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

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Summary

- Primary succession models focus on aboveground vascular plants. However, the prevalence of mosses and lichens, i.e. cryptogams, suggests they play a role in soil successions. Here, we explore whether effects of cryptogams on belowground microbes can facilitate progressive shifts in sand dune succession.
- We linked aboveground vegetation, belowground bacterial and fungal community, and soil chemistry in six successional stages in Arctic inland sand dunes: bare sand, grass, moss, lichen, ericoid heath and mountain birch forest.
- Compared to the bare sand and grass stages, microbial biomass and the proportion of fungi increased in the moss stage, and later stage microbial groups appeared despite the absence of their host plants. The microbial communities of the lichen stage resembled the communities in the vascular plant stages. Bacterial community correlated better with soil chemistry than with vegetation, whereas the correlation of fungi with vegetation increased with vascular vegetation.
- Distinct bacterial and fungal patterns of biomass, richness, and plant-microbe interaction showed that the aboveground vegetation change structured the bacterial and fungal community differently. The nonalignment of aboveground vs. belowground changes suggests that cryptogams can drive succession towards vascular plant dominance through microbially mediated facilitation in eroded Arctic soil.

Keywords: Arctic soil, bacterial community, cryptogam, erosion, 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 (plant species pool present, order of arrival, species interactions). Although focusing on plants, most models of terrestrial primary succession overlook bryophyte communities (e.g. Connell and Slatyer, 1977; Baker and Walford, 1995), whereas 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).

Ecosystem succession includes stochastic elements for instance through priority effects and, on the other hand, deterministic influences such as competition and facilitation (Måren *et al.*, 2018). Cryptogams, here mosses and lichens, may have diverse but unexplored competitive and facilitative effects on the development of vascular plant and belowground microbial communities. Firstly, dispersal of cryptogams 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 carbon 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). Moss rhizoids and litter provide carbon to the belowground soil (Bowden, 1991), and mosses can release carbon also in drying and wetting cycles (Wilson and Coxon, 1999). Many lichens characteristic of primary succession fix atmospheric nitrogen in specific structures called cephalodia (Vitousek, 1994), whereas moss leaves are a habitat of N_2-fixing bacteria (DeLuca *et al.*, 2002; Arróniz-Crespo *et al.*, 2014). 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).

Despite the fact that microbes are the first colonizers of any barren surface and modify soil chemistry during succession, there are only few models predicting successional trajectories of bacteria and fungi (Jackson, 2003; Fierer *et al.*, 2010 Dini-Andreote *et al.*, 2015; Tripathi *et al.*, 2018; Ortiz-Álvarez *et al.*, 2018). Belowground 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 the vegetation (Brown and Jumpponen, 2014; Jiang *et al.*, 2018). 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 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 an ecosystem based on the biomass production by the resident plants is associated with changes in the microbial community (Bardgett and Walker, 2004; Edwards *et al.*, 2006; Blaalid *et al.*, 2012; Knelman *et al.*, 2012). Along succession, the 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). These results do not, however, fully predict vegetation development in succession. We suggest that belowground changes associated with cryptogams are a key factor in the ecosystem shift from early to late succession. We link belowground community development with that aboveground and expect the interaction strength to vary and that the community changes do not take place in complete synchrony as is inferred in facilitation models (Brooker *et al*., 2008).

Ecosystems are declining due to soil erosion and loss of vegetation cover (Montgomery, 2007; Vanwalleghem *et al.*, 2017), and it is critical to identify the key factors promoting shifts between non-vegetated and vegetated stages in succession. Identifying microbial and plant communities that signal for the shift from autotrophic to mainly heterotrophic microbial biomass production can help identify factors that drive the shift back to vascular plant cover, soil stabilization, and a productive ecosystem. One example of areas where vegetation has

been lost due to environmental changes and lack of recovery are Arctic inland sand dunes. 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 basin, including moss- and lichen-dominated areas. In temperate inland dunes, the cryptogam stages have been identified as pioneer vegetation stages of the succession (Sparrius *et al*., 2012). Previous work comparing the start and end points of the Arctic inland dune succession proposed that bacteria and fungi may show distinct successional patterns (Poosakkannu *et al.*, 2017). The role of the cryptogam stages as part of successional trajectories above- and belowground has not been elucidated, although the prevalence of lichens and mosses suggests they are important.

In this study, we explored whether the belowground and aboveground community changes take place in synchrony in Arctic inland sand dune succession. We hypothesized that, compared to bare soil, the cryptogam-covered stages contain belowground soil microbial community more similar to the stages covered by vascular plants. Such nonalignment of belowground vs. aboveground communities could indicate that the cryptogam stages drive succession towards vascular plant dominance through microbially mediated facilitation. Secondly, we compared the belowground responses of two microbial groups – bacteria and fungi - to successional stages defined by aboveground vegetation. We hypothesized that i) in comparison to fungi, bacteria would be more influenced by cryptogam-associated changes in the soil chemical environment because of their limited saprophytic capacity and potential autotrophy, and ii) as powerful and obligate decomposers, fungal community would respond particularly to the appearance of vascular vegetation and consequently organic matter accumulation. Such a pattern would indicate differences in the way vegetation change and plant-microbe interaction structure bacterial vs. fungal communities along the succession. By linking the aboveground vegetation, the belowground microbial communities, and the

associated soil chemical environment, we aimed to define the multi-faceted role of cryptogams in Arctic 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. 1a). Although the soil in all the stages was aeolian sand, in three stages the largest part of the surface was mostly exposed sand (92-100 % sand), while in the other three stages vegetation covered the sand (0-52 % sand). The sand-exposed stages (‘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 the 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).

158 We sampled soil in each successional stage in four of the basins in July 2011. To take into
159 account that plants and microbes vary at different spatial scales, the soil samples were
160 collected in triplicate. The samples were taken from the depth of 0-10 cm with a 12.5 cm ×
161 12.5 cm soil corer (72 samples in total: six successional stages × four basins × three soil cores
162 per 1×1 m study plot). Soil was sieved with a 4-mm sieve and frozen at -20°C.

163

164 **Soil chemistry and temperature**

165 Total C and N of soil organic matter, soil P, Ca, K, Na, Fe, Mn, Al, and Mg contents, and pH
166 were measured as detailed in Methods S1. Soil temperature at the 10 cm depth was recorded
167 every two hours with Hobo Temp External H08-002-02 loggers coupled with TMC6-HD soil
168 temperature sensor during 2009-2015. We defined maximum temperature as the average
169 temperature of the warmest month (July) and the minimum temperature as the average of the
170 two coldest months (January and February).

