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Soil bacteria and archaea change rapidly in the first century of Fennoscandian boreal forest development

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ABSTRACT

Using a gradient of changing ground age, caused by glacial isostatic adjustment, we compared systems that spanned ages from 25 to 560-years-old. Illumina sequencing was applied to determine archaeal and bacterial composition, investigating how different phylogenetic groups change as ecosystems develop. Bacterial communities dramatically changed during early ecosystem development ($p < 0.001$), evidenced by significant compositional shifts between 25 and 115 year-old-soils. Although significant differences did occur in the three later aged sites, they did not change as much. This was consistent with vegetation that shifted from meadow (25 year) to alder dominated forest (115 year), to ecosystems containing spruce. Correlation networks revealed that the microbial communities became more interconnected in older age ecosystems with a two-fold increase in network density. Species richness had the opposite trend with a decreased number of species: 781 operational taxonomic units (OTUs) in the youngest ecosystem to 366 OTUs in the oldest ecosystem. The observed shifts in community composition are consistent with other reported ecosystem gradients, but here we show that not only does composition change, but as ecosystems age the network connectivity increases indicating potentially more social interactions among microbes or increasingly stringent plant-microbe-soil interactions.

1. Introduction

Glacial isostatic adjustment along the coast lines of northern Sweden and Finland provide a unique system to study microbial community change with soil development and ecosystem age. The age of new ground rising out of the sea is calculated from the known rate of rebound and the height over the ocean. These environments have previously been studied to understand soil biogeochemistry (Merilä et al., 2006; Wallander et al., 2009; Vincent et al., 2013; Blaško et al., 2015) plant succession (Svensson and Jeglum, 2003) on mineral soils, as well as on peat soils (eg. Merilä et al., 2006; Larmola et al., 2014). In this paper we focus exclusively on mineral soils. As these ecosystem ages and plants colonize, there is an increase in soil organic matter (SOM), a decrease in soil pH, and an increase in organic N and soil C/N ratio. Although total organic N increases, plant N decreases along with net and gross N mineralization (Blaško et al., 2015).

Plant species richness increases within the first 100 years of soil development, but decreases and remains constant between 200 and 600 years (Svensson and Jeglum, 2003). Norway spruce (*Picea abies* L.) colonizes at approximately ~150 years and remains dominant. Both spruce and later Scots pine (*Pinus sylvestris* (L.)) rely on ectomycorrhizal (ECM) fungi to supply needed N. There is evidence that ECM and associated microorganisms are N-sinks (Högberg et al., 2007a), sequestering N inside mycelium and potentially exacerbating N limitation to plants (Näsholm et al., 2013). Microbial N-immobilization could thus be one reason why boreal forests become N limited. Microbial studies reveal linkages between vegetation and microbial community composition (Merilä et al., 2002, 2010). In line with this Blaško et al. (2015) observed an increase in fungal:bacterial ratio with increasing age, and microbial composition of the fungi and bacteria differed in the early development stages according to phospholipid fatty acid (PLFA) analysis.

Although plant communities have often been studied along chronosequences, fewer studies have examined microbial communities (Fierer et al., 2010). The microbes most extensively studied are the fungal saprobes (Dighton and White, 2017) and arbuscular and ECM fungi (Johnson et al., 1991; Jumpponen and

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Egerton-Warburton, 2005; Chagnon et al., 2013). In one study, fungi appeared to change largely in connection to plant succession, but bacteria were correlated to edaphic changes (Cline and Zak, 2015). Additionally, soil bacterial communities, from a dune system in the Great Lakes region of Michigan, USA, changed rapidly within the first 450 years of soil development but then stabilized and remain similar for the next 500 years (Williams et al., 2013). Archaeal community composition also changed during early succession, for example composition differences were observed in deglaciated soils ranging in age from 2 years to 167 years (Zumsteg et al., 2012).

We focused on the bacterial and archaeal communities under vegetation on mineral soils, querying how these populations would respond to increased ecosystem development. The chronosequence allowed us to ask: Does ecosystem development lead to differences in the interactions between individual OTUs? Network analysis, a mathematical approach used to visualize and test for interactions or related activities within a complex community (Faust and Raes, 2012), was used to look for these interactions. For example, co-occurrence networks were used in one study to confirm the co-occurrence of N-fixing bacteria and wood decay fungi (Hoppe et al., 2014) and in another study rhizosphere bacterial communities become increasingly connected as plants matured throughout the growing season (Shi et al., 2016). We used co-occurrence, taxonomic association (Hartmann et al., 2014), and bi-partite networking (Bowen et al., 2013) to test three hypotheses along the uplift chronosequence: 1) There will be rapid changes in the bacterial and archaeal community in the early stages of ecosystem development when soil C and N is accumulating and N cycling rates are higher, but communities will become less variable with time; 2) Bacterial and archaeal community composition will vary with ecosystem age due both to loss and recruitment of OTUs over time; 3) The community will be connected over time due to either increased biotic and environmental filtering or interaction.

2. Materials and methods

2.1. Study location

The study sites are located in the Gulf of Bothnia (N 63°44′, E 20°35′) on Bjuren island, a nature reserve just off of Sweden's coast. The rate of isostatic rebound in this area is 8.16 mm per year (Ekman, 1996; Vestøl, 2006). Five ecosystem ages were designated along each of three transects: 1) a meadow that was 25 ± 12 years-old with short grasses and rushes (e.g., *Agrostis stolonifera* var. *Bottnica*, *Juncus balticus*); 2) a 115-year-old site dominated by grey alder (*Alnus incana* (L.) *Moench*) (112 ± 13 years) which forms root nodule symbioses with N_2-fixing actinobacteria of the genus *Frankia* (Huss-Danell, 1997); 3) a 150-year-old ecosystem (151 ± 15 years) containing Norway spruce (*Picea abies* (L.)) Karst forest; 4) a Norway spruce forest that was 215 ± 24 years-old; and 5) a 560-year-old (564 ± 31 years) Norway spruce and Scots pine (*Pinus sylvestris* (L.)) forest (Visual abstract). The soils along each transect were sandy and silty glacial deposits (FAO) with many boulders. The soils with continuous organic mor-layer with F- and H-horizons (150-, 215-, and 560-year-old ground) are classified as Haplic Podzol (FAO classification). For more detailed descriptions of plant community composition and soil characteristics, see Blaško et al. (2015).

Soils were sampled along each of the three transects by marking a temporary sampling plot (10 m × 15 m) and removing five composite soil cores from randomly selected locations within each sampling plot. The 25- and 115-year-old plots, which lacked a continuous organic layer, were sampled by removing the five cm of the uppermost soil layer using a spade and a frame (30 cm × 30 cm). One to two soil samples were composited to make a single soil sample. The three more developed sites contained organic horizons, and F + H layers of c. 10 cm depth were sampled by removing three soil cores (10 cm diameter corer) and compositing those cores to make one sample. This was repeated to yield five composite samples per ecosystem age. The five representative soil samples were to be analyzed at each ecosystem age in each transect ending up in three mean values. Soils were collected in August and kept on ice for transport back to the lab in Umeå, Sweden (Blaško et al., 2015). Chemical parameters such as pH, Total C and total N have been reported previously (Blaško et al., 2015). A subsample (1–7 g) of each soil was freeze dried, ground, and stored at −80 °C until shipment on dry-ice to College Park, MD.

2.2. DNA extraction, quantification, and sequencing

Soil DNA was extracted using the MoBio "Powerlyzer" Powersoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA) according to manufacturer's instructions, with the exception of using a FastPrep-24 Instrument (MP Biomedical, Solon, OH) set at 5.5 m/s for 45 s for cell lysis (settings recommended by the manufacturer). Purified DNA was quantified by fluorimetry using a Qubit 2.0 (Life Technologies, Carlsbad, CA).

Soil DNA used in Q-PCR was diluted to 2.0 ng μL^{-1} and 2 μL of each dilution was used in triplicate Q-PCR reactions. Genes coding for bacterial 16S rRNA were used to quantify the total bacteria and archaea using the universal primer set 505F-806R (Caporaso et al., 2012). Quantification was based on a plasmid standard containing a cloned fragment of the amplicon from an *E. coli* pure culture. Thermocycling conditions were 95 °C for 5 min, followed by 40 cycles of 95 °C for 5 s/55 °C for 15 s/72 °C for 10 s, and a final melting cycle to produce a melting curve for quality control. All runs had standard efficiencies curves of $r^2 > 0.99$ and efficiencies 97–105%. An additional soil standard curve was constructed using a pool of all soil extracts and then diluted. The efficiency of this curve was 102%. In order to ensure that various inhibitors in soil extracts did not affect quantification, 1 μL of each soil extract was added together to make a single composite sample representing all samples. This composite sample was used to make a dilution series that was run against the plasmid dilution series (Hargreaves et al., 2013). In this case, the efficiency and slope of the plasmid standard and composite soil were within 5% of each other and no correction for any inhibitory effects on the PCR reaction was made.

Soil DNA was diluted to 5 ng μL^{-1} for amplicon sequencing of the bacterial and archaeal 16S rRNA. The V4 - V5 region of the 16S rRNA gene was amplified using the same universal primers 505F-806R that was used in Q-PCR (Caporaso et al., 2012). The primers contained Illumina sequencing adapters: 5′ TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG – Forward Primer – 3′, and 5′ GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G – Reverse Primer – 3′ (16S Metagenomic Sequencing Library Preparation Guide). Samples were amplified in duplicate and prepared for sequencing according to Illumina protocol (16S Metagenomic Sequencing Library Preparation Guide). Following clean-up of the PCR products with AMPure XP beads (Beckman Coulter, Pasadena, CA), 8 base pair (bp) nucleotide indexes were added using a Nextera XT Index Kit (Illumina, San Diego, CA). Samples were then cleaned a second time with AMPure XP beads (Beckman Coulter, Pasadena, CA) and all 75 amplified products were pooled and quantified. Each amplification was diluted to 1 ng μL^{-1} and 2 μL of each individual dilution was combined into a single pooled sample. The pooled sample was sent to the Center for Genome Research and Biocomputing at Oregon State University. A spike of 5% PhiX (Illumina, San Diego, CA) was added to the sample. A paired-end 250 bp v2 MiSeq kit was used for sequencing (Illumina, San Diego, CA). All sequences have been deposited in the NCBI database under the BioProject accession number: PRJNA374731.