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Vertical distribution of fungi in hollows and hummocks of boreal peatlands

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ABSTRACT

Hollows and hummocks of boreal peatlands differ in water table position, pH, plant community composition and biochemical properties that might affect the structure of their fungal communities. The community composition of fungi at three depths (0–5 cm, 15–20 cm, 30–35 cm) in hollows and hummocks of a nutrient-poor fen in northern Ontario, Canada were assessed by Illumina sequencing of 28S amplicons. Our metabarcoding results revealed statistically distinct fungal community composition between hollows and hummocks. Hollows contained a more diverse fungal community than hummocks. However, the middle horizon of hollows and the bottom horizon of hummocks were comparable in terms of fungal biodiversity. These layers were identified as the areas bearing the most diverse community composition of fungi, most likely driven by their similarly respective distance from the water table position. This optimum area is expected to be most affected following water table drawdown under future climate change conditions.

1. Introduction

Hollows (wet depressions) and hummocks (drier raised areas) are common micro-topographical features of northern peatlands that develop through a variety of feedbacks arising from different *Sphagnum* species associated with this micro-topography (Belyea and Clymo, 2001). These *Sphagnum* dissimilarities include differences in moss shoot growth and height (i.e. acrotelm thickness) (Belyea and Clymo, 2001), which interacts with water table position (Rydin, 1985; Weltzin et al., 2000; Rydin and McDonald, 2013), nutrient availability, and pH (Clymo, 1986). In addition to these micro-environmental conditions, hollow and hummock formations can be characterized by differences in key ecosystem processes driven by their plant species composition (Wallën and Smalmer, 1992; Glime, 2007). In particular, rates of decomposition have been cited as key controls and indicators of hollow-hummock formation (Clymo, 1965; Rudolph and Johnk, 1982; Wallën et al., 1988; Turetsky et al., 2008), where decomposition is slower on raised hummock areas than in hollow depressions (Rochefort et al., 1990), which is attributable to the inherent decomposability of the different constituent species (Clymo, 1965; Rudolph and Johnk, 1982; Turetsky et al., 2008), and the emergent microclimate (moisture, temperature) of the micro-topographies themselves. Underlying this process are the microbial (fungal and bacterial) communities that perform decomposition, which are also influenced by the physical micro-environmental conditions of the hollow-hummock (e.g. peat wetness, oxygen availability, plant litter deposition etc.). For example, hollows that are often submerged in through-flow do not experience the strong acidifying effect of *Sphagnum* seen in hummocks, leading to higher pH in hollows, and consequently higher microbial activities compared to hummocks (Clymo, 1986).

Among microbial communities of boreal peatlands, fungi, with their heterogeneous physiology, metabolic activities, and ecological functions, are recognized as key decomposers of complex carbon polymers in these ecosystems (Myers et al., 2012). Although the majority of fungi isolated and identified from peatlands are aerobic (Andersen et al., 2012), isolation of fungi from the anaerobic, lower peat layers suggests a range of tolerances to anoxic conditions (Thormann et al., 2004). Microbial activities can differ by depth and among different peatland types (Fisk et al., 2003; Myers et al., 2012). Changes in community composition of different groups of bacteria (Kotiaho et al., 2013; Deng et al., 2014), and fungi (Peltoniemi et al., 2009; Preston et al., 2012) along depth and micro-topographical gradients of boreal peatlands have been

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demonstrated.

Dissimilarities in fungal community composition between these two micro-topographies are expected, since oxygen availability and pH varies from hollows to hummocks, as does vegetation — hollows have more easily decomposable mosses compared to hummocks, which also have an abundance of vascular plants (Clymo, 1965; Rudolph and Johnk, 1982; Wallën et al., 1988; Turetsky et al., 2008). Many fungi are intricately linked with aboveground (plant) communities through mycorrhizal associations, as plant pathogens or as decomposers, utilizing root exudates as labile carbon sources in the rhizosphere, and plant litter inputs as substrate for decomposition (Hooper et al., 2000; Pistón et al., 2016). These differences in aboveground plant community composition are likely to be reflected in belowground communities (Hooper et al., 2000). In this study we applied different approaches to describe microscale- and depth-dependent variations of fungal community composition within a well-characterized nutrient-poor fen of northern Ontario, Canada. We specifically tested the hypotheses that: (1) hummock fungal communities will be less diverse and more homogeneous than their counterparts in hollows; (2) root-associated fungi will be more abundant in hummocks than hollows; and (3) fungal richness within each micro-topography will decrease with depth.

2. Materials and methods

2.1. Study site

The study site located at White River, northern Ontario, Canada (48.21°N, 85.21°W) is characterized as a nutrient-poor, sparsely forested fen occupying approximately 4.5 ha, and covered by *Sphagnum* mosses (*Sphagnum angustifolium*, *Sphagnum cuspidatum*, *Sphagnum fallax*, *Sphagnum fuscum* and *Sphagnum magellanicum*). Other vegetation identified at the site includes black spruce (*Picea mariana*), and tamarack (*Larix laricina*), leatherleaf (*Chamaedaphne calyculata*), Labrador tea (*Rhododendron groenlandicum*), small cranberry (*Vaccinium oxycoccos*), and *Carex magellanica* and *Carex disperma*. The study area has obvious micro-topographical variations of hollows and hummocks, each with different vegetation, and hydrological regime (McLaughlin and Webster, 2013). Hollows are mostly covered by *S. magellanicum*, whereas hummocks are mostly covered by *S. fuscum*, and also have significantly higher abundances of leatherleaf, cranberry and sedges. Trees such as black spruce and tall shrubs are more common in the lawn area between hollow and hummock (McLaughlin and Webster, 2013).

Water table fluctuations in the study site occur following changes in weather conditions or stream flow (Webster and McLaughlin, 2010). Hollows can range from a moist condition at the top (0–5 cm) to fully saturated at the lowest peat layer that we sampled (30–35 cm below the surface). The latter is in the catotelm (anoxic) area of hollow microscale. In contrast, hummocks are always dry at the top (0–5 cm), often dry in the middle (15–20 cm) depending on water fluctuations, and moist, but not necessarily saturated, in the bottom layer (30–35 cm). All of these layers are within the acrotelm (oxic) area in hummock microscale of the study site (Fig. 1). While the peat at this site may extend to depths of 104–127 cm (McLaughlin and Webster, 2013), the lowest sampling area we report is at 30–35 cm depth, from the top of either the hollow or the hummock; we refer to this as the bottom layer of our peat collection. The bottom layer of our peat differed in absolute depth below average ground height as hummocks were on average 10.9 cm (±5.8 SD) taller than hollows. For a more complete description of the site, see Webster and McLaughlin (2010) and McLaughlin and Webster (2013).

2.2. Sampling

In August 2013, 12 peat monoliths were harvested from two different micro-topographies (six monoliths from each of hollows and hummocks, with a distance of 2.0–2.5 m between monoliths) (Fig. 1). The monoliths were cut and the intact cores were removed using shovels. The diameter and depth of these monoliths were approximately 25 and 40 cm, respectively. To obtain undisturbed samples, each monolith was cut down the centre with a sterilized saw, and samples (each approximately 25 g fresh weight) were collected from different depths (upper 0–5 cm, middle 15–20 cm, bottom 30–35 cm) of these monoliths (36 field samples in total). All samples were transferred into air-tight plastic bags, and frozen in the field using dry ice to capture the fungal community under natural state. The frozen samples were stored at −80 °C until they were processed.

2.3. DNA extraction and PCR procedures

All the frozen peat samples were lyophilized for 48 h, homogenized by hand, and roughly 0.5 g (dry weight) of each sample was ground in liquid nitrogen to a fine powder from which approximately 25 mg (dry weight) was used for DNA extraction using the Zymo Soil DNA isolation kit (Zymo Research Corporation). The concentration of extracted DNAs was assessed using NanoDrop (NanoDrop 2000, Thermo Scientific), and normalized to 20 ng μL^{-1}, then kept at −20 °C until PCR amplifications.

PCR amplifications were done using two sets of primers, LSU200A-F (AACKGCGAGTGAAGCRGYA)/LSU476A-R (CSATCACTSTACTTGTKCGC) to target Ascomycota diversity and LSU200-F (AACKGCGAGTGAAGMGGGA)/LSU481-R (TCTTTCCCTCACGGTACTTG) to capture all other major groups of fungi such as Basidiomycota, Chytridiomycota, Zygomycota, Glomeromycota and Neocallimastigomycota — hereafter, we refer to this group as the 'Basidiomycota group' because a large proportion of fungi in this group belonged to Basidiomycota. The size of amplicons generated by these primers is approximately 300 bp (Asemaninejad et al., 2016). Illumina MiSeq adapters, and unique barcode sequences of 8-nucleotides were fitted to the 5' ends of forward and reverse primers (for full details see Asemaninejad et al., 2016). PCR reactions were performed in a total volume of 25 μL, with 20 ng of template DNA, 5 μM each of forward and reverse primers, and 12.5 μL of ToughMix (Quanta Biosciences), and run in a 'T300' Thermocycler (Biometra). Optimal PCR conditions for 'LSU200-F/LSU481-R' included initial denaturation for two minutes at 94 °C, followed by 29 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 62 °C and elongation for 18 s at 72 °C. For 'LSU200A-F/LSU476A-R' primers the optimal PCR condition was 94 °C for two minutes, followed by 29 cycles of 94 °C for 30 s, 55 °C for 30 s in first cycle, and 62 °C for the rest of cycles, elongation for 18 s at 72 °C (Asemaninejad et al., 2016). The concentration of PCR products was measured using a Qubit fluorometer with the dsDNA HS kit (Life Technologies), and then normalized. The normalized PCR products were sent to the London Regional Genomics Centre (Robarts Research Institute, London, Canada) for paired-end sequencing on an Illumina MiSeq sequencer (2 × 300 bp V3 chemistry).

2.4. Bioinformatic analysis

For each primer set, two MiSeq runs were carried out producing four multiplexed runs. A custom MiSeq pipeline (https://github.com/ggloor/miseq_bin/tree/master) was used for Bioinformatics analysis of the FASTQ data produced from each run (for full description of methods see Asemaninejad et al., 2016). PANDAseq (https://github.com/neufeld/pandaseq) was used to align