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Testing life history and trait-based predictions of AM fungal community assembly

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ABSTRACT

Arbuscular mycorrhizal (AM) fungal disturbance response is thought to be moderated by life history strategies (LHS). Research suggests that disturbance-tolerant taxa may be represented by fungi in the Glomerales, those in culture collections, and by cosmopolitan taxa due to their generalist growth habit. The corollary is that these taxa should be less common in undisturbed systems. Although widely accepted, these ideas originate from research conducted in previously disturbed systems. Whether they hold up to comparisons of disturbed versus undisturbed systems remains to be seen.

We addressed this question by surveying logged and intact sites within forests dominated an AM fungal host (western redcedar; *Thuja plicata*). We predicted that old-growth sites would host fewer taxa from the Glomerales, fewer cultured taxa, and fewer cosmopolitan taxa compared to logged sites.

Contrary to our predictions, the logged and intact sites did not differ with respect the putative disturbance-tolerant taxa. However, taxonomic composition differed, driven primarily by variation in relative abundance rather than loss or gain of taxa. Multiple analyses of indicator taxa revealed no consistent indicators of either undisturbed or disturbed habitats.

Based on these findings, the current paradigm for a phylogenetically based LHS of AM fungi warrants re-examination.

1. Introduction

Plant and animal community ecologists have long relied on theories concerning life history strategies (LHS) to better understand the process of community assembly (e.g. Pianka, 1970; Grime, 1974), and their success has inspired similar approaches among microbial ecologists. Using an approach based on r- and K-selected traits (Pianka, 1970), Hart et al. (2001) suggested that disturbance can shape AM fungal communities via fungal LHS: AM fungal with 'ruderal' traits (r-selected) should be promoted in disturbed systems, whereas AM fungi with 'disturbance intolerant' or 'stress tolerant' traits (K- selected) should prevail in undisturbed systems. They further suggested that propagule type (root fragment, hypha or spore) plus growth rate/extent may distinguish AM fungi into "r" and "K" selected taxa. Chagnon et al. (2013) applied Grime's (1974) Competitive-Stress Tolerant-Ruderal (C, S, R) framework to AM fungi and suggested additional traits (e.g. hyphal healing ability, host identity and host carbon requirements) should discriminate AM fungi with more "r" traits from those with "S" or "K" traits. For brevity, we henceforth use "K" to signify LHS associated with both disturbance intolerance (K) and stress tolerance (S).

Among the most common traits associated with "r" strategists include: reproduction via spores, roots and hyphal fragments (Klironomos and Hart, 2002), superior hyphal healing capacity (Avio et al., 2006; Voets et al., 2006), rapid growth (Hart and Reader, 2002), and low nutrient exchange ratio (Chagnon et al., 2013). Ruderal fungi are considered to display broad host range (Lekberg et al., 2007; Bennett et al., 2013) and have generalist growth requirements (Lekberg et al., 2007). As such, "r" strategists may be most evident among readily cultured taxa (Sýkorová et al., 2007) and among globally cosmopolitan taxa (Ohsowski et al., 2014). In contrast, "K" strategists should be difficult to culture due to specific host and substrate requirements. These specialist habits should in turn render "K" strategists less common overall, but perhaps more locally prevalent in pristine or non-managed systems (Ohsowski et al., 2014).

Many researchers have suggested the Glomeraceae exhibit "r" strategies, while members of the Diversisporaceae exhibit "K" strategies. This family-level distinction is supported by studies

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showing some degree of trait conservation among AM fungal families (Hart and Reader, 2002; Maherali and Klironomos, 2007; Powell et al., 2009). Further, many studies have suggested Glomeraceae dominance in disturbed sites (e.g. Johnson et al., 1991; Helgason et al., 1998; Douds and Millner, 1999; Hart and Reader, 2004; Oehl et al., 2009; Sikes et al., 2012; Melo et al., 2014). Taken together, the above information leads to the expectation that the relative prevalence of "r" strategist AM fungi should decrease from disturbed to undisturbed systems, while the converse should be true of "K" strategist AM fungi.

These trait-based LHS concepts and characterizations of AM fungi have gained wide acceptance in the mycorrhizal literature. However, their use may warrant reconsideration. First, their origins lie primarily in research conducted in previously disturbed systems (e.g. McGonigle and Miller, 1996; Johnson et al., 1991; Helgason et al., 1998; Jansa et al., 2002; Jasper, 2007; Oehl et al., 2009). While recent studies have examined a broader range of habitats (Kivlin et al., 2011; Öpik et al., 2013; Hart et al., 2014; Martinez Garcia Laura et al., 2015; Davison et al., 2015), some of these pertained to non-AM fungal dominant systems (Wubet et al., 2003; Öpik et al., 2008), or to systems in which the disturbance history was unclear (Kivlin et al., 2011; Öpik et al., 2013; Davison et al., 2015). Truly "undisturbed" systems, in which succession proceeds uninterrupted over millennia, are rarely studied, but arguably provide the most appropriate reference condition (or "control") when, evaluating hypotheses concerning disturbance. Second, it is not clear whether traits commonly ascribed to ruderal AM fungi (Glomeraceae, cultured, and cospmopolitan) are true indicators of the ruderal LHS. Given the paucity of information about fungal traits in general (Aguilar-Trigueros et al., 2015) it is important to test the generality of these assumptions.

Here, we report the results of a study in which we compared the AM fungi communities of clear-cut forest sites with those of truly undisturbed sites within ancient (ca. 4500 years) *Thuja* forests. Our primary interest was in testing assumptions about associations between AM fungi LHS and disturbance. Specifically, we tested the following predictions (i) compared to old growth sites, logged sites will exhibit a greater richness of "r" strategists (Glomerales taxa, cultured taxa, and cosmopolitan taxa), and (ii) OTU composition will differ significantly between old growth and logged sites. We then examined which taxa underlie compositional differences among old growth and logged sites.

2. Materials & methods

2.1. Study sites

Samples were collected in July 2013 from three remote sites (sites "A", "B", and "C") in the world's only temperate inland rainforest, located in the Interior Cedar Hemlock ecozone of British Columbia, Canada (Table S1). This region has been dominated by redcedar (*Thuja plicata* Donn *ex* D. Don), an exclusively AM host, for approximately 4500 years (Hebda, 1995). We selected sites meeting the following criteria: *Thuja plicata* dominant, primary forest adjacent to a recently logged site (less than 25 years), as well as similar elevation and soil type (Table S1). Sites were selected using HectaresBC (hectaresbc.org), a geo-spatial database for the province of British Columbia.

2.2. Sampling design

Because logging roads impose disturbance into pristine, uncut forests, we established three 100 m transects through old growth (henceforth "OG") and logged ("L") forests, each separated by 50 m in order to assess disturbance attributable to road building. Transects began on the logging road and extended into the forest, perpendicular to the road. Soil cores (15 cm) were taken every 25 m, starting with the road edge (0 m) and sampling into the forest, for a total of 30 samples per site (15 old, 15 cut), and 90 samples across all sites. We assumed that the "distance from road" factor corresponded to a gradient of decreasing disturbance (100m = least disturbed). Preliminary analyses of both our biotic and abiotic data showed that roadside samples were dramatically different from others, and that distance from road had minimal effect after 25 m (not shown). As our primary interest was in the disturbance related to logging, we based our analyses on samples 25–100 m from the road, and did not consider distance to road further. Thus, the final dataset included 12 samples for each site x disturbance combination, except one site (site B), for which one sample was incomplete (n = 11). At each sampling point along the transect, we estimated percent plant cover and plant species richness from a photograph taken at a set height in order to capture a uniform area at each site (see below). Soils were stored on ice until transported to the lab and were subsequently stored at 4 °C.

2.3. Abiotic conditions and plant communities

Soils were dried overnight then sieved to remove debris (2 mm). A subsample was shipped to the Analytical Chemistry Laboratory of the BC Ministry of the Environment in Victoria, BC. where it was analysed for Total C and N on a Flash 2000 combustion elemental analyzer (Thermo Instruments). Available P was measured colourimetrically after a 1 min shake using Bray P-1 extractant at a ratio of 2.5 g soil to 10 mL solution. Moisture content was determined on a separate sub-sample and air-dried analysis values were converted to oven-dry on this basis. Soil pH was measured using a ratio of 5 g soil to 5 mL water.

Percent plant cover was determined using the program Sample Point (samplepoint.org). 100-point grids were overlaid on overhead pictures of a 1×2 m area corresponding to soil sample locations. Grid points were assigned one of two categories: soil (ground) or vegetation (plant cover) and percentage of plant cover were calculated for each sample. We also estimated plant species richness by identifying plant morphotypes from photographs.

2.4. Molecular methods

DNA was extracted from 500 mg dried and sieved soil using the FastDNA SPIN Kit for Soil (MP Biomedicals) as per the manufacturer's instructions. Glomeromycotan sequences were amplified from soil DNA extracts using polymerase chain reaction (PCR) and the widely-used primers NS31 and AM1, which target a 550-bp central fragment of the SSU rRNA gene in Glomeromycota (Helgason et al., 1998). While there is some debate as to whether this region offer sufficient variation to resolve finer taxonomic differences (Bruns and Taylor, 2016), it remains the most widely used gene target with the most comprehensive representation in public databases (Hart et al., 2015). These primers were linked to 454-sequencing adapters and linkers Primer A (NS31) and Primer B (AM1), with unique 10 bp identification barcodes (Roche Technical Bulletin No. 09005) assigned to each sample. Each reaction contained 11.75 ul of H_20, 5.0 ul of 25 mM 5 x GoTaq Flexi buffer (Promega), 3.5 ul of 25 mM MgCl2, 0.5 ul of 25 mM dNTPs, 1.0 ul of 10 mg/mL Bovine Serum Albumin (BSA), 1.0 ul of each primer (10uM), 0.25ul of GoTaq DNA Polymerase (Promega) and 1.0 ul of DNA template for a final volume of 25 ul per reaction. Reactions were performed using a C1000 Thermal Cycler (Bio-Rad Laboratories) using the following program: 105 °C hot lid start, 95 °C for 3 min; 35 cycles of 95 °C for 30 s, 69 °C for 30 s, 72 °C for 1 min; 72 °C for 5 min. PCR was performed in triplicate (for a total of 270 reactions), and amplification was confirmed through visualization on a 1% agarose gel. Samples were pooled for sequencing, resulting