



Research paper

Selecting fungal disturbance indicators to compare forest soil profile reconstruction regimes



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ARTICLE INFO

Keywords:

Soil reclamation
Fungal community
ITS2 sequencing
Disturbance
Indicator

ABSTRACT

Physical disturbance of soil profiles, even at shallow depths, is a ubiquitous consequence of anthropogenic landscape modification, with short-term impacts on important ecological guilds of fungi. DNA-based methods for surveying community composition are widely incorporated into studies attempting to explain fungal responses to forest ecosystem disturbances. Here we compare fungal community composition between three distinct soil profile manipulations (20 cm depth) and undisturbed control plots in a sub-boreal spruce forest in the Central Interior region of British Columbia, Canada. Fungal community composition differences were tracked by internal transcribed spacer 2 (ITS2) amplicon sequencing, with comparisons drawn using genus-level annotations. Non-metric multidimensional scaling (NMDS) analysis indicated that disturbed-sample community compositions were similar to controls at 0-months and distinct from controls at 5- and 12-months post disturbance, but did not indicate clustering of samples according to disturbance regime. We used Linear Discriminant Effect Size (LEfSe) analysis to identify fungal genera that consistently indicate disturbed or undisturbed (control) treatments across 5- and 12-month sampling times. Four fungal genera (*Exophiala*, *Hyphodontiella*, *Mastigobasidium*, and *Umbelopsis*) were detected with higher ranges of relative abundance in all disturbance regimes when compared to control plots. Deliberate mixing of LFH into mineral horizon soils stimulated multiple genera that were more frequently detectable in replicate plots at 12 months, when compared to undisturbed and immediately re-assembled plots. Four ectomycorrhizal genera (*Amphinema*, *Cortinarius*, *Piloderma*, and *Russula*) were identified as strong indicators of control plot soils. A single genus, *Capronia*, was identified as differentially abundant between stockpiled LFH and immediately replaced LFH. Our results are consistent with declines in ectomycorrhizal fungal abundance and increases in saprotroph abundance previously reported in DNA-based community profiling studies of forest soil disturbance. This investigation demonstrates that bulk soil sampling can be used to evaluate soil-handling regimens to understand fungal community disruption/recovery and highlights LEfSe as an approach to indicator selection in DNA-dependent biodiversity surveys.

1. Introduction

Biodiversity monitoring relies on accurate observation of organisms at varying taxonomic levels of classification over large geographical areas. Traditional approaches to monitoring fungal populations in forest ecosystems have focused on macrofungal surveys of fruiting bodies or belowground ectomycorrhizal (EcM) root tips (Geml et al., 2014; Halme et al., 2012). This approach misses fungi that develop fruiting bodies infrequently or not at all, leading to inaccurate or patchy occurrence/relative abundance data (Halme et al., 2012).

Metabarcoding-based surveys exploit sequences of target DNA regions (coding or intergenic) that have sufficient levels of intraspecific diversity to discriminate fungal organisms at phylum down to species level (Cristescu, 2014; Thomsen and Willerslev, 2015). The internal transcribed

spacer 2 (ITS2) region has been widely used for profiling fungal community composition (Lindahl et al., 2013) in forest soils studies that have focused on vertical distributions in soil profiles (Baldrian et al., 2012; Lindahl et al., 2007; Santalahti et al., 2016), response to ecosystem disturbances (Glassman et al., 2015; Pec et al., 2016; Stursová et al., 2014; Sun et al., 2015), and linking organic matter cycling to fungal taxonomic classifications (Bödeker et al., 2016; Purahong et al., 2016; Talbot et al., 2014; Treseder et al., 2016). DNA samples can be isolated from a variety of sample materials (fruiting bodies, soils, water, ectomycorrhizal root tips, etc.), and the availability of public, curated sequence databases enable broad taxonomic detection by metabarcoding approaches. Further, these attributes also make DNA-metabarcoding an appealing option in biodiversity monitoring contexts for interpreting fungal community inventories according to functional guild associations. For example, ITS2 sequencing

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<http://dx.doi.org/10.1016/j.ecolind.2017.09.021>

Received 30 May 2017; Received in revised form 8 September 2017; Accepted 11 September 2017

Available online 10 October 2017

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investigations of forest soils post-disturbance indicate that ectomycorrhizal (EcM) fungi dominate the community composition in forests where soils have returned to being suitable habitats for these symbionts and where their connection to aboveground photosynthetic carbon has been restored (Kyaschenko et al., 2017; Saravesi et al., 2015).

While EcM fungi reliably indicate recovery of forest soils several years after disturbance, fungal biodiversity monitoring of soil disturbance on a shorter time-scale (weeks to months) are more useful for evaluating land reclamation and soil handling when disturbance/land use change occurs rapidly. An example of this is the practice of stockpiling and replacing LFH soil in reclaiming mining sites (Naeth et al., 2013) or as an organic cap over subsoil-refilled areas where pipelines are installed (Soon et al., 2000), which has been shown to improve the establishment and diversity of plant communities (Naeth et al., 2013). Preservation of local microbial inhabitants like EcM fungi can contribute to the efficacy of salvaged LFH in promoting plant health. Beneficial fungi and other aerobic microorganisms can be compromised by suboptimal topsoil storage due to the development of *in situ* anaerobic conditions (Harris et al., 1989) or because prolonged stockpile storage delays recovery of EcM density. Therefore DNA metabarcoding can be useful to assess how soil-handling influences beneficial and/or pathogenic fungi abundance, and to estimate the extent of soil profile recovery based on community composition.

Interpreting a DNA-derived inventory of fungi to identify ecological indicator taxa can be difficult because of extensive taxon richness, as frequently observed with forest soils (Lindahl et al., 2013). Applying indicator species analysis (Dufrene and Legendre, 1997) methods to a taxonomic inventory is useful for summarizing or inferring organismal associations to ecological attributes of a sampling area. Similar to indicator species analysis, the linear discriminant analysis effect size (LEfSe) algorithm (Segata et al., 2011) facilitates detection of taxa that differentiate communities/samples/treatments (defined as “classes”) by testing for class differences per taxon with non-parametric methods, and determines an effect size for differentiating taxa by linear discriminant analysis. The LEfSe algorithm initially applies a Kruskal-Wallis rank sum test to each taxon in the community profile inventory between classes, followed by a test for “biological consistency” where the Wilcoxon rank sum test is applied to evaluate whether significant differences between taxon distribution are maintained in subclass (if present in the dataset) comparisons across classes. The final LDA effect size calculation for differentiating taxa is applied only to community members in the inventory that had significantly different distributions consistent across class and subclass comparisons.

The data categorizations of subclass and class by the LEfSe algorithm lend themselves to ecological indicator detection, since class and subclass categories could easily be mapped onto bio-monitoring datasets where land use, anthropogenic disturbance, or site are defined at the start of the investigation. Further, the identification of class-differentiating taxa by LEfSe can be useful as a starting point for linking taxa to ecological guilds or successional processes. LEfSe analysis has already been employed to identify fungal communities associated with different *Panax notoginseng* cropping systems (Dong et al., 2016), fungi associated with plants in the built environment (Mahnert et al., 2015), and to determine fungal community compositional changes along a pH gradient in soils from the high arctic (Zhang et al., 2016) in addition to the extensive use of this analysis tool in archaeal- and bacterial-specific microbiome investigations (Dareng et al., 2016; Mandal et al., 2016; Suchodolski et al., 2015; Sun et al., 2016; Venkataraman et al., 2016; Wang et al., 2012).

We applied LEfSe analysis in a site-scaled field experiment in a sub-boreal spruce forest in the central interior region of British Columbia to compare soil fungal community composition between three different shallow soil disturbance regimens, and to compare stockpiled LFH organic forest floor material to LFH directly replaced on the physically disturbed plots. Disturbance and non-disturbance-indicating genera were detected by pairwise comparisons of disturbance regimens to undisturbed control plots. We used 5- and 12-month sampling data as subclasses to ensure detection

of indicator genera minimally impacted by seasonal variation. Sampling at multiple times post-disturbance also allowed us to compare dynamics of fungal genera increase, recovery, or suppression relative to control plots.

2. Methods

2.1. Site description

Two field sites were selected in 2014 for this study, both located approximately 60 km northeast of Prince George, British Columbia (Site 1: 54.2007 N, –123.1600 W; Site 2: 54.1992 N, –123.1606 W), and both belonged to the SBSmk1 subzone (Mossvale, moist, cool) of the SBS zone, based on the BC Biogeoclimatic Ecosystem Classification (Beaudry et al., 1999). The experimental plots were established in a predominantly old-growth stand of interior spruce and lodgepole pine with pockets of subalpine fir, trembling aspen and black spruce scattered throughout the forest stand. A plant diversity assessment, prior to applying soil disturbance treatments, indicated minimal differences between sites and total number of tree, shrub and herb species ranged from 44 to 45 species (Table A1).

2.2. Soil classifications and depth profile

Two soil pits were excavated to allow for detailed profile descriptions and soil horizon sampling and subsequent analysis; each of the pits was located in the center of the 3 blocked areas (see 2.4) within a site. Soils at both sites were classified as Eluviated Dystric Brunisols within the Canadian System of Soil Classification (SCWG, 1998). Site 1 (Table A2a) has a sandy loam texture in the Ae horizon that changes to a loamy sand/sandy texture in the B horizon. Site 2 (Table A2b) has a loam texture in the Ae horizon and sandy loam texture in B horizon material.

2.3. Soil physical and chemical characterization

Gravimetric moisture content was determined via mass loss following oven-drying at 105 °C for 24 h (Kalra and Maynard, 1991). Particle size analysis (% sand, silt, and clay) was determined using the Bouyoucos Hydrometer Method (Kroetsch and Wang, 2007). Soil pH was determined in water (1:1 soil-to-liquid ratio for mineral soil, 1:2 for forest floor material) using an electronic pH meter (Kalra and Maynard, 1991). Ergosterol was isolated from soils by KOH/methanol extraction and quantification as a proxy for fungal biomass (Seitz et al., 1977) by high performance liquid chromatography (Chiocchio and Matković, 2011; Newell et al., 1988). Total carbon (Skjemstad and Baldock, 2008) and nitrogen (Rutherford et al., 2008) were determined by dry combustion using an elemental analyzer. Available nitrate-N and ammonium-N were determined colorimetrically, following soil extraction with 2N KCl (Maynard et al., 2008). Bray extraction method was used to estimate available phosphorus (Kalra and Maynard, 1991) followed by spectrophotometric quantification. Effective cation exchange capacity (CEC) was determined by the BaCl₂ method as described by Hendershot et al. (Hendershot et al., 2008). With the exception of gravimetric moisture content, all analyses described in this section were performed at the Analytical Chemistry Laboratory, B.C. Ministry of Environment, Knowledge Management Branch (Victoria, B.C., Canada). Kruskal-Wallis tests were run in R version 3.1.2 (R Core Development Team, 2014) to test for significant differences in the distribution of ergosterol values between disturbance regimes, but within sampling times and sampling depths. Post hoc pairwise comparisons were made between treatments for significant Kruskal-Wallis ($p < 0.05$) test results using the Holm-corrected Dunn's test in R package PMCMR (Pohlert, 2014).

2.4. Field plot experimental design and sample collection

Plots were demarcated and four soil physical disturbance regimes were applied on May 12–14, 2014 using a randomized block design in each of

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