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Cryptogams signify key transitions 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 communities, and soil chemical properties 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. Microbial communities of the lichen stage resembled the communities in the vascular plant stages. Bacterial communities correlated better with soil chemical variables than with vegetation and vice versa for fungal communities. The correlation of fungi with vegetation increased with vascular vegetation.
- Distinct bacterial and fungal patterns of biomass, richness, and plant-microbe interactions showed that the aboveground vegetation change structured the bacterial and fungal community differently. The asynchrony 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 plants 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 N₂-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 chemical properties 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 during early succession are considered to be driven by soil chemical properties, 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 more easily 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; Blaallid *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; Tschirko *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 expect that linking belowground community development with that aboveground will show 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 (Sparrus *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 communities more similar to the stages covered by vascular plants. Such asynchrony 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 communities 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 community, 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).

We sampled soil in each successional stage in four of the basins in July 2011. To take into account that plants and microbes vary at different spatial scales, the soil samples were collected in

triplicate. The samples were taken from the depth of 0-10 cm with a 12.5 cm × 12.5 cm soil corer (72 samples in total: six successional stages × four basins × three soil cores per 1×1 m study plot). Soil was sieved with a 4-mm sieve and frozen at -20°C.

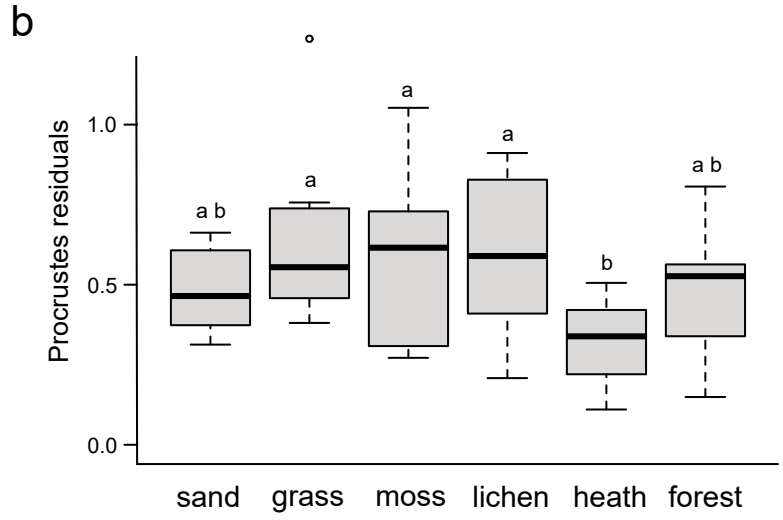
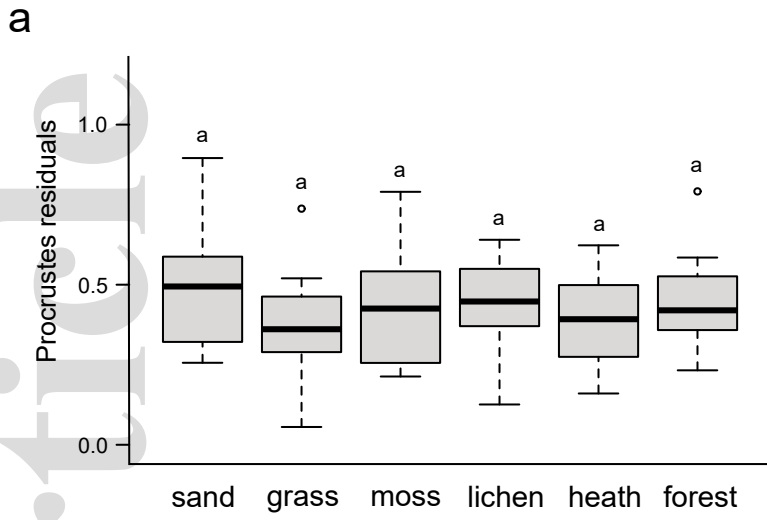
Soil chemical variables and temperature

Total C and N of soil organic matter, soil P, Ca, K, Na, Fe, Mn, Al, and Mg contents, and pH were measured as detailed in Methods S1. 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).

Phospholipid 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, 16:1 ω 9, i17:0, a17:0/17:1 ω 8c (eluted in the same peak), 17:0, cyclo-17:0, 18:1 ω 7c/ ω 9t (eluted in the same peak) and cyclo-19:0 were used to represent bacterial biomass and PLFA 18:2 ω 6 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.

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