171

172 **Phospholipid fatty acid (PLFA) analysis**

173 Phospholipid fatty acids were extracted as described previously (Francini *et al.*, 2014) from
174 freeze-dried soil (15 g for sand and grass stages, 10 g for moss, 8 g for lichen, 5 g for heath
175 and 3 g for forest stage corresponding to OM content). Briefly, lipids were extracted with
176 chloroform:methanol:citrate buffer (Bligh and Dyer, 1:2:0.8 v/v/v), fractionated using silica
177 columns (Bond Elut LRC, Varian), and phospholipids were methylated with mild alkaline
178 methanolysis. The resulting fatty acid methyl esters were analyzed by gas chromatography
179 and PLFAs identified based on a bacterial fatty acid standard mixture (Supelco, Bellefonte,
180 PA, USA) and fatty acids from reference strains. For community analysis, data was expressed
181 as peak area percentage of a single PLFA of the total area of the profile, and for biomass
182 analysis as nmol of PLFAs per gram dry weight (gdw) of soil or per gram OM. The sum of
183 PLFAs i15:0, a15:0, 15:0, i16:0, 16:1ω9, i17:0, a17:0/17:1ω8c (eluted in the same peak),
184 17:0, cyclo-17:0, 18:1ω7c/ω9t (eluted in the same peak) and cyclo-19:0 were used to
185 represent bacterial biomass and PLFA 18:2ω6 fungal biomass. Sum of all these PLFAs was
186 used to represent total microbial biomass. Biomass of selected bacterial groups was

187 calculated by multiplying the total microbial biomass of a sample with the relative abundance
188 of the bacterial group in the sample based on 16S rRNA gene sequencing.

189

190 **DNA extraction and PCR**

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

195

196 Bacterial and fungal PCRs were carried out as two-step PCR where the second step
197 introduced barcodes and adapters for Ion Torrent sequencing. The first step of bacterial 16S
198 rRNA gene PCR (V1-V2 region) was carried out with primers 27F (5'-
199 AGAGTTTGATCCTGGCTCAG-3') and 338R (5'-TGCTGCCTCCCGTAGGAGT-3').The
200 first step of intergenic transcribed region (ITS) PCR for fungi was carried out with primers
201 fITS7 (5'-GTGARTCATCGAATCTTTG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-
202 3') (Ihrmark *et al.*, 2012), where the fITS7 primer contained an M13 adapter for the second
203 step (Mäki *et al.*, 2016).

204

205 The second step primers introduced barcodes and Ion Torrent adapters A and P1. For
206 bacterial 2nd step PCR, the forward primer was adapter A-barcode-27F, and the reverse
207 primer adapter P1-338R. For the fungal 2nd step PCR, the forward primer was adapter A-
208 barcode-M13 adapter and the reverse primer adapter P1-ITS4. Details for PCR are given in
209 Methods S1. After the second PCR, the duplicate products were pooled, purified with
210 Agencourt AMPure XP purification system (Beckman Coulter), and quantified with Qubit
211 fluorometer and Qubit dsDNA HS kit. Equimolar amounts (10 ng) of products were pooled
212 for sequencing.

213

214 **Ion Torrent sequencing and sequence analysis**

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

240

241 **Statistical analyses**

242 Chemistry and microbial biomass were compared among the successional stages using linear
243 mixed models with R package nlme and function lme with successional stage as a fixed
244 factor and deflation basin as a random blocking factor. Chemistry and biomass values except
245 pH and Al were log transformed to normalize their distribution.

246

247 Bacterial and fungal sequence data were converted to relative abundances of OTUs and
248 square root transformed to achieve Hellinger transformation that reduces the weight of OTUs
249 with low counts and zeros. Only OTUs with 5 ≥ reads were included. Variation of
250 community structure was visualized as non-metric multidimensional scaling (NMDS) plots
251 with function metaMDS using the Bray-Curtis distance measure. Dispersion was visualized
252 in the NMDS plots as ellipses based on standard deviation of sample scores using the
253 ordiellipse function. Multivariate dispersion between the successional stages was tested with
254 betadisper (Bray-Curtis distances). Permutational analysis of variance (PERMANOVA,
255 Anderson, 2001; McArdle and Anderson, 2001) with the adonis2 function (Bray-Curtis
256 distances) was used to compare community structure between the successional stages and in
257 response to environmental variables. In the univariate model for successional stage, the stage
258 was nested within deflation basin. The multivariate PERMANOVA model for environmental
259 variables included OM-pH gradient and in addition Al and minimum soil temperature, which
260 did not correlate with OM and pH (Pearson $|r|<0.7$) but differed with successional stage. The
261 OM-pH gradient was defined with detrended correspondence analysis, and the first axis
262 scores were used as a variable in PERMANOVA. Impact of the individual environmental
263 variables was determined with marginal tests. Transitions between successional stages were
264 quantified by calculating average distances of the successional stages from bare sand for
265 bacterial and fungal community structure (Bray-Curtis distances), vegetation (Bray-Curtis
266 distances of square root transformed data) and soil chemistry (Euclidean distances). Using the
267 same distance measures, we carried out Procrustes analysis with the function procrustes to
268 correlate microbial community structure with vegetation, and microbial community structure
269 with soil chemistry (Lisboa *et al*., 2014). The analysis was based on the first four axes of
270 NMDS analysis of bacterial community, fungal community, vegetation and soil chemistry
271 (stress for all ordinations ≤0.07). The strength and the significance of the Procrustes
272 correlation was tested with the function protest (Peres-Neto and Jackson, 2001). Residuals
273 from the Procrustes analysis were used to compare the strength of the plant-microbe and soil
274 chemistry-microbe correlation along the succession by comparing the successional stages
275 with linear mixed models as above for soil chemistry. To separate the effects of vegetation
276 and soil chemistry on the microbial community, we carried out partial Procrustes analysis
277 where the variation due to soil chemistry was first partitioned out by multiple regression of
278 vegetation and microbial data against soil chemistry, and the residuals were then used in
279 Procrustes analysis (Peres-Neto and Jackson, 2001; Lisboa *et al*., 2014). All multivariate

280 analyses were carried out with the vegan package (v.2.4.2, Oksanen *et al.*, 2017) in R.
281 Significance tests were based on 999 permutations.

282

283 We identified bacterial and fungal OTUs characteristic to specific successional stages or
284 combinations of stages by indicator species analysis with the package indicspecies using the
285 function multipatt and the association index IndVal.g which corrects for unequal site numbers
286 (De Cáceres and Legendre, 2009). Only OTUs that occurred in the subsampled data at least
287 12 times, i.e., once in each sample of a successional stage, were included in the analyses. The
288 combinations of stages included in the analyses were each successional stage on its own, all
289 six stages together, all stages except sand, all stages except forest, all stages except sand and
290 grass, all stages except heath and forest, sand+grass, moss+lichen, moss+grass, lichen+heath,
291 heath+forest, sand+grass+moss, moss+lichen+heath, and lichen+heath+forest. Instead of
292 using all possible combinations, we selected the combinations to limit the number of tests and
293 to focus on the combinations most relevant for the aims of the study. OTUs were considered
294 indicator OTUs if their indicator value was >0.3 and $p < 0.05$. Significance was evaluated
295 with a permutation test (999 permutations). P values were adjusted for multiple comparisons
296 with function p.adjust (method "fdr"). Generalist OTUs present in all successional stages
297 were defined at indicator values >0.5, because no p values are generated for taxa present in
298 all sites. The identified indicator OTUs were grouped based on their taxonomic classification
299 at order (bacteria) or class (fungi) level. Unclassified OTUs were included in these
300 comparisons by re-clustering all reads assigned to unclassified bacteria or fungi in mothur at
301 75% sequence similarity to represent phylum level similarity for bacteria and class level
302 similarity for fungi (Tedersoo *et al.*, 2014).

303

304 To evaluate responses of bacterial and fungal indicator taxa to environmental variables, we
305 used relative abundances of indicator OTUs grouped at order level (bacteria) and class level
306 (fungi), including those unclassified 75% similarity groups that represented >0.4% of all
307 unclassified OTUs. The tested environmental variables were those that best explained
308 microbial community variation (OM, pH, Al for bacteria; OM, pH, minimum temperature for
309 fungi). The analysis was carried out with the function traitglm in the package mvabund (v.
310 3.13.1, Wang *et al.*, 2012). The function fits fourth corner models to relate sites and their
311 traits to species and species traits. When used without species trait data, it becomes a

312 predictive model of responses of different OTUs to variation of environmental factors
313 (Brown *et al.*, 2014). The method used was glm1path, fitting a generalized linear model with
314 LASSO penalty, which means that fourth corner coefficients of OTUs not decreasing
315 Bayesian information criterion (BIC) are forced to 0, i.e. no response to environmental
316 variation, to simplify the model (Brown *et al.*, 2014).

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

318

319 # Results

320 **Soil chemistry and microbial biomass along Arctic sand dune succession**

321 The increasing aboveground vegetation cover from bare sand to mountain birch forest was
322 mirrored belowground in increasing soil OM content and decreasing pH (Fig. 1; Pearson
323 correlation r=-0.78, p< 0.001). Organic matter content was low in the sand, grass, and moss
324 stage soils (0.4±0.2%, mean±SD) but increased to 0.9±0.3% in the lichen stage and up to
325 4.5% ± 1.7% in the forest stage (Fig. 1b, Table S1). pH was highest in the grass stage (5.8 ±
326 0.2) and lowest in the forest stage (4.7 ± 0.1) (Fig. 1c). Soil contents of N, P, K, Ca, Mg, Fe
327 and S increased with increasing OM (Table S1).

328

329 Microbial biomass measured as PLFAs per soil dry weight followed closely the OM-pH
330 gradient (Fig. 1e, Fig. S1). The moss stage had similar OM content as bare sand, but its
331 microbial biomass was higher than in the other sandy stages. When related to soil OM,
332 microbial biomass in the moss stage reached a level similar to the lichen and vascular plant
333 stages (Fig. 1d). Similarly, a shift to a higher proportion of fungal to bacterial biomass
334 occurred in the moss stage and was maintained during the later successional stages (Fig. 1f).
335 Overall, the proportion of fungi was highest in the heath stage.

336

337 **Microbial community structure**

338 Bacterial and fungal community structure based on sequencing (Fig. 2) and PLFA (Fig. S2)
339 followed the order of the vegetation stages on the OM-pH gradient and differed with
340 successional stage (PERMANOVA bacteria r^2=0.58 P<0.001, fungi r^2=0.59 p<0.001). The

341 moss stage grouped with the other sandy stages (sand, grass) with partly overlapping
342 communities, whereas the lichen stage grouped with the vascular plant stages (heath, forest)
343 (Fig. 2a, 2c). It should be noted that multivariate dispersion also differed between the
344 successional stages (betadisper p=0.007 bacteria, p=0.001 fungi). The highest bacterial OTU
345 richness (Fig. 1g) and Shannon diversity (Table S1) were detected in the heath stage, whereas
346 fungal richness and diversity were highest though very variable in bare sand (Fig. 1g, Table
347 S1). The soil parameter that best explained the community variation of bacteria and fungi was
348 the OM-pH gradient, especially in the vegetated stages (Table S2).

349

350 To compare successional shifts in bacteria, fungi, vegetation, and soil chemistry, we
351 calculated distance measures from bare sand to the other successional stages (Fig. 3). The
352 largest shift in bacterial and fungal community structure was detected from moss to lichen
353 stage (Fig. 3). Soil chemistry changed most markedly from heath to forest. Vegetation
354 showed increasingly larger shifts in later succession, with the largest shift from heath to
355 forest (Fig. 3). To determine if the microbial communities correlated with vegetation and soil
356 chemistry, we carried out Procrustes analysis (Fig. S3). The bacterial community correlated
357 better with soil chemistry than vegetation (bacteria-vegetation r=0.71, bacteria-soil chemistry
358 r=0.80, p=0.001 for both). The fungal community correlated tighter with vegetation than with
359 soil chemistry (fungi-vegetation r=0.77, fungi-soil chemistry r=0.71, p=0.001 for both).
360 When the variation due to soil chemistry was partitioned out, correlation with vegetation
361 remained with lower correlation coefficients (bacteria-vegetation r=0.51, fungi-vegetation
362 r=0.52, both p=0.001). We then used the Procrustes residuals to compare the strength of the
363 relationship between the plant and microbial community along the succession. The strength
364 of the vegetation-bacteria correlation did not vary with successional stage (Fig. 4). In
365 contrast, vegetation determined fungal community composition more strongly in the heath
366 stage than in the other stages (Fig. 4)

367

368 **Bacterial taxonomic distribution with succession**

369 Overall, the most abundant bacterial phyla or classes were Alphaproteobacteria,
370 Actinobacteria, and Acidobacteria (Fig. 2b, Fig. S4). The most abundant bacterial OTU
371 belonged to the candidate phylum WD272 (WPS-2). This phylum was the fourth or fifth most

372 abundant phylum in the sandy and lichen stages (on average 4.6-8.1%), decreasing in the
373 heath (3.5%) and forest (0.9%) (Fig. 2b, Fig. S4). When related to microbial biomass, the
374 abundance of WD272 increased in the moss stage (Fig. S5). WD272 showed abundant
375 indicator OTUs for the sandy stages but also less abundant forest-specific indicators (Fig. 5).
376 Most indicator groups specific for the sandy stages were strongly associated with low OM
377 content (Cytophagales, Deinococcales, Acidobacteria subgroup 4, Pseudonocardiales
378 (Actinobacteria), Oligoflexales (Deltaproteobacteria), unclassified group 9) (Fig. 5).
379 Cyanobacteria, three Chloroflexi groups and unclassified group 2 were indicators for the
380 moss and lichen stages and associated with both low OM and low pH. Another sign that the
381 moss stage was a key transition stage for bacterial community was that it showed unclassified
382 clusters typical to both sandy and vegetated stages (Fig. S6a). Most indicator groups for the
383 forest stage were indicators also for the other vegetated stages. For example, Acidobacteria
384 subgroup 2 associated with low pH and subgroup 6 associated with high OM appeared in the
385 moss and lichen stages and had indicators also in the heath and forest stages (Fig. 6). The
386 overall abundance of Acidobacteria increased with decreasing pH (Pearson correlation $r = -$
387 0.81, $p<0.001$).

388

389 **Fungal taxonomic distribution with succession**

390 The fungi at class level were dominated by Ascomycetes, followed by Basidiomycetes,
391 unclassified fungi, and smaller shares of Zygomycota, Rozellomycota, Chytridiomycota, and
392 Glomeromycota (Fig. 2d, Fig. S7). The most abundant Ascomycota class was Leotiomycetes,
393 followed by unclassified ascomycetes and Dothideomycetes. Bare sand had the highest
394 relative abundance of unclassified fungi up to 35% (Fig. 2d, Fig. S7), and several unclassified
395 clusters were specific to the sandy stages (Fig. S6b) and associated with high pH or low OM
396 (Fig. 6). Other strong indicators of sandy stages included Helotiales and Thelebolales
397 (Leotiomycetes), and Polysporales and Boletales (Agaricomycetes). Lecanoromycetes,
398 containing lichenized fungi, was most abundant in the moss stage (Fig. 2d). Similarly to
399 bacteria, the fungal groups that were the best indicators of the moss and lichen stages were
400 associated with low OM and low pH or both, including Lecanoromycetes (Lecanorales,
401 Agyriales, Ostropomycetidae), Agaricomycetes (Cantharellales, Atheliales, Agaricales), and
402 Dothideomycetes (Pleosporales, Capnodiales) (Fig. 6). The most abundant fungal OTU
403 overall and one of the fungal generalists (Table S3) was classified as *Pezoloma ericae*

404 (Leotiomycetes; also known as *Rhizoscyphus ericae*). This OTU was particularly dominant in
405 the heath (34±5% of reads). Its relative abundance was lowest in bare sand (2±1%) and the
406 grass stage (12±14%), but in the moss and lichen stages it increased to 26±16%. In the forest,
407 its relative abundance was lower than in the cryptogam-dominated stages (17±14%).

408

409 **Discussion**

410 We hypothesized that the belowground and aboveground succession in Arctic sand dunes are
411 not completely synchronous. Specifically, we hypothesized that the cryptogam-dominated
412 stages of an Arctic sand dune succession involve key belowground components that facilitate
413 transitions towards vascular vegetation. Supporting this hypothesis, the bacterial and fungal
414 communities in the lichen stage soil resembled the communities in the later, vascular plant
415 dominated stages. In the other cryptogam stage studied – the moss stage – the overall
416 microbial communities overlapped with the earlier, sandy stages. Nevertheless, indicative of
417 belowground transitions preceding aboveground succession, microbial biomass per OM and
418 the proportion of fungi increased in the moss stage, and later stage microbial groups appeared
419 despite the absence of their presumed host plants. The higher correlation of bacterial
420 community with soil chemistry than with vegetation and the increase in the strength of plant-
421 fungal interaction with vascular vegetation supported the hypothesis on the different
422 influences of vegetation succession on bacteria and fungi.

423

424 The dune sand is a harsh habitat with low carbon and nitrogen availability. The initial
425 microbial colonizers must be able to tolerate the oligotrophic conditions either by using the
426 low levels of allocthonous organic matter (Hodkinson *et al.*, 2002) or as autotrophs
427 (Nemergut *et al.*, 2007; Fierer *et al.*, 2010). Comparison of biomass per OM with biomass per
428 soil dry weight shows that the microbial biomass increased in the moss stage when OM
429 potentially available for heterotrophs still remained low. A characteristic group for the early
430 stages from bare sand to lichen was candidate phylum WD272, currently known as WPS-2
431 and recently named *Candidatus* Eremiobacterota, 'desert bacterial phylum', which are
432 proposed to be autotrophs able to use atmospheric H_2 and carbon monoxide (CO) as energy
433 sources (Ji *et al.*, 2017; Holland-Moritz *et al.*, 2018) . Another potential H_2- and CO-
434 oxidizing group in the moss and lichen stages were members of Chloroflexi classified as

Ktedonobacteria (King and King, 2014; Islam *et al.*, 2019). The increased occurrence of WD272 and Chloroflexi in the moss and lichen stages suggests that they may form a basis of biomass accumulation in the Arctic sand dune succession. These microbes may therefore facilitate development of cryptogam cover and heterotrophic microbial community (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, known as N_2-fixing early colonizers and components of biocrusts. Another distinctive indicator for the cryptogam stages were Chloroflexi, which in dryland and grassland ecosystems occur in moss and lichen biocrusts and in sub-crust soil (Kuske *et al.*, 2012; Navarro-Noya *et al.*, 2014; Maier *et al.*, 2018). In a temperate inland dune succession, a thick *P. piliferum* cover increased microbial biomass compared to bare sand (Sparrius and Kooijman, 2013). In our ecosystem, *P. piliferum* biomass was small in the moss stage but nonetheless affected the microbial biomass and communities. Therefore *P. piliferum* appeared to have a disproportionate effect on 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 the soil microbial community structure, it seems that in these Arctic dunes the grass *Deschampsia flexuosa* may not drive ecosystem succession. Based on our results, we propose that *P. piliferum* could participate in the physical stabilization of Arctic inland dunes and direct succession towards vascular vegetation. Verifying this proposition requires further studies on *P. piliferum* with emphasis on the role of rhizoids.

Indicative of nonaligned belowground vs. aboveground succession, lichen-forming fungi (Lecanoromycetes, Ascomycetes) were detected in the moss stage soil before the succession stage where they formed lichens. Furthermore, the relative abundance of the dominant fungal OTU *Pezoloma ericae* increased in the moss stage to levels similar to those in the later

467 stages. We interpret this increase as a possible sign of fungal community shift at the moss
468 stage towards a status where vascular plant establishment is possible. *Pezoloma ericae* forms
469 mycorrhiza with ericoid plants such as the dominant heath species *Empetrum nigrum* (Read,
470 1991; Smith and Read, 2008). However, no known host plants of *P. ericae* were detected in
471 the moss stage. In addition to ericoid mycorrhiza, *P. ericae* forms mycorrhiza-like
472 associations in liverwort rhizoids (Pressel *et al.*, 2010; Kowal *et al.*, 2018), and it has been
473 found as an Antarctic moss endophyte (Zhang *et al.*, 2013). However, no liverworts were
474 visible among the moss at our site, and no association of *P. ericae* with *Polytrichum* rhizoids
475 is known.

476

477 Supporting the hypothesis of different controls, bacteria and fungi showed distinct richness
478 and biomass patterns along the succession. The proportion of fungi in the PLFA data,
479 assumed to represent the living biomass (Zhang *et al.*, 2019), increased already in the moss
480 stage. Bacterial richness peaked in the heath and fungal richness in the bare sand, and neither
481 correlated with ecosystem productivity or community maturity defined as soil OM or plant
482 richness. In general, richness and diversity link to ecosystem functions through the complex
483 interaction between diversity and ecosystem stability and productivity (Loreau and de
484 Mazancourt, 2013). Plant richness peaks in the middle or late succession (Bonet and Pausas,
485 2004; Peyrat and Fichtner, 2011; Prach *et al.*, 2014). For microbes, few general patterns of
486 richness or diversity with primary succession have emerged. In very young soils, richness is
487 often low and then increases and stabilizes or even decreases in later stages (Jackson, 2003;
488 Nemergut *et al.*, 2007; Fierer *et al.*, 2010; Williams *et al.*, 2013; Jiang *et al.*, 2018). Primary
489 succession gradients where both bacteria and fungi have been studied show conflicting
490 patterns with each other but generally agree on different trajectories for bacterial and fungal
491 richness (Brown and Jumpponen, 2014; Cutler, *et al*., 2014; Dini-Andreote, *et al*. 2014; Dini-
492 Andreote, *et al*. 2016; Jiang, *et al*. 2018). In our case, fungal richness remained rather stable
493 under vegetation covered stages, while bacterial richness had a unimodal response. What can
494 be concluded from above and the present study is that fungi and bacteria do not follow the
495 same trajectories during succession and are not equally shaped by the same factors.

496

497 The low bacterial richness in the bare sand stage can be related to the oligotrophic conditions
498 (i.e. low environmental resource availability); 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 the bare sand together with the low fungal biomass, however, is unpredicted by theories that species richness should increase with the increase in resources and environmental heterogeneity (Stein *et al*., 2014; Cline *et al*., 2018). Some of the fungal richness in bare soil 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 or specialized.

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 plant *E. nigrum*. We suppose that the high bacterial richness is promoted by resources and niches provided by fungal mycelium and *Empetrum* litter, despite its high phenolic content and proposed adverse properties on microbial activity (Wardle *et al.*, 1998; Gallet *et al.*, 1999). In the forest, where the highest microbial richness would be expected if richness correlated positively 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 along succession.

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, vegetation and microbes 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. No measured soil

chemistry variable changed in parallel with the large bacterial community shift between moss and lichen stages. 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 stage 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 relation to vegetation and soil chemistry in a less studied Arctic soil ecosystem. Successional dynamics may not be linear nor deterministic, which makes the space-for-time substitution in mosaics of repeated plant communities that are thought to represent chronosequences challenging (Walker *et al*., 2010). In our case, the soil organic matter helps link vegetation development in the lichen, heath and forest with time. However, the moss and grass stages are equally devoid of soil organic matter. The microbial community composition in the grass stage suggests that this stage is not well connected to the progressive successional pathway from bare sand to mountain birch forest. Our results highlight the importance of considering not only vascular plants but also cryptogams, i.e. mosses and lichens and particularly the potential dune keystone species *Polytrichum piliferum*, when defining the plant-microbe interactions in succession. Taken together, the results indicate that the belowground transition towards the later stages starts before the establishment of vascular vegetation. This means that the belowground succession may have a conditioning effect on the vascular plant succession in the early primary succession. This work complements evidence that bacterial and fungal richness are not necessarily related to soil ecosystem productivity or change in plant species richness (Waldrop *et al.*, 2006; Porazinska *et al.*, 2018; Delgado-Baquerizo and Eldridge, 2019). Soil chemistry and the effects of plants on microbial resources and niches seem to be important factors driving succession. However, the key transitions in soil chemistry, vegetation and microbial community did not align, suggesting additional drivers such as competition between microbial taxa. 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 stage is crucial for restoration of vegetation on cold-climate soils suffering from erosion and vegetation loss under changing environment.

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Author contributions

MMK conceived the research and led the field work, HJ and MM conducted PLFA analyses, HJ conducted DNA work, analyzed all the data and performed the statistical analyses, MT provided expertise and resources for Ion Torrent sequencing, HJ and MMK wrote the manuscript, and all authors commented on the manuscript.

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867 ## Figure legends

868 **Figure 1** (a) Six successional stages defined in deflation basins of an Arctic inland sand dune
869 area, (b) organic matter, (c) pH, (d) microbial biomass based on phospholipid fatty acids
870 (PLFAs) per soil organic matter (OM) content, (e) microbial biomass per gram soil dry
871 weight (dw), (f) ratio of fungal to bacterial biomass based on PLFA, and (g) bacterial 16S
872 rRNA gene (upper row, darker colours) and fungal ITS amplicon (lower row, lighter colours)
873 OTU numbers in sand dune successional stages. Data are averages of four deflation basins
874 and three soil cores per basin. Ends of whiskers represent minimum and maximum values
875 excluding outliers, and outliers are shown as separate points. Different letters indicate
876 significant differences at $p<0.05$.

877

878 **Figure 2** Community structure of bacteria (a) as a non-metric multidimensional scaling
879 (NMDS) plot (stress=0.10) and (b) at phylum level (class level for Proteobacteria)
880 taxonomical classification based on 16S rRNA gene amplicon sequencing, and community
881 structure of fungi (c) as an NMDS plot (stress=0.13) and (d) at class-level taxonomical
882 classification based on ITS2 region amplicon sequencing. Data points represent three soil
883 cores from four deflation basins per sand dune successional stage. Shapes of the symbols
884 represent separate deflation basins. In b and d, data are averages of four deflation basins and
885 three soil cores per basin. For values of individual samples and the groups combined in
886 'other', see Fig. S4 and S7.

887

888 **Figure 3** Average distances of successional stages from bare sand for bacterial, fungal, and
889 plant communities and for soil chemistry. Different letters indicate significant differences at
890 $p<0.05$. Ends of whiskers represent minimum and maximum values excluding outliers, and
891 outliers are shown as separate points.

892

893 **Figure 4** Strength of correlation of vegetation with (a) bacterial community and (b) fungal
894 community determined as residuals of partial Procrustes analysis when variation due to soil
895 chemistry was partitioned out. Low values of the residuals indicate stronger correlation.

Different letters indicate significant differences between the sand dune successional stages at $p<0.05$.

Figure 5 Bacterial indicator OTUs for successional stages grouped at order level. Grey shading shows where the successional stage changes, and each group is placed in the stage where it was the most abundant (vertical successional stage labels; for., forest). Only groups with total abundance of indicator OTUs >0.05% are shown. The heatmap on the right shows fourth corner coefficients (coeff.) describing the association of the indicator OTU groups with environmental variables (env. variables). Positive coefficient indicates that the microbial group occurred at high values of the environmental factor and negative coefficients indicate occurrence at low values of the environmental factor. Ind. OTUs, number of indicator OTUs; rel. abund., relative abundance; OM, organic matter.

Figure 6 Fungal indicator OTUs for successional stages grouped at class level. Grey shading shows where successional stage changes, and each group is placed in the stage where it was the most abundant (vertical successional stage labels: lich., lichen). 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 (coeff.) describing the association of the indicator OTU groups with environmental variables (env. variables). Positive coefficient indicates that the microbial group occurred at high values of the environmental factor and negative coefficients indicate occurrence at low values of the environmental factor. Ind. OTUs, number of indicator OTUs; rel. abund., relative abundance; OM, organic matter, minT, minimum soil temperature.

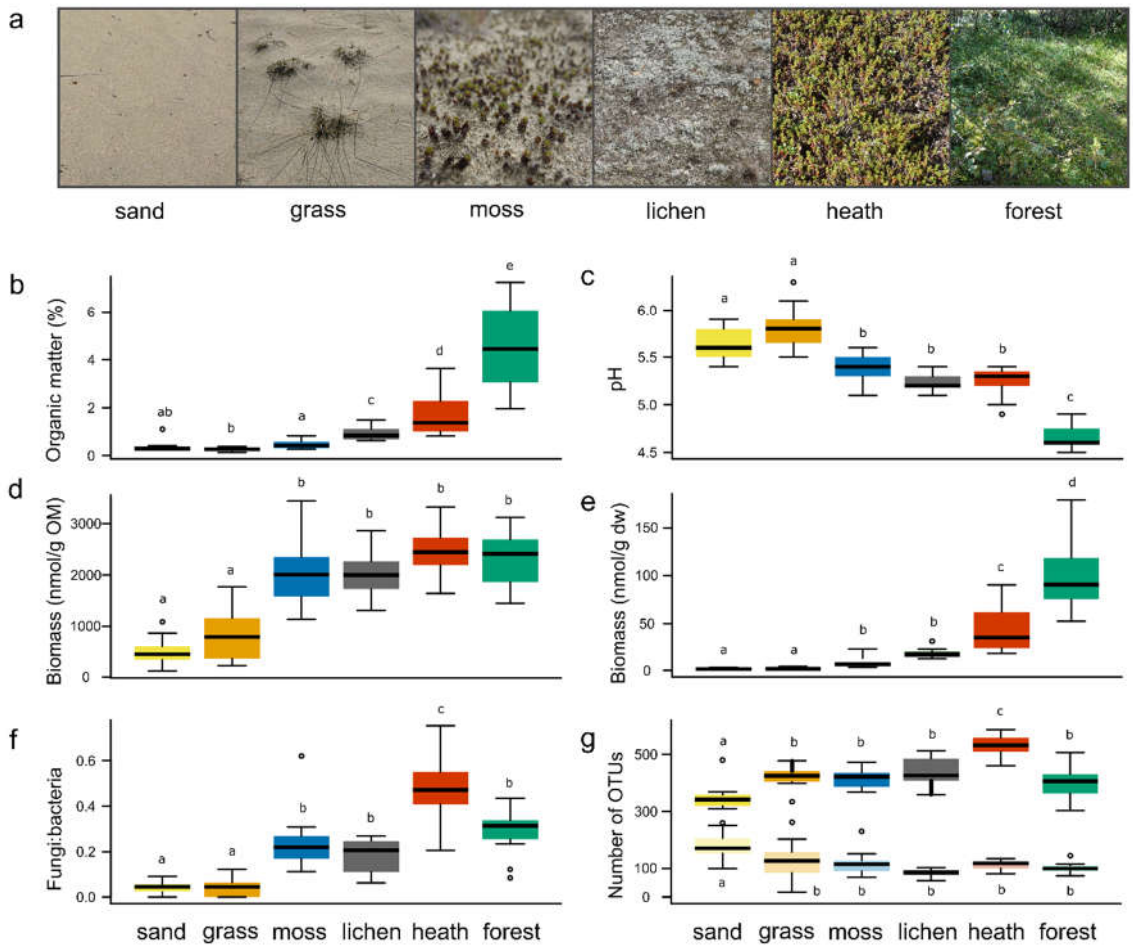
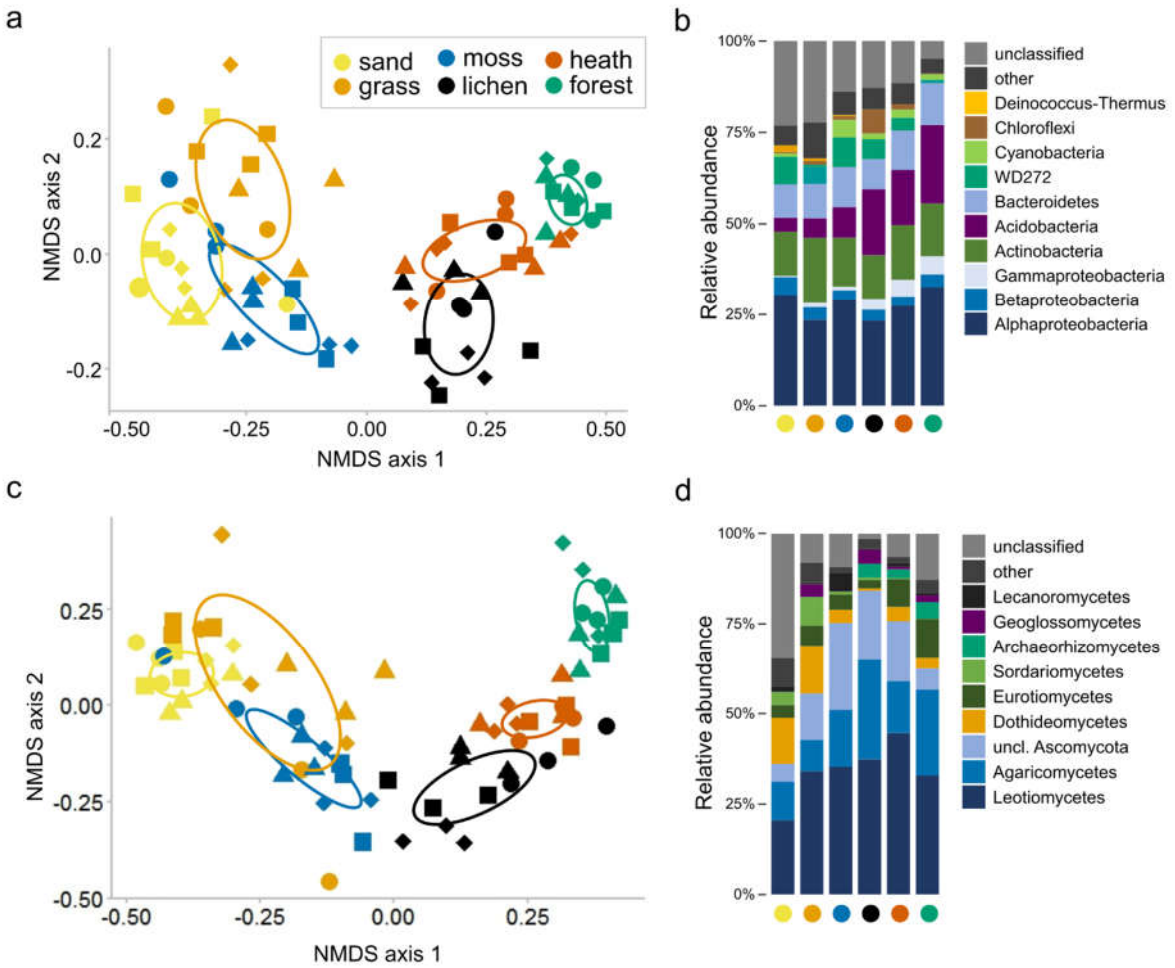


Figure 1 (a) Six successional stages defined in deflation basins of an Arctic inland sand dune area, (b) organic matter, (c) pH, (d) microbial biomass based on phospholipid fatty acids (PLFAs) per soil organic matter (OM) content, (e) microbial biomass per gram soil dry weight (dw), (f) ratio of fungal to bacterial biomass based on PLFA, and (g) 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 soil cores per basin. Ends of whiskers represent minimum and maximum values excluding outliers, and outliers are shown as separate points. Different letters indicate significant differences at $p<0.05$.



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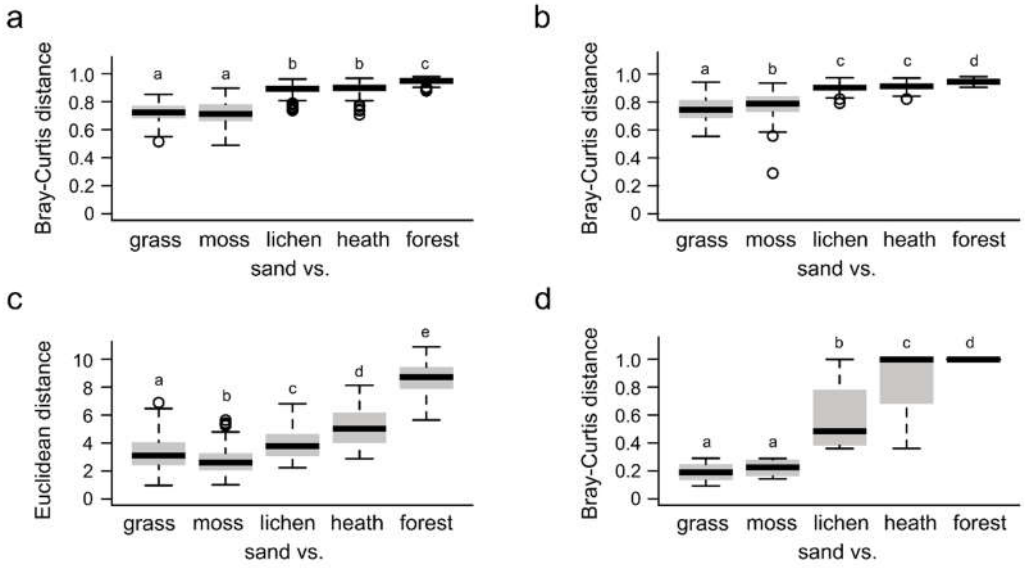

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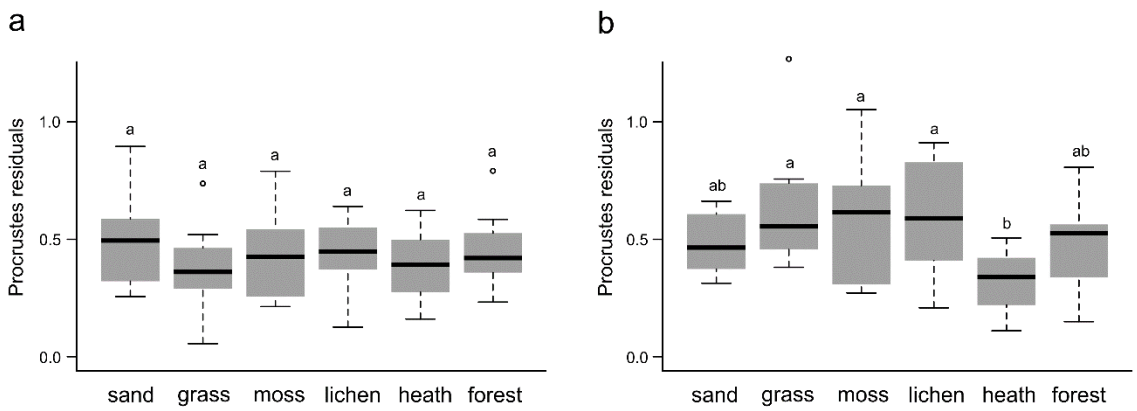

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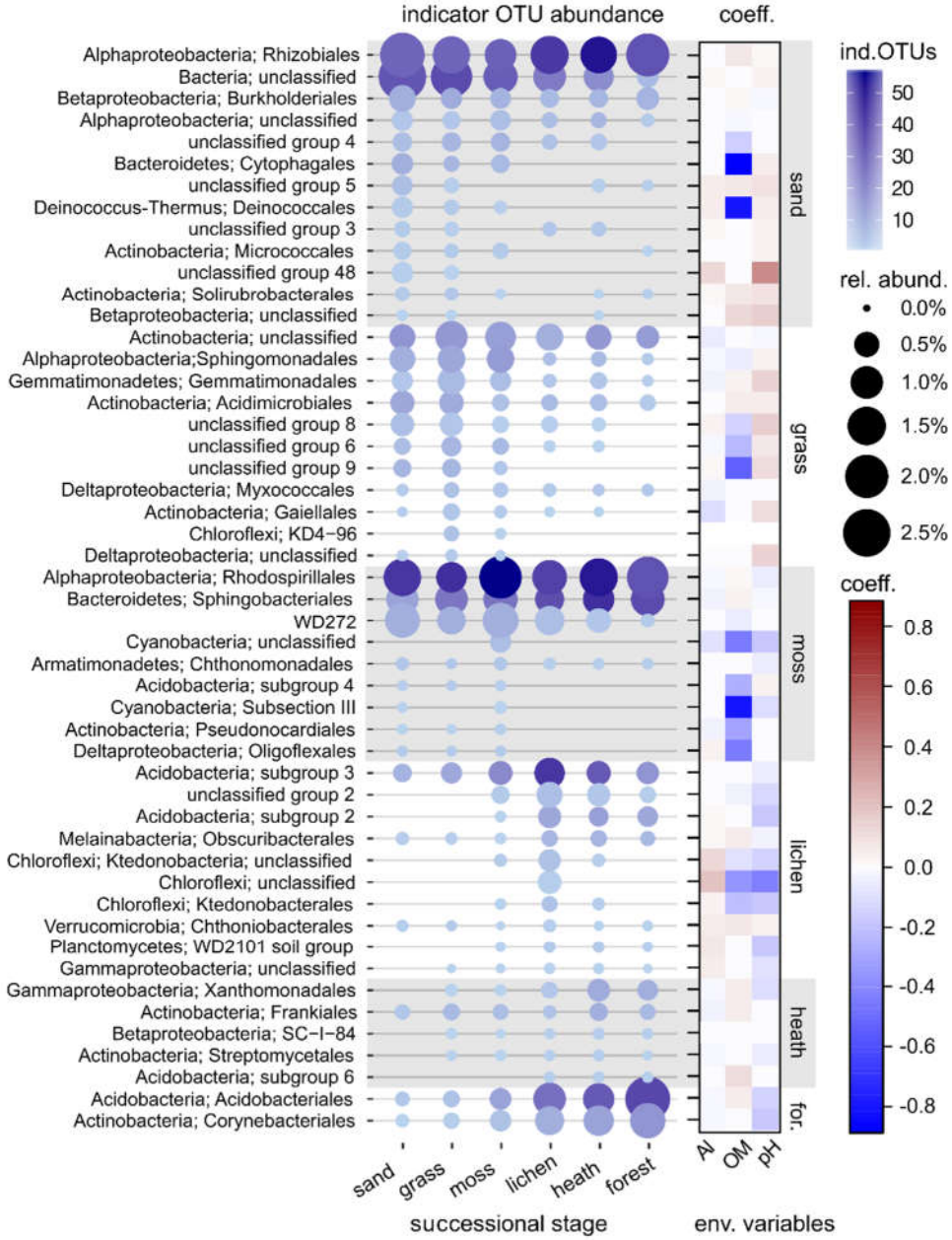

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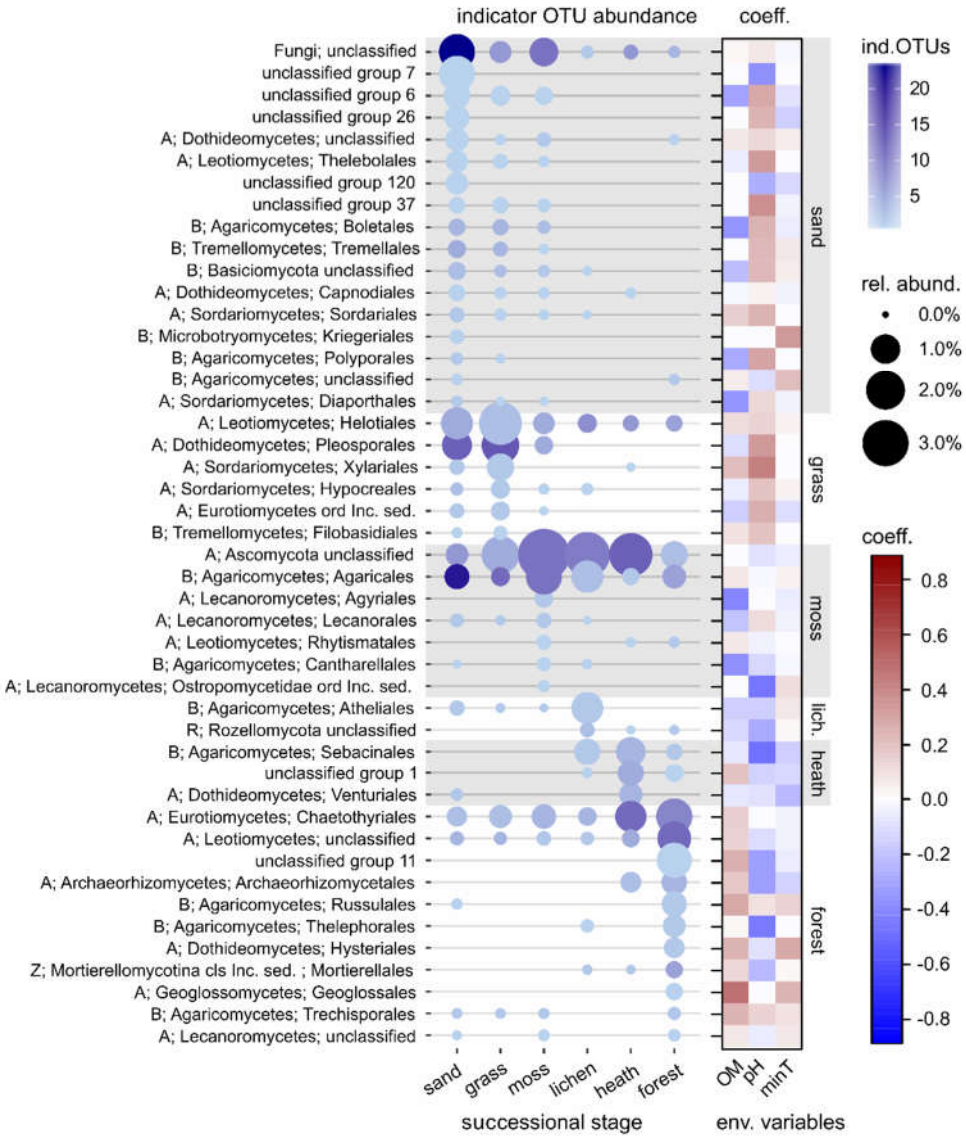

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