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Impacts of experimentally accelerated forest succession on belowground plant and fungal communities

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ABSTRACT

Understanding how soil processes, belowground plant and fungal species composition, and nutrient cycles are altered by disturbances is essential for understanding the role forests play in mitigating global climate change. Here we ask: How are root and fungal communities altered in a mid-successional forest during shifts in dominant tree species composition? This study utilizes the Forest Accelerated Succession ExperimenT (FASET) at the University of Michigan Biological Station (UMBS) as a platform for addressing this question. FASET consists of a 39-ha treatment in which all mature early successional aspen (*Populus* spp.) and paper birch (*Betula papyrifera*) were killed by stem-girdling in 2008. Four years after girdling, neither overall fungal diversity indices, plant diversity indices, nor root biomass differed between girdled (treated) and non-girdled (reference) stands. However, experimental advancement of succession by removal of aspen and birch resulted in 1) a shift in fungal functional groups, with significantly less ectomycorrhizal fungi, 2) a trend toward less arbuscular mycorrhizal fungi, and 3) a significant increase in the proportion of saprotrophs in girdled stands. In addition to shifts in functional groups between treated and untreated stands, ectomycorrhizal fungi proportions were negatively correlated with NH_4^+ and total dissolved inorganic nitrogen (DIN) in soil. This research illustrates the propensity for disturbances in forest ecosystems to shift fungal community composition, which has implications for carbon storage and nutrient cycling in soils under future climate scenarios.

1. Introduction

Determining how carbon (C) and nitrogen (N) cycles are altered by disturbances is essential to our understanding of the future states of the world's forests. Soil processes are among the least well-known components of these cycles (Carney et al., 2007; Norby and Zak, 2011). Nitrogen is particularly important in forest ecosystems, as it is most often the nutrient limiting primary production (Lebauer and Treseder, 2017; Norby and Zak, 2011; Vitousek and Howarth, 1991). Because N availability (N_{avail}) has differential effects on tree species growth and functional groups, it strongly influences plant community composition and forest C sequestration (Norby et al., 2010). Plant and fungal communities play key roles in soil processes impacted by disturbances and successional shifts (Chapman et al., 2005; Courty et al., 2010a; Horton and Bruns, 2001; Kaye and Hart, 1997; Lilleskov and Bruns, 2001).

As plant community composition shifts through time (*i.e.*, during succession), competition for N among individuals of different plant species in N-limited systems increases (Tilman, 1990; Vitousek and Howarth, 1991). This competition can be exacerbated as N becomes more limited and plants reliance on previously “inaccessible” N increases (Luo et al., 2004). Plants have evolved various strategies to compete for nutrients, including varying above ground vs. below ground allocation. Previous research has shown that the proportion of carbon allocation to roots can remain constant across a nitrogen gradient, but that fine root turnover rates increase as N_{avail} increases (Hendricks et al., 1993; Nadelhoffer et al., 1985).

An additional adaptation manifests in mycorrhizal associations between plant roots and fungi that enables plants to acquire more nutrients and water than they might otherwise attain without a fungal symbiont (Kirk et al., 2004; Smith and Read, 2008). Mycorrhizal associations can enhance plant competitive abilities and accelerate belowground nutrient cycling. Two dominant forms of mycorrhizae in forests are ectomycorrhizal fungi (EM) and arbuscular-mycorrhizal fungi (AM). AM fungi are the most ancient form of mycorrhizal symbiont, have plant symbionts belonging to all phyla, and are characterized by the formation of arbuscules in plant roots, which function to transfer nutrients and carbon between the mycorrhizal symbiont and plant host (Smith and Read, 2008). EM fungi form a mantle, or sheath, enclosing the plant root and grow within the root cortex to form a Hartig net where nutrient transfer occurs. EM mine soils for inorganic

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nutrients and assimilate amino acids and amino sugars from soil solution (Schimel and Bennett, 2004). Controversy exists, however, as to how widespread phylogenetically and to what extent EM may function to break down soil organic matter (SOM) via protease, lignocellulase and peroxidase enzymes, and transfer nutrients to the plant host (Averill et al., 2