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Bacterial diversity and community structure along different peat soils in boreal forest

Hui Sun[a,*], Eeva Terhonen[a], Kaisa Koskinen[b], Lars Paulin[b], Risto Kasanen[a,c], Fred O. Asiegbu[a]

[a] Department of Forest Sciences, University of Helsinki, P.O. Box 27, FIN-00014 Helsinki, Finland
[b] DNA Sequencing and Genomics Lab, Institute of Biotechnology, University of Helsinki, P.O. Box 56, FIN-00014 Helsinki, Finland
[c] Finnish Forest Research Institute (Metla), Vantaa Research Unit, P.O. Box 18, FIN-01301 Vantaa, Finland

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ABSTRACT

Bacteria in peat forest soil play important role in global carbon cycling. The distribution of bacteria population in different peat soils as a whole and how forest management practices alter the bacterial populations are still poorly known. Using pyrosequencing analysis of 16S rRNA gene, we quantified the diversity and community structure of bacteria in eight peat forest soils (pristine and drained) and two mineral forest soils from Lakkasuo, Finland with either spruce-dominant or pine-dominant tree species. In total, 191,229 sequences which ranged from 15,710 to 22,730 per sample were obtained and affiliated to 13 phyla, 30 classes and 155 genera. The peat forest soils showed high bacterial diversity and species richness. The tree species seems to have more strong impact on the bacterial diversity than the type of peat soil, which drives the changes in bacterial community structure. The dominant taxonomic groups across all soils (>1% of all sequences) were *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Planctomycetes* and *Verrucomicrobia*. The relative abundance of bacteria phylum and genus differed between soil types and between vegetation. Significant differences in relative abundance of bacteria phyla were only found for *Gemmatimonadetes* and *Cyanobacteria* between the pristine and the drained peat forest soils. At genus level, the relative abundance of several genera differed significantly between the peat soils with same or different tree species, including *Burkholderia*, *Caulobacter*, *Opitutus*, *Mucilanginibacter*, *Acidocella*, *Mycobacterium*, *Bradyrhizobium*, *Dyella* and *Rhodanobacter*.

1. Introduction

Boreal peatlands play important roles in global climate through the long-term net sequestration of carbon dioxide in organic peat soils. Peat contains substantial reservoirs of carbon and nitrogen, accumulating as much as one-third of the global terrestrial carbon pool (Gorham, 1991; Limpens et al., 2008). They also contribute to the beta diversity with their assemblages of specialized and unique plants and animals (Andersen et al., 2012) as well as methane (CH_4) emission in global climate regulation (Minkkinen et al., 2002), in which the methane–oxidizing bacteria are involved (Jaatinen et al., 2005).

Microorganisms in peatlands can control the turnover of organic carbon to contribute to global carbon cycling (Winsborough and Basiliko, 2010). They are instrumental in nutrient mineralization and uptake, which can feedback on plant productivity and overall ecosystem functioning (Andersen et al., 2012). Bacteria are the most abundant group of soil microorganisms (Roesch et al., 2007) and are among the first organisms to colonize dead wood and metabolize especially the easily accessible substrates (Schmidt et al., 2007; de Boer and van der Wal, 2008). Fungi are considered to be important decomposers in peat soil and have received the most attention (Thormann et al., 2001, 2002; Thormann, 2011; Jaatinen et al., 2008; Myers et al., 2012). However, a recent study has suggested that bacteria could be more active in aerobic decomposition than fungi in the oxic upper layers across many peatland types (Winsborough and Basiliko, 2010). Most of the studies in boreal peatlands have focused on some particular functional groups of bacteria relevant for litter decomposition or methane oxidation (Jaatinen et al., 2005; Yrjälä et al., 2011; Straková et al., 2012; Dobrovol'skaya et al., 2012). There are, however, uncertainties about the relative contribution of each of the different microbial groups to decomposition and nutrient cycling processes. Also, the distribution of bacteria population in different peat soils as a whole is still poorly known.

The composition and function of certain bacterial community in peat soil has been shown to vary according to nutrient regimes (Nilsson and Rülcker, 1992; Jaatinen et al., 2005) and prevailing plant communities (Fisk et al., 2003; Thormann et al., 2004). The

* Corresponding author. Tel.: +358 9 191 59880; fax: +358 9 191 58100.
E-mail address: hui.sun@helsinki.fi (H. Sun).

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structure of bacterial communities in soil also seems to be driven by the physical and chemical properties of soil (Fierer and Jackson, 2006) and the land use types (e.g. forestland vs. grassland) (Nacke et al., 2011). Forest management (e.g. drainage) can change the physical and chemical properties of peat soil as well as the vegetation communities, which, in turn, alters the microbial environment in peat soil (Laine et al., 1995). In addition, the diverse litter input probably also has a great impact on the microbial community structure in boreal forest (Straková et al., 2012) and other soil types (Fierer et al., 2011). Certain bacterial communities in a boreal peatland, such as *Actinobacteria*, showed close correlation with the type of peat soil and changed significantly following forest management (long-term drainage) (Jaatinen et al., 2007, 2008). Recent study also suggested that local environmental conditions may be a stronger driver of microbial community composition than geographical distance (Andersen et al., 2012).

Previous authors have shown that molecular methods provide powerful tools for detecting bacterial and fungal structure and diversity in environmental samples as well as evaluating the response of microbes to environmental changes (Fierer et al., 2007; Allison and Martiny, 2008; Weedon et al., 2012). The recent advanced sequencing technology, such as high throughput pyrosequencing, would widen the view of microbial diversity and allow us to better understand the microbial community structure. In this study, we applied pyrosequencing of the V1–V3 16S rRNA gene to analyze the diversity and community structure of bacteria in peat forest soil. We selected eight peat forest plots differing in nutrient regimes, ground vegetation and peat management. In addition, we compared the observed result with two mineral forest plots in boreal forest, which were selected based on the soil nutrient and vegetation difference. The hypothesis is that both forest type and plant type play important roles in shaping of bacterial community. The aim of the study is to investigate the bacterial diversity and composition in peat forest soils as a whole and to provide baseline to understand the relative contribution of each groups of bacteria in a changing environment (e.g. forest management).

2. Materials and methods

2.1. Site description and sampling

The research was carried out at Lakkasuo (61°48′ N, 24°19′ E, ca. 150 m above sea level), a boreal peatland in Central Finland, where a large variety of Finnish peat site types can be found. The characteristics of the ten selected study plots are listed in Table 1. Among the selected plots, four pristine peat (PP) plots, four drained pristine (DP) plots and two mineral soil (MS) plots were included. The forest tree species in each plot is either dominated by Scots pine (*Pinus sylvestris* L.) or Norway spruce (*Picea abies* (L.) H. Karst.). A detailed description of Lakkasuo and the study plots can be found elsewhere (Laine et al., 2004). Samples were taken at the beginning of August 2010. Three replicate soil cores (3 cm × 3 cm × 5 cm depth) were taken from the topsoil of the peat or mineral soil after removing the litter layer from each of the 10 sampling plots. The distance between each replicate sample was 10 m. The samples were taken to the laboratory and stored at −20 °C until further processing.

2.2. DNA extraction, amplification of 16S rRNA genes and pyrosequencing

Genomic DNA was extracted from 0.5 g of the homogenized soil per sample using the 'PowerSoil™ DNA Isolation Kit' (MoBio Laboratories, Carlsbad, CA, USA) as recommended by the manufacturer. A total of 30 extracted DNA samples were quantified with a Nanodrop-1000 spectrometer (NanoDrop Technologies, Wilmington, DE, USA) according to the manufacturer's protocol and adjusted to a final concentration of 10 ng μl-1.

The bacterial primers 27 F (5′- Axxxxxx AGAGTTTGATCMTGGCTCAG-3′) and 519R (5′-B GTATTACCGCGGCTGCTG-3′) were used to generate 16S rRNA gene fragments of ca. 500 bp from the variable regions 1–3, where A and B represent the two pyrosequencing adapters, respectively, (CGTATCGCCTCCCTCGCGCCATCAGAGCAGC and CTATGCGCCTTGCCAGCCCGCTCAG) were the primer pairs, and xxxxxx was designed for the sample identification barcoding key that allowed assignment of each individual sequence read to its original sample. A total of 100 ng of template DNA was used for a 50 μl PCR amplification reaction. The following thermal cycling scheme was used: 98 °C for 30 s (pre-denaturation), 28 cycles of 10 s at 98 °C (denaturation), 59 °C for 30 s (annealing) and 72 °C for 30 s (extension), followed by 10 min at 72 °C (final extension). Possible amplification of contaminants was determined with a negative PCR control in which the template DNA was replaced with sterile H_2O. These remained free of PCR amplicons.

The presence of PCR products was determined by analyzing 5 μl of product on 1.5% agarose gel to confirm DNA amplicons. Amplicons were purified using Agencourt AMPure XP beads (Beckman Coulter) to remove amplification primers and reaction buffer. After purification, DNA concentration was determined by Nanodrop ND-1000 spectrophotometer and equal amounts (~ 100 ng) of all amplicons were mixed in a single tube. The amplicons were sequenced at the Institute of Biotechnology (Helsinki University, Finland) using the 454 GS-FLX Titanium protocol (454 Life Sciences/Roche Diagnostics, CT, USA), which yields reads length of ~400 bp.

2.3. Analysis of pyrosequencing data

Raw pyrosequencing reads were extracted, pyrodenoised and quality trimmed using Mothur software (Schloss et al., 2009). Sequences were removed if they contained: (i) ambiguous (N) bases; (ii) homopolymers longer than eight nucleotides; (iii) average quality score lower than 25; (iv) chimeras (Chimera uchim command in Mothur); and (v) fewer than 200 nucleotides. Tag and primer sequences were removed from all the sequence reads. Under these conditions, 51% of the raw sequence reads passed through the quality control. All trimmed, high-quality pyrosequencing reads that were used in this study have been deposited in the Sequence Read Archive (SRA) at the European Bioinformatics Institute (EBI, accession No. ERP001576).

Sequences were aligned to the SILVA alignment database (Pruesse et al., 2007) and pairwise genetic distance matrix was calculated. They were clustered into operational taxonomic units (OTUs) defined by a 3% distance level using the average neighbor algorithm in Mothur Ver. 1.23.1 (Schloss et al., 2009).

Species richness was estimated using a rarefaction curve as well as the richness estimators Chao (Chao, 1984) and ACE (Chao and Lee, 1992), which does not take into account the abundances of the species. Species diversity was estimated with Shannon index, which took not only the species richness into account but also the species abundance, by using the Mothur program. The sequences from three sample replicates in each plot were pooled together; the sequences were analyzed to estimate the overall bacterial diversity and richness in each plot.

To correct for differences in survey effort between samples, subsampled sequences with same size were analyzed for normalization. The number of sequences from the smallest size among all individual samples or among pooled samples in each plot was randomly selected and used for calculation of species richness and diversity as well as for bacterial structure comparison between communities.