004, Edwards *et al.*, 2006, Blaalid *et al.*, 2012, Knelman *et al.*, 2012). Along succession, fungi to bacteria ratio generally increases as soil organic matter increases and pH decreases (Pennanen *et al.*, 2001, Tscherko *et al.*, 2004, Bardgett and Walker, 2004, Bardgett *et al.*, 2007, Baldrian *et al.*, 2008). These results do not, however, identify the only factors responsible for vascular plant establishment. As early colonizers, cryptogams with their influence on soil chemical and physical properties and biotic interactions could be an additional key factor in the ecosystem shift from early to late succession.

In the world where ecosystems are declining due to soil erosion and loss of plant cover (Montgomery, 2007, Vanwalleghem *et al.*, 2017), it is critical to identify the key shifts between non-vegetated and vegetated stages in ecosystem succession. Identifying the microbial and possibly plant communities that signal for the shift from autotrophic to allotrophic helps identify the key factors that drive the shift back to vascular plant cover, soil stabilization and a productive ecosystem. Arctic inland sand dunes are an example of areas where vegetation has been lost due to environmental changes and lack of recovery. The dunes have formed from wind-blown sand after the last ice age (Koster, 1988). Plants colonized the dunes later on, but today large areas lack vascular plant cover. Forest fires, insect outbreaks, and overgrazing may have caused the ecosystem degradation (Seppälä, 1995). In our study

site in Northern Finland, wind erosion has carved deflation basins into the dunes. Inside the basin, primary succession vegetation stages have developed, and dune slopes surrounding the basins are covered by mountain birch forest, the climax stage in the present climate. Eroding inland sand dunes provide an excellent opportunity to study primary succession due to the mosaic of closely situated successional stages within deflation basins. Previous work comparing the start and end points of succession proposed that Arctic sand dune bacteria and fungi may show distinct successional patterns (Poosakkannu *et al.*, 2017).

In this study, we compared two cryptogam-dominated stages with non-vegetated and vascular plant covered stages in an eroded Arctic dune ecosystem. We hypothesized that the cryptogam-covered stages contain soil microbial community more similar to stages covered by vascular plants and thereby drive succession towards vascular plant dominance through microbially mediated facilitation. Secondly, we compared the belowground responses of two microbial groups - fungi and bacteria - to successional stages defined by aboveground characteristics. We hypothesized that bacteria would be more prominent and diverse in the early successional stages and fungi in the later stages with vascular vegetation. By linking the aboveground vegetation, the belowground microbial communities, and the associated soil chemical environment, we aimed to define the role of cryptogam stages in the Arctic dune ecosystem succession.

Materials and methods

Study site and sampling

The study site is located in an inland aeolian (i.e. wind-deposited) dune area in Northern Fennoscandia (68° 29′ 16″ N, 24° 42′ 13″ E). The 1981–2000 average annual temperature in the region was -1.3 °C, with extreme air temperatures between +30 °C and -52 °C and the average annual precipitation 550 mm (Pirinen *et al.*, 2012). We defined six successional stages in the dunes based on vegetation (Fig. S1). Although the soil in all the stages was aeolian sand, in three the largest part of the surface was mostly exposed sand (92-100 %), while in the other three vegetation covered the sand (0-52 % sand). The sand-exposed ‘sandy stages’ were: 1) sand: bare loose sand without plants or visible soil crust, 2) grass: bare loose

sand with the grass *Deschampsia flexuosa* and without visible soil crust, and 3) moss: bare loose sand with the moss *Polytrichum piliferum*. The stages with extensive vegetation cover (‘vegetated stages’) were 4) lichen: the main species lichens *Stereocaulon* spp. and *Cladonia* spp., and mosses *P. piliferum* and *Racomitrium ericoides*, 5) heath: the dominant species dwarf shrub crowberry *Empetrum nigrum* with *Cladonia* spp. and/or *Stereocaulon* spp., and 6) forest: mountain birch (*Betula pubescens* subsp. czerepanovii) forest, the main species in study plots the shrubs *E. nigrum* and *Vaccinium vitis-idaea*,the moss *Pleurozium schreberi*, *D. flexuosa*, and the herb *Linnaea borealis*. The sand, grass, moss, and lichen stages are located at the bottom or on the inner slopes of the deflation basins and heath at the edge of the basins. The remnant mountain birch forests are located around the basins at the crest of the dune or on the outer slope. Permanent study plots of 1 × 1 m were established in 2008 and included each of the six vegetation stages in seven deflation basins. The vegetation in the plots was recorded in detail in 2009 by point frequency method (100 points within 50 × 50 cm, presence/absence and frequency of species). The plots have been photographed every year from 2010 to 2018 and the vegetation has not changed notably.

We sampled soil in each successional stage in four of the basins in July 2011. The soil samples were taken in triplicate from the depth of 0-10 cm with a soil corer (72 samples in total: six successional stages × four basins × three replicates). Soil was sieved with a 4-mm sieve and frozen at -20°C.

Soil chemistry and temperature

Total C and N of soil organic matter were analysed on a LECO CHN 1000 elemental analyser (LECO Corporation, USA). For analyzing soil P, Ca, K, Na, Fe, Mn, Al, and Mg contents, soils were extracted with acid (pH 4.65) 1 M ammonium acetate (15 ml soil sample /150 ml extraction solution) and the elements were determined with an ICP emission spectrometer iCAP 6500 DUO 2011 (Thermo Scientific, United Kingdom). The dry matter content of the soil was determined by drying the samples (105 ºC) and organic matter (OM) content analyzed by loss on ignition (550 ºC) using LECO TGA-701 analyzer (LECO Corporation, USA). pH was determined in 1:2 (w:v) soil:water suspensions (10 g soil and 20 ml H2O). Soil temperature at the 10 cm depth was recorded every two hours with Hobo Temp External H08-002-02 loggers coupled with TMC6-HD soil temperature sensor during 2009-2015. We

defined maximum temperature as the average temperature of the warmest month (July) and the minimum temperature as the average of the two coldest months (January and February).

Phospholipic fatty acid (PLFA) analysis

Phospholipid fatty acids were extracted as described previously (Francini *et al.*, 2014) from freeze-dried soil (15 g for sand and grass stages, 10 g for moss, 8 g for lichen, 5 g for heath and 3 g for forest stage corresponding to OM content). Briefly, lipids were extracted with chloroform:methanol:citrate buffer (Bligh and Dyer, 1:2:0.8 v/v/v), fractionated using silica columns (Bond Elut LRC, Varian), and phospholipids were methylated with mild alkaline methanolysis. The resulting fatty acid methyl esters were analyzed by gas chromatography and PLFAs identified based on a bacterial fatty acid standard mixture (Supelco, Bellefonte, PA, USA) and fatty acids from reference strains. For community analysis, data was expressed as peak area percentage of a single PLFA of the total area of the profile, and for biomass analysis as nmol of PLFAs per gram dry weight (gdw) of soil or per gram OM. The sum of PLFAs i15:0, a15:0, 15:0, i16:0, 16:1ω9, i17:0, a17:0/17:1ω8c (eluted in the same peak), 17:0, cyclo-17:0, 18:1ω7c/ω9t (eluted in the same peak) and cyclo-19:0 were used to represent bacterial biomass and PLFA 18:2ω6 fungal biomass. Sum of all these PLFAs was used to represent total microbial biomass. Biomass of selected bacterial groups was calculated by multiplying the total microbial biomass of a sample with the relative abundance of the bacterial group in the sample based on 16S rRNA gene sequencing.

DNA extraction and PCR

DNA was extracted from 0.85 g (0.4 g for forest stage) of soil with Powersoil DNA extraction kit (MoBio). Bead beating was carried out with a FastPrep instrument at 5.0 m s^{-1} for 40 s. DNA concentration was determined with Qubit fluorometer and Qubit dsDNA HS kit (Invitrogen).

Bacterial and fungal PCR was carried out as two-step PCR where the second step introduced barcodes and adapters for Ion Torrent sequencing. The first step of bacterial 16S rRNA gene

PCR (V1-V2 region) was carried out with primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 338R (5'-TGCTGCCTCCCGTAGGAGT-3'). The 25-µl reactions were carried out in duplicate and contained 1 × buffer, 200 µM dNTPs, 0.4 µM each primer, 0.4µg µl-1 BSA, 0.75 U DreamTaq DNA polymerase (Thermo Fisher Scientific), and 1-2 µl of template DNA (depending on concentration). The cycling conditions were initial denaturation at 95 °C for 3 min, followed by 30 cycles of 95 °C for 30 s, 53 °C for 30 s, and 72 °C for 45 s, and a final extension of 72 °C for 7 min. The first step of intergenic transcribed region (ITS) PCR for fungi was carried out with primers fITS7 (5'-GTGARTCATCGAATCTTTG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (Ihrmark *et al*., 2012), where the fITS7 primer contained an M13 adapter for the second step (Mäki *et al*., 2016). Reaction composition was the same as in bacterial PCR but including additional $MgCl_2$ to total concentration of 3 mM. The cycling conditions were initial denaturation at 94 °C for 3 min, followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, and a final extension of 72 °C for 7 min.

The second step primers introduced barcodes and Ion Torrent adapters A and P1. For bacterial 2nd step PCR, the forward primer was adapter A-barcode-27f, and the reverse primer adapter P1-338R. For the fungal 2nd step PCR, the forward primer was adapter A-barcode-M13 adapter and the reverse primer adapter P1-ITS4. Second step PCR reactions (25 µl) for both bacteria and fungi contained 1 × buffer, 200 µM dNTPs, 0.3 µM each primer, 0.5 U DreamTaq DNA polymerase, and 1 µl (bacteria) or 2 µl (fungi) of 1:10-diluted first step PCR product as template. The cycling conditions were as in the first step PCR but with 7 cycles. After the second PCR, the duplicate products were pooled, purified with Agencourt AMPure XP purification system (Beckman Coulter), and quantified with Qubit fluorometer and Qubit dsDNA HS kit. Equimolar amounts (10 ng) of products were pooled for sequencing. The fungal product pool was size-separated in precast 1% Pippin Prep gel (Sage Science) to remove oversized products > 400 bp, which occurred in some of the samples. These products were cloned and sequenced to confirm they were not of fungal origin (data not shown).

Ion Torrent sequencing and sequence analysis

Pooled bacterial and fungal PCR products were sequenced after emulsion PCR with the Ion OneTouch system and Ion OT2 400 kit (Life Technologies) on Ion 314 chips with Ion PGM Sequencing 400 Kit (Life Technologies) according to the manufacturer's instructions. The sequences were processed in mothur software package v. 1.38 (Schloss *et al.*, 2009) following the relevant parts of MiSeq SOP protocol (https://mothur.org/wiki/MiSeq_SOP; accessed in January 2017; (Kozich *et al.*, 2013). Sequences were quality-filtered using average quality of 25 and a window size of 50 bases, a minimum sequence length of 200 bp, a maximum length of 400 bp for bacteria and 410 bp for fungi, and further settings maxhomop=6, maxambig=0, pdiffs=1, and bdiffs=0. Bacterial sequences were aligned against Silva database v. 1.23 (Quast *et al.*, 2013). Chimeras were detected with Uchime (Edgar *et al.*, 2011) in mothur. Fungal ITS sequences were identified, ITS2 region was extracted and non-fungal sequences were removed with ITSx (Bengtsson-Palme *et al.*, 2013). After quality filtering, alignment for bacteria, removal of chimeras and non-target sequences, we had 121711 bacterial sequence reads and 279885 fungal reads. Reads were preclustered with setting diffs=2 (bacteria) or diffs=1 (fungi). The sequences were clustered into 97% operational taxonomic units (OTUs) using the average neighbor method for bacteria, and the vsearch algorithm for fungi, yielding 5242 bacterial and 1742 fungal OTUs after removal of singletons. Bacterial OTUs were classified against the Silva v. 1.23 database and fungi against the Unite database (Kõljalg *et al.*, 2013). Data was subsampled to the read number of the sample with the lowest number of reads, which was 1055 reads for bacteria and 1605 reads for fungi. Shannon diversity index and coverage were calculated in mothur. Coverage was 76 ± 6% for bacteria and 97 ± 1% for fungi. The sequence data were submitted to NCBI under BioProject accession PRJNA471306.

Statistical analyses

Chemistry and microbial biomass were compared with the successional stage using linear mixed models with R package nlme with successional stage as a fixed factor and deflation basin as a random blocking factor. Chemistry and biomass values except pH and Al were log transformed.

Bacterial and fungal sequence data were converted to relative abundances of OTUs and square root transformed. Only OTUs with 5 ≥ reads were included. Variation of community structure was visualized as non-metric multidimensional scaling (NMDS) plots with function metaMDS using Bray-Curtis distance measure. Multivariate dispersion between the successional stages was tested with betadisper (Bray-Curtis distances). Permutational analysis of variance (PERMANOVA, (Anderson, 2001, McArdle and Anderson, 2001) with adonis (Bray-Curtis distances) was used to compare community structure between the successional stages and with environmental variables (OM, pH, and in addition Al, Na and minimum and maximum temperature which did not correlate with OM and pH but differed with successional stage). We used Mantel test to estimate the correlation between microbial community structure and soil chemistry (Euclidean distances) and microbial community structure (Bray-Curtis distances) and vegetation (Bray-Curtis distances of square root transformed data). All multivariate analyses were carried out with the vegan package (v.2.4.2, (Oksanen *et al.*, 2017) in R. Significance tests were based on 999 permutations.

We identified bacterial and fungal OTUs characteristic to specific successional stages or combinations of stages by indicator species analysis with package indicspecies using function multipatt and correction for unequal site numbers (54). Only OTUs that occurred in the subsampled data at least 12 times, i.e., once in each sample of a successional stage, were included in the analyses. The combinations of stages included in the analyses were each successional stage on its own, all six stages together, all stages except sand, all stages except forest, all stages except sand and grass, all stages except heath and forest, sand+grass, moss+lichen, moss+grass, lichen+heath, heath+forest, sand+grass+moss, moss+lichen+heath, and lichen+heath+forest.(De Cáceres and Legendre, 2009). Instead of using all possible combinations, we selected the combinations to limit the number of tests and to focus on the combinations most relevant for the aims of the study. OTUs were considered indicator OTUs if their indicator value was >0.3 and $p < 0.05$. Significance was evaluated with a permutation test (999 permutations). P values were adjusted for multiple comparisons with function p.adjust (method “fdr”). Generalist OTUs present in all successional stages were defined at indicator values >0.5, because no p values are generated for taxa present in all sites. The identified indicator OTUs were grouped based on their taxonomic classification at order (bacteria) or class (fungi) level. Unclassified OTUs were included in these comparisons by re-clustering all reads assigned to unclassified bacteria or fungi in mothur at 75% sequence

similarity to represent phylum level similarity for bacteria and class level similarity for fungi (Tedersoo *et al.*, 2014).

To evaluate responses of bacterial and fungal indicator taxa to environmental variables, we used relative abundances of indicator OTUs grouped at order level (bacteria) and class level (fungi), including those unclassified 75% similarity groups that represented >0.4% of all unclassified OTUs. The tested environmental variables were those that best explained microbial community variation (OM, pH, Al for bacteria; OM, pH, minimum temperature for fungi). The analysis was carried out with the function traitglm in the package mvabund (v. 3.13.1, (Wang *et al.*, 2012) and function traitglm. The function fits fourth corner models to relate sites and their traits to species and species traits. When used without species trait data, it becomes a predictive model of responses of different OTUs to variation of environmental factors (57). The method used was glm1path, fitting a generalized linear model with LASSO penalty, which means that fourth corner efficients of OTUs not decreasing Bayesian information criterion (BIC) are forced to 0, i.e. no response to environmental variation, to simplify the model (Brown, A. M. *et al.*, 2014) (see supplementary materials for details).

All statistical analyses were carried out in R v.3.2.5. or v.3.4.0 (RCoreTeam, 2018).

Results

Soil chemistry and microbial biomass along Arctic sand dune succession

The increasing aboveground vegetation cover from bare sand to mountain birch forest was mirrored belowground in increasing soil OM content and decreasing pH (Pearson correlation $r=-0.78$, $p< 0.001$, Fig. 1). Organic matter content was low in the sand, grass, and moss stage soils (0.4±0.2%, mean±SD) but increased to 0.9±0.3% in the lichen stage and up to 4.5% ± 1.7% in the forest stage (Fig. 1A, Table S1). pH was highest in the grass stage (5.8 ± 0.2) and lowest in the forest stage (4.7 ± 0.1) (Fig. 1B). Soil contents of N, P, K, Ca, Mg, Fe and S correlated with OM (Table S1).

Microbial biomass measured as PLFAs per soil dry weight followed closely the OM gradient (Fig. 1D). The moss stage had similar OM content as bare sand, but its microbial biomass was higher than in the other sandy stages. When related to soil OM, microbial biomass in the moss stage reached a level similar to the lichen and vascular plant stages (Fig. 1C). Similarly, a shift to a higher proportion of fungal to bacterial biomass occurred in the moss stage (Fig. 1E). Overall, the proportion of fungi was highest in the heath stage.

Microbial community structure

Bacterial and fungal community structure based on sequencing (Fig. 2) and PLFA (Fig. S2) followed the order of the vegetation stages on the OM-pH gradient and differed with successional stage (PERMANOVA bacteria r^2=0.40 P<0.001, fungi r^2=0.38 p<0.001). The moss stage grouped with the other sandy stages (sand, grass) with partly overlapping communities, whereas the lichen stage grouped with the vascular plant stages (heath, forest) (Fig. 2). It should be noted that multivariate dispersion also differed between the successional stages (betadisper p=0.007 bacteria, p=0.001 fungi). The highest bacterial OTU richness (Fig. 1F) and Shannon diversity (Table S1) were detected in the heath stage, whereas fungal richness and diversity were highest though very variable in bare sand (Fig. 1F, Table S1).

The largest shift in bacterial community structure was detected from moss to lichen stage (Fig. 3). In contrast, the extent of fungal community change from stage to stage remained rather constant. Plant community composition showed increasingly larger shifts with succession, with the largest shift from lichen to heath. Soil chemistry changed most markedly from heath to forest. Overall, microbial community structure correlated better with soil chemistry than with plant species composition (Mantel test; bacteria chemistry r=0.71 vs. vegetation r=0.53; fungi r=0.40 vs. r=0.25, p=0.001 for all). Community variation of bacteria and fungi was best explained by OM content (Table 1), especially in the vegetated stages, which comprised the largest shifts in OM content. In the sandy stages, pH, OM, and Al content explained the highest share of bacterial and fungal variation.

Bacterial taxonomic distribution with succession

Overall, the most abundant bacterial phyla or classes were Alphaproteobacteria, Actinobacteria, and Acidobacteria (Fig. 4A, Fig. S3). The most abundant bacterial OTU belonged to the candidate phylum WD272 (WPS-2). This phylum was the fourth or fifth most abundant phylum in the sandy and lichen stages (on average 4.6-8.1%), decreasing in the heath (3.5%) and forest (0.9%) (Fig. 4A, Fig. S3). When related to microbial biomass, the abundance of WD272 increased in the moss stage (Fig. 5). WD272 showed abundant indicator OTUs for the sandy stages but also less abundant forest-specific indicators, which possibly explains why WD272 indicators could not be related to environmental variables (Fig. 6). On the other hand, most indicator groups specific for the sandy stages, such as Cytophagales, Deinococcales, Acidobacteria subgroup 4, Pseudonocardiales (Actinobacteria), Oligoflexales (Deltaproteobacteria) and unclassified group 9 were strongly associated with low OM content. Cyanobacteria, three Chloroflexi groups and unclassified group 2 were indicators for the moss and lichen stages and associated with both low OM and low pH, which reflected their occurrence at the pH shift in the succession. Another sign that the moss soil was a bacterial transition stage was that it showed unclassified clusters typical to both sandy and vegetated stages (Fig. S5A). Most indicator groups for the forest stage were indicators also for the other vegetated stages. For example, Acidobacteria subgroup 2 associated with low pH and subgroup 6 associated with high OM appeared in the moss and lichen stages and had indicators also in the heath and forest stages. Other bacterial indicators in the vegetated stages associated with low pH, high OM or both included Planctomycetes (WD2101 soil group), Frankiales and Streptomycetales (Actinobacteria), and Zanthomonadales (Gammaproteobacteria). Acidobacteriales (Acidobacteria) and Corynebacteriales (Actinobacteria) occurred throughout the gradient but increased in relative abundance towards the forest and were associated with low pH. The overall abundance of Acidobacteria increased with decreasing pH (Pearson correlation $r = -0.81$, $p<0.001$).

Fungal taxonomic distribution with succession

The fungi at class level were dominated by Ascomycetes, followed by Basidiomycetes, unclassified fungi, and smaller shares of Zygomycota, Rozellomycota, Chytridiomycota, and Glomeromycota (Fig. 4B, Fig. S4). The most abundant Ascomycota class was Leotiomycetes, followed by unclassified ascomycetes and Dothideomycetes. Bare sand had the highest

relative abundance of unclassified fungi up to 35% (Fig. 4B, Fig. S4), and several unclassified clusters were specific to the sandy stages (Fig. S5B) and associated with high pH or low OM (Fig. 7). Other strong indicators of sandy stages included Helotiales and Thelebolales (Leotiomycetes), and Polysporales and Boletales (Agaricomycetes). Lecanoromycetes, containing lichenized fungi, was most abundant in the moss stage (Fig. 4B). Similarly to bacteria, the fungal groups that were the best indicators of the moss and lichen stages were associated with low OM and low pH or both, including Lecanoromycetes (Lecanorales, Agyriales, Ostropomycetidae), Agaricomycetes (Cantharellales, Atheliales, Agaricales), and Dothideomycetes (Pleosporales, Capnodiales). The most abundant fungal OTU overall and one of the fungal generalists (Table S2) was classified as *Pezoloma ericae* (Leotiomycetes; also known as *Rhizoscyphus ericae*) known to form mycorrhiza with ericoid plants such as the dominant heath species *Empetrum nigrum* (Read, 1991, Smith and Read, 2008). Accordingly, this OTU was particularly dominant in the heath (34±5% of reads). Its relative abundance was lowest in bare sand (2±1%) and the grass stage (12±14%), but in the moss and lichen stages it increased to 26±16% despite the lack of known host plants. In the forest, its relative abundance was lower than in the cryptogam-dominated stages (17±14%). Venturiales (Dothideomycetes) was an indicator of the heath stage. More general indicator groups for the vegetated stages associated with low pH, high OM or both were Archaeorhizomycetes, Mortierellales (Zygomycotina), Rozellomycota, Russulales, Sebacinales and Thelophorales (Agaricomycetes), and unclassified groups 1 and 11. Hysteriales (Dothideomycetes) and Geoglossales (Geoclossomycetes) showed high OM associated indicator OTUs exclusive for the forest stage.

Discussion

We hypothesized that the cryptogam-dominated stages of an Arctic sand dune succession represent key ecosystem transitions towards vascular vegetation. Supporting this hypothesis, the bacterial and fungal communities in the lichen stage resembled the communities in the later vascular plant dominated stages. Although the moss stage microbial communities overlapped with the earlier sandy stages, the shift towards vascular plant covered soils started with the appearance of moss: Microbial biomass per OM and the proportion of fungi increased in the moss stage, and later stage microbial groups appeared despite absence of their presumed host plants.

The dune sand is a harsh habitat with low carbon and nitrogen availability. The initial microbial colonizers must be able to tolerate the oligotrophic conditions either by using the low levels of allocthonous organic matter (Hodkinson *et al.*, 2002) or as autotrophs (Nemergut *et al.*, 2007, Fierer *et al.*, 2010). Carbon fixed by these initial colonizers then allows the establishment of the first heterotrophs. However, comparison of biomass per OM with biomass per soil dry weight (Fig. 1) shows that the microbial biomass increased in the moss stage when OM potentially available for heterotrophs still remained low. A characteristic group for the early stages from bare sand to lichen was candidate phylum WD272, currently known as WPS-2 and recently named *Candidatus* Eremiobacteraeota, 'desert bacterial phylum' (Ji *et al.*, 2017). Studies on mosses, volcanic ice caves and Antarctic desert soils have proposed members of WD272 to be autotrophs fixing carbon with ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo; (Tebo *et al.*, 2015, Ji *et al.*, 2017, Holland-Moritz *et al.*, 2018). Based on metagenome-assembled genomes, WD272 members have been proposed to use atmospheric H_2 and carbon monoxide (CO) as energy sources (Ji *et al.*, 2017) or to be anoxygenic photoautotrophs (Holland-Moritz *et al.*, 2018). As another potential trace gas oxidizing group, Chloroflexi in the moss and lichen stages were classified as Ktedonobacteria, a class with several CO-oxidizers (King and King, 2014). Many Chloroflexi can use H_2 and CO to persist under low nutrient conditions (Islam *et al.*, 2019). The potential of WD272 and Chloroflexi to survive in the harsh early stages as autotrophs or trace gas oxidizers and their increased biomass in the moss and lichen stages (Fig. 5) leads us to speculate that they form a basis of biomass accumulation in the Arctic sand dune succession. These microbes may therefore facilitate cryptogam cover development and further vascular vegetation development (Connell and Slatyer, 1977, Brooker *et al.*, 2008).

Many moss stage microbial groups that were rare or absent in the earlier sandy stages are linked to biological soil crusts. Biocrusts are consortia of microbes, mosses, and lichens that are recognized as the major stabilizing factor in early successional soils worldwide (Weber *et al.*, 2016). The rhizoids of *Polytrichum* mosses themselves stabilize soil, facilitate N_2 fixation, and provide OM to the soil (Bowden, 1991). The moss stage showed the highest proportion of Cyanobacteria, N_2-fixing early colonizers and components of biocrusts (Weber *et al.*, 2016), and of Lecanoromycetes (lichenized fungi, Ascomycetes). Lichens were therefore detected in the belowground soil before they were detected on the soil surface.

Chloroflexi were distinctive indicators for the cryptogam stages, and in dryland and grassland ecosystems they occur in moss and lichen biocrusts and in sub-crust soil (Kuske *et al.*, 2012, Navarro-Noya *et al.*, 2014, Maier *et al.*, 2018). Although *Polytrichum* biomass was small in the moss stage, *Polytrichum piliferum* appeared to have a disproportionate effect at the ecosystem level, suggesting it is a keystone plant species in Arctic sand dunes. The mechanism could be soil stabilization by rhizoids and moss-associated, soil crust-forming microbes. Traditionally, grasses are credited with the initial physical stabilization of sand in dune succession (e.g. (Olson, 1958, Ellenberg, 2009). Inspecting soil microbial community structure, it seems that in these Arctic dunes the grass *Deschampsia flexuosa* may not drive the belowground ecosystem succession. Based on the results, we speculate that *Polytrichum* mosses could participate in the physical stabilization of Arctic inland dunes and direct succession towards vascular plant cover. Verifying this proposition requires further studies on dune *Polytrichum* and its rhizoids.

The relative abundance of the dominant fungal OTU *Pezoloma ericae* increased in the moss stage to levels similar to those in the later stages despite no host plants detected. In addition to ericoid mycorrhiza, *P. ericae* forms mycorrhiza-like associations in liverwort rhizoids (Pressel *et al.*, 2010, Kowal *et al.*, 2018), and it has been found as an Antarctic moss endophyte (Zhang, T. *et al.*, 2013). However, no liverworts were visible among the moss at our site, and no association of *P. ericae* with *Polytrichum* rhizoids is known. Nevertheless, we interpret the increase of *P. ericae* in the moss stage as a possible sign of fungal community shift towards a stage where vascular plant establishment is possible. An alternative explanation would be that propagules of *P. ericae* accumulate as the early succession proceeds.

We hypothesized that bacteria would be more prominent and diverse in early succession and fungi in late succession but found no clear support for this. The proportion of fungi in PLFA data, assumed to represent living biomass (Zhang, Y. *et al.*, 2019), increased already in the moss stage. Bacterial richness peaked in the heath and fungal richness in the bare sand, and neither correlated with ecosystem productivity defined as plant richness or soil OM. In general, richness and diversity link to ecosystem functions through the general effects of diversity on ecosystem stability and productivity (Loreau and de Mazancourt, 2013). Plant

richness peaks in the middle or late succession (Bonet and Pausas, 2004, Peyrat and Fichtner, 2011, Prach *et al.*, 2014). For microbes, few general patterns of richness or diversity with primary succession have emerged. In very young soils, richness is often low and then increases and stabilizes or even decreases in later stages (Jackson, 2003, Nemergut *et al.*, 2007, Fierer *et al.*, 2010, Williams *et al.*, 2013, Jiang *et al.*, 2018). However, primary succession gradients where both bacteria and fungi have been studied show conflicting patterns. In glacier forefield successions, bacterial richness has increased or remained stable and fungal richness remained stable through succession (Brown, S. P. and Jumpponen, 2014, Jiang *et al.*, 2018). In a lava flow succession, fungal richness increased but bacterial richness was not related to terrain age (Cutler *et al.*, 2014). In a salt marsh chronosequence, fungal richness was not consistently stable with soil age due to a dominant OTU at one stage, whereas bacterial richness first remained stable and then decreased (Dini-Andreote *et al.*, 2014, Dini-Andreote *et al.*, 2016). Our results are best in line with studies showing stable microbial richness through primary succession, as shown for fungi in a dune succession (Roy-Bolduc *et al.*, 2015).

The low bacterial richness in the bare sand stage can be related to the oligotrophic conditions; richness already increased with the scattered presence of the grass *D. flexuosa*. Pioneering plants can influence bulk soil when plants are of sufficient size (Tscherko *et al.*, 2004, Edwards *et al.*, 2006, Miniaci *et al.*, 2007). The high fungal richness in bare sand together with the low fungal biomass may represent dormant fungi, spores or hostless biotrophic fungi (Jumpponen, 2003, Rime *et al.*, 2015). On the other hand, our observation that the unclassified fungi can be grouped into class level clusters that occurred consistently in replicate samples and across the sandy successional stages also hints that the fungal community in bare sand may be in one way or other selected.

The high bacterial richness in the heath coincided with the highest fungal biomass and highest occurrence of *P. ericae*, presumably forming mycorrhizae with the dominant ericoid *E. nigrum*. We speculate that the high bacterial richness could be promoted by resources and niches provided by fungal mycelium and *Empetrum* litter, despite its high phenolic content and proposed adverse properties on microbial activity (Gallet *et al.*, 1999, Wardle *et al.*, 1998). In the forest, where the highest microbial richness would be expected if richness

correlated with OM, plant species richness and productivity, bacterial diversity was lower than in the heath, possibly due to the environmental filtering effect of the low pH in the forest soil (Tripathi *et al.*, 2018). The varied responses of bacterial and fungal richness and diversity to primary succession highlight the need to cover successional gradients with different parent material, vegetation and soil chemistry to understand microbial community patterns with succession.

Both bacterial and fungal community variation were better explained by soil chemistry than by plant community composition. Effects of soil chemistry on microbial communities can override biotic factors such as plant richness (Chen *et al.*, 2016, Jiang *et al.*, 2018), and the role of vegetation is emphasized in successional gradients where soil chemistry does not change markedly (Knelman *et al.*, 2012, Cutler *et al.*, 2014). Soil chemistry and vegetation are strongly intertwined in succession, and the present gradient from bare sand to mountain birch forest represents the common pattern where organic matter increases and the soil acidifies with increasing plant cover (Odum, 1969, Merilä *et al.*, 2010). However, the largest microbial community shifts did not align with the largest shifts in soil chemistry or vegetation (Fig. 3). No measured soil chemistry variable changed markedly with the large bacterial community shift between moss and lichen stages (Fig. 3). Both cryptogam stages showed indicator microbial groups associated with low OM and low pH. These results suggest that the microbial shift from moss to lichen is due to unmeasured effects of the lichen crust formation. Such factors could be better long-term moisture retention by the lichen crust than the sparse mosses in the moss stage, microenvironments provided by lichen crumbs in the belowground soil (de los Ríos *et al.*, 2011) or the different chemical composition and secondary metabolites of mosses versus lichens.

Conclusions

This study on inland sand dunes illuminates bacterial and fungal succession in a less studied Arctic soil ecosystem. Our results highlight the importance of considering not only vascular plants but also cryptogams, i.e. mosses and lichens and particularly the potential dune key stone species *Polytrichum piliferum*, when defining the plant-microbe interactions in succession. Taken together, the results indicate that the microbiological transition towards the

later stages with vascular vegetation and soil stabilization starts at the moss stage. The biomass representing early stage microbes increased and soil crust-associated microbes and later stage fungi appeared, although the plant cover and OM were still low. Whether the sand stage microbes initiate the transition by facilitating the appearance of moss or whether the sand microbes linger and proliferate in the soil after the moss has appeared should be determined in future experiments. This work adds to evidence that bacterial and fungal richness are not necessarily related to soil ecosystem productivity or successional change in plant species richness (Waldrop *et al.*, 2006, Porazinska *et al.*, 2018, Delgado-Baquerizo and Eldridge, 2019). Furthermore, soil chemistry and the effects of plants on microbial resources and niches are important factors driving succession. However, the key transitions in soil chemistry, plant community and microbial community did not align, suggesting additional unrecognized drivers. Bacterial and fungal indicators identified in this work, including novel unclassified diversity, can signpost ecosystem transitions towards vascular plant cover. Understanding the drivers of the shift from bare to vegetated is crucial for restoration of vegetation on cold-climate soils suffering from erosion and vegetation loss under changing environment.

Acknowledgements

We thank Helena Jauhiainen, Riitta Nissinen and Manoj Kumar for help with sampling and DNA extraction, Anita Mäki for assistance with sequencing, and Outi Manninen for the vegetation inventory. This work was funded by Maj and Tor Nessling Foundation and the Academy of Finland (project 287545 to M.-M.K.).

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Figure legends

Figure 1 (A) Organic matter, (B) pH, (C) microbial biomass based on phospholipid fatty acids (PLFAs) per soil organic matter (OM) content, (D) microbial biomass per gram soil dry weight (dw), (E) ratio of fungal to bacterial biomass based on PLFA, and (F) bacterial 16S rRNA gene (upper row, darker colours) and fungal ITS amplicon (lower row, lighter colours) OTU numbers in sand dune successional stages. Data are averages of four deflation basins and three replicates per basin. Ends of whiskers represent minimum and maximum values excluding outliers. Different letters indicate significant differences at $p<0.05$.

Figure 2 Non-metric multidimensional scaling (NMDS) plots of (A) bacterial communities based on 16S rRNA gene amplicon sequencing (stress=0.10) and (B) fungal communities based on ITS2 region amplicon sequencing (stress=0.13) in sand dune successional stages. Data points represent three replicate samples from four deflation basins per successional stage.

Figure 3 Average distances of successional stage pairs for bacterial, fungal and plant communities and for soil chemistry. Different letters indicate significant differences at $p<0.05$. Ends of whiskers represent minimum and maximum values excluding outliers. Shading indicates the largest transitions.

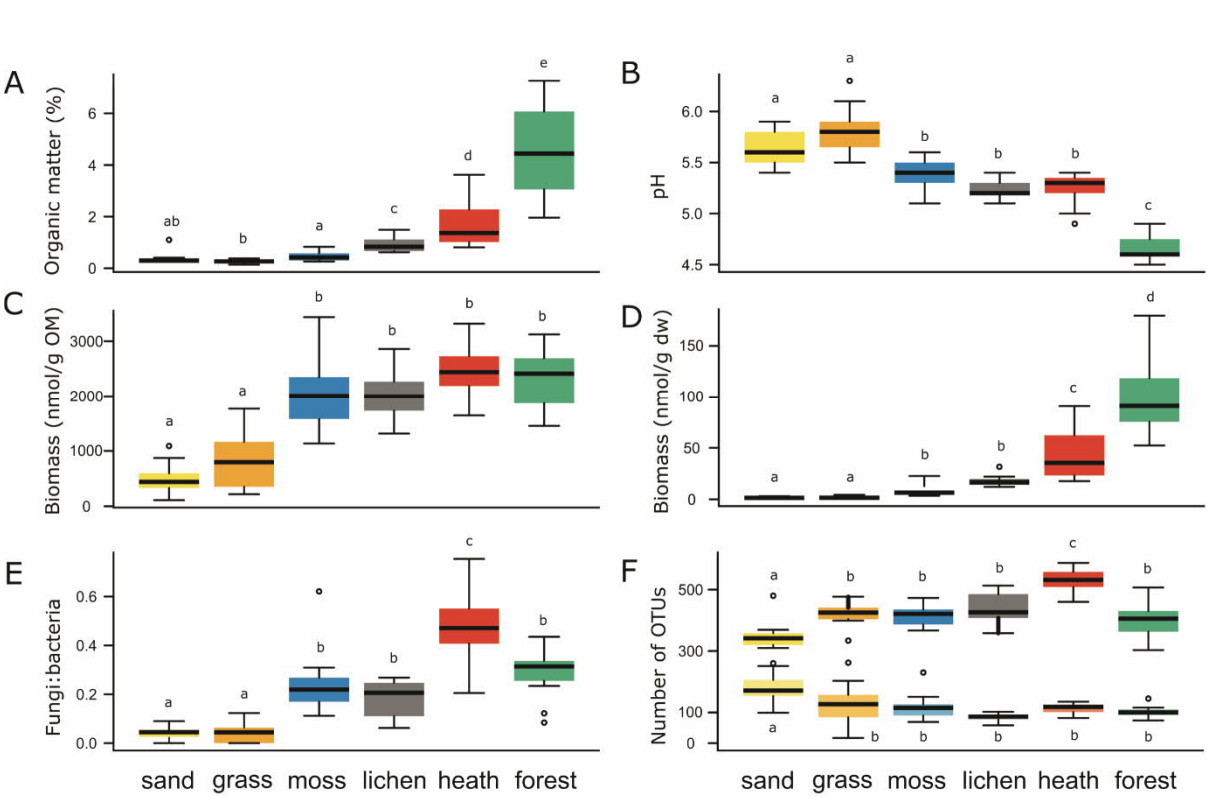
Figure 4 (A) Phylum level (class level for Proteobacteria) community structure of bacteria based on 16S rRNA gene sequencing and (B) class-level community structure of fungi based on ITS2 region sequencing with sand dune successional stage. Data are averages of four deflation basins and three replicates per basin. For values of individual samples and groups combined in ‘other’, see Fig. S3 and S4.

Figure 5 Biomass of selected early and cryptrogam stage bacteria with sand dune successional stage. Relative abundances from sequencing data were converted into biomass by multiplying with the total microbial PLFA concentration. Horizontal lines indicate mean values. Labelled points are outliers that would be outside the plot area.

Figure 6 Bacterial indicator OTUs for successional stages grouped at order level. Thicker horizontal lines show where the successional stage changes, and each group is placed in the stage where it was the most abundant. Only groups with total abundance of indicator OTUs >0.05% are shown. The heatmap on the right shows fourth corner coefficients describing the association of the indicator OTU groups with environmental variables (env. variables). Ind. OTUs, number of indicator OTUs; rel. abund., relative abundance; OM, organic matter.

Figure 7 Fungal indicator OTUs for successional stages grouped at class level. Thicker horizontal lines show where successional stage changes, and each group is placed in the stage where it was the most abundant. A, Ascomycota; B, Basidiomycota; R, Rozellomycota; Z, Zygomycota; cls, class; ord, order; Inc. sed., Incertae sedis. Only groups with total abundance of indicator OTUs >0.05% are shown. The heatmap on the right shows fourth corner coefficients describing the association of the indicator OTU groups with environmental variables (env. variables). Ind. OTUs, number of indicator OTUs; rel. abund., relative abundance; OM, organic matter, minT, minimum soil temperature.

Figures



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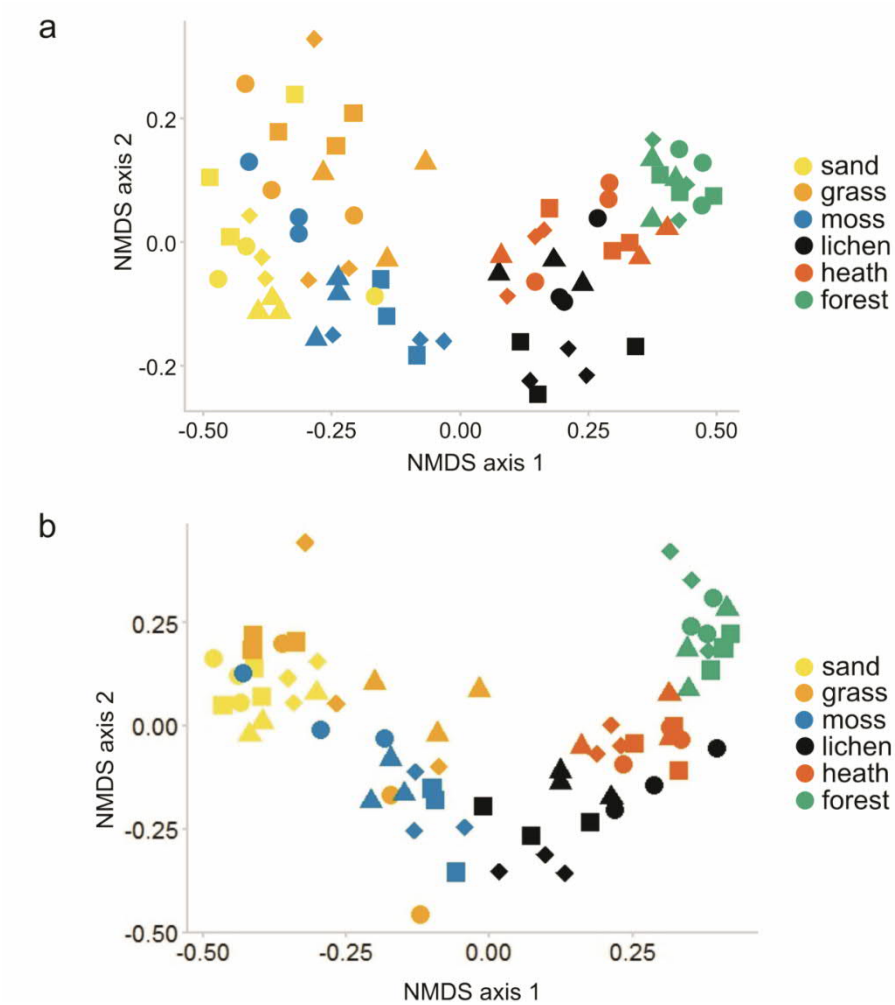

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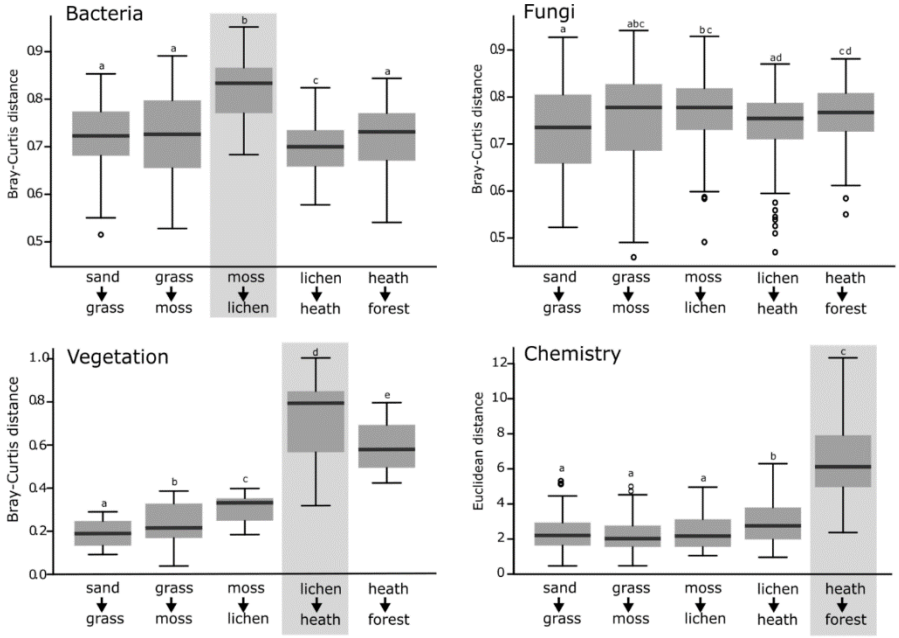

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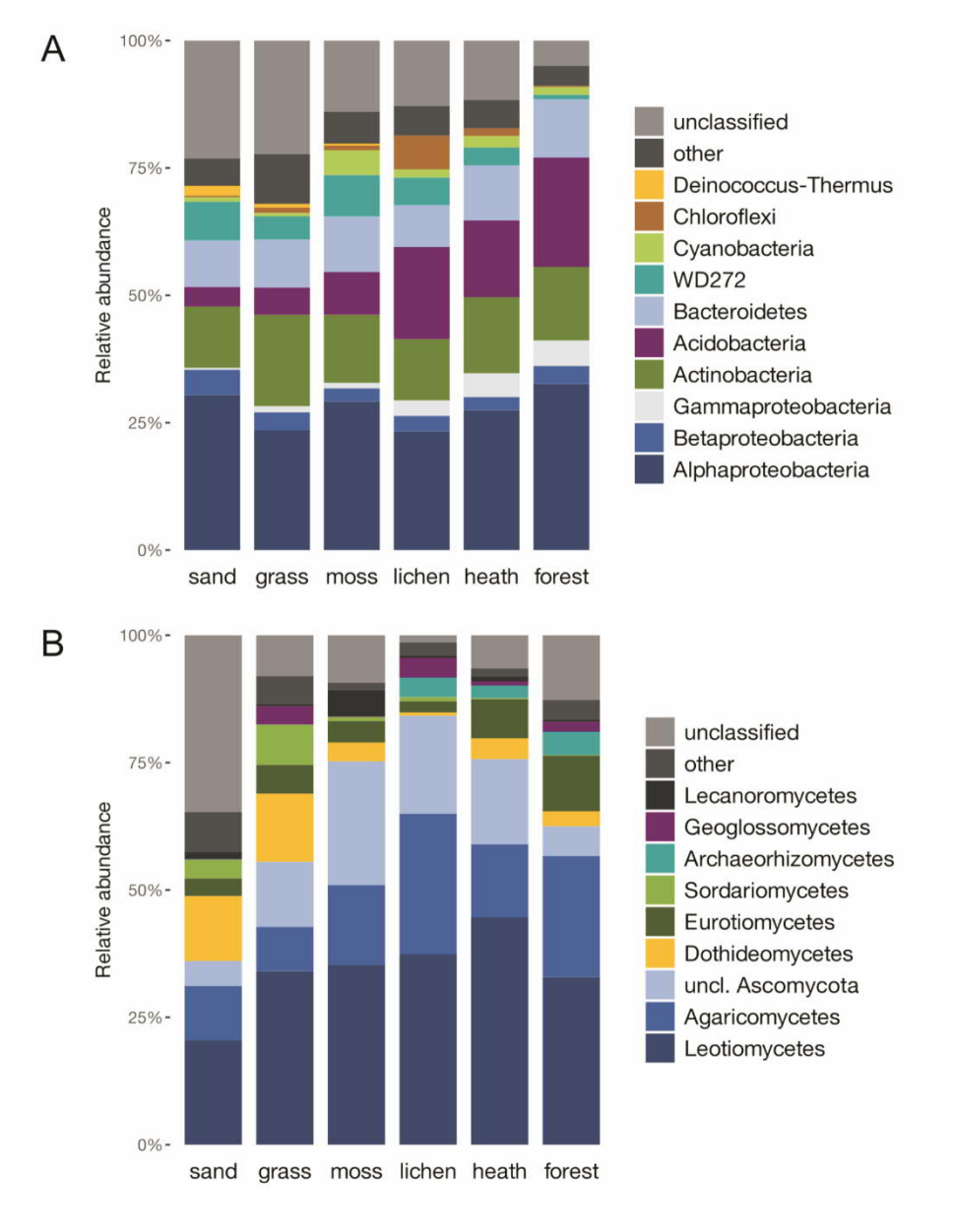

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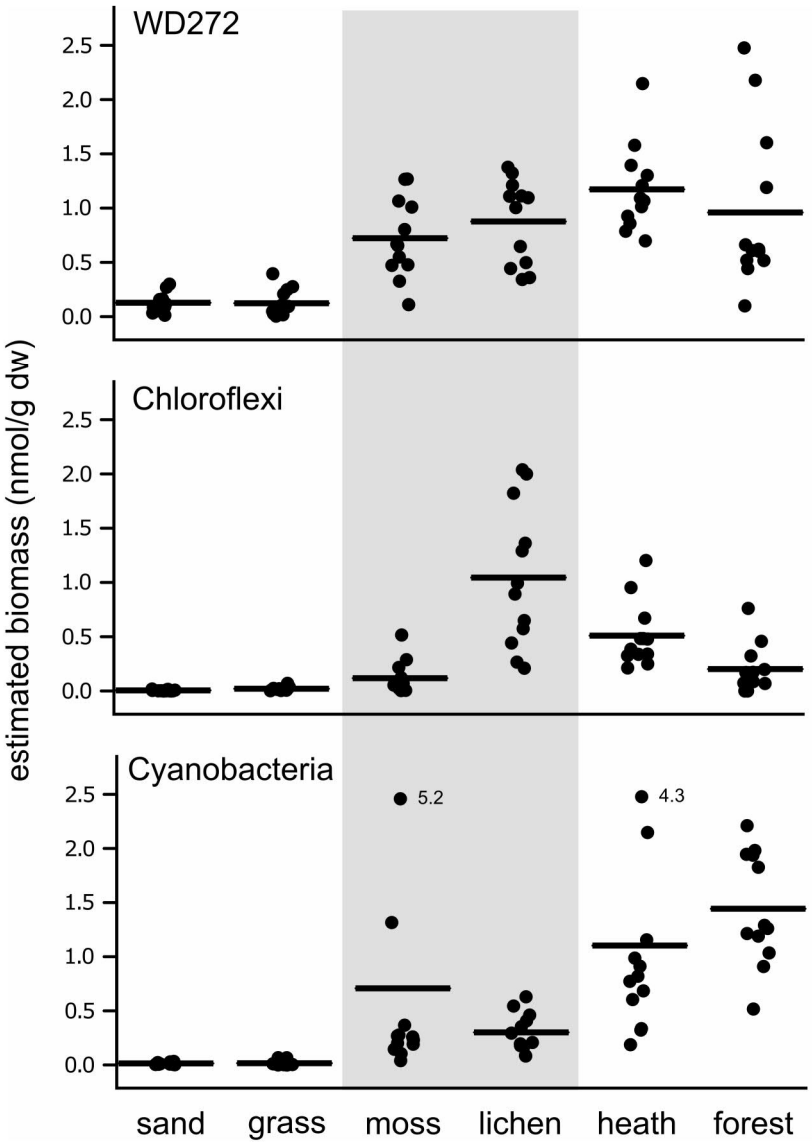

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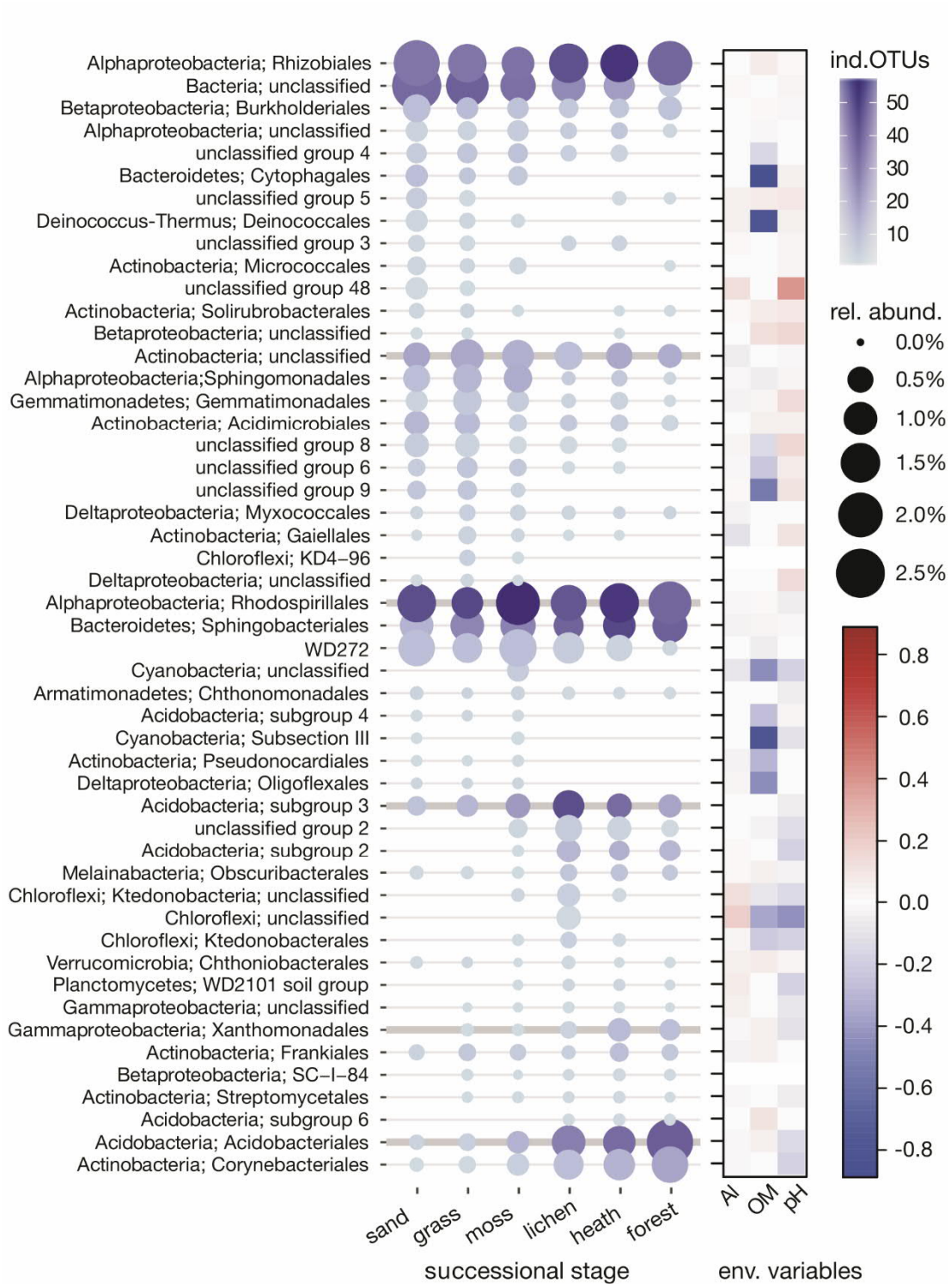

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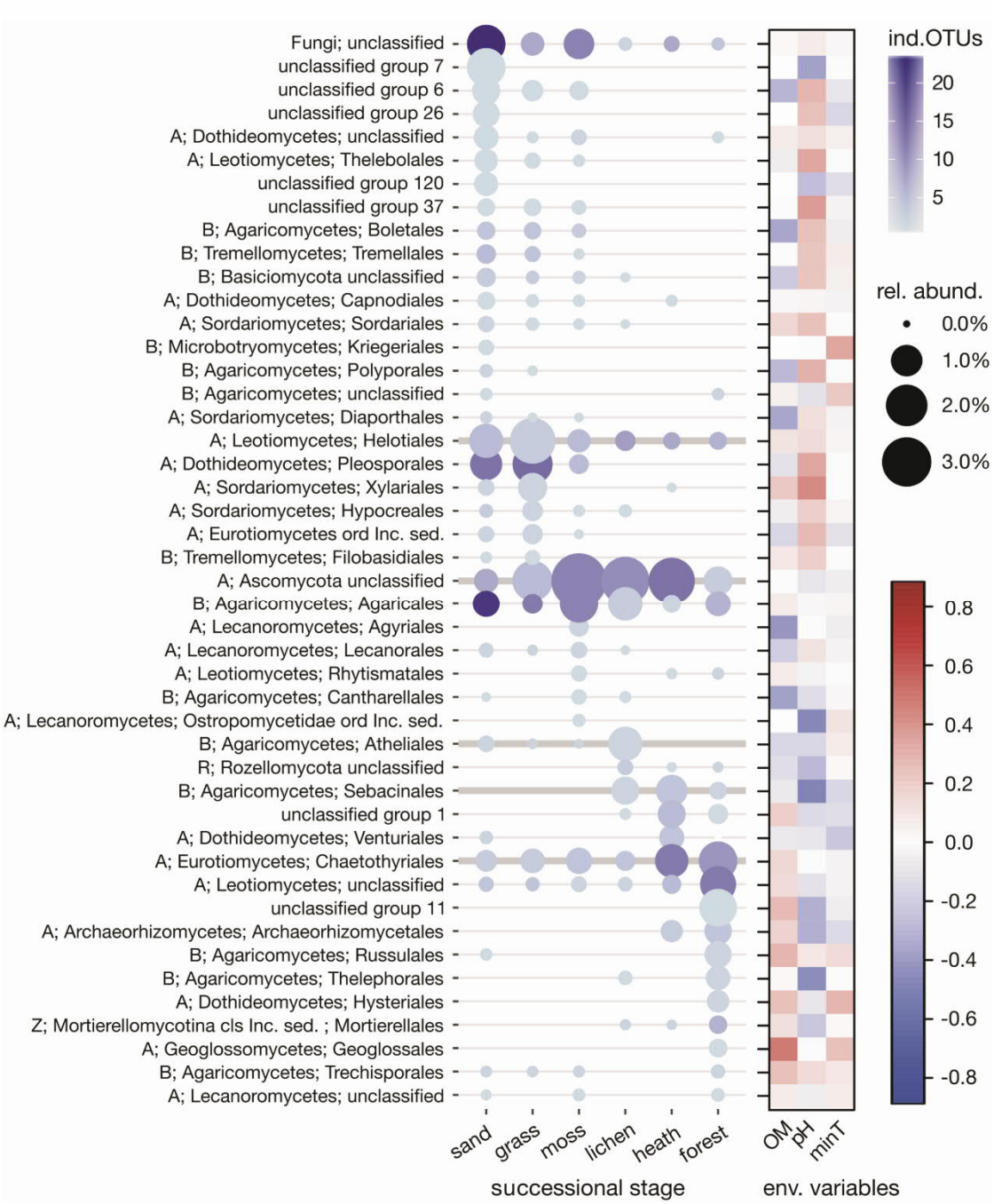

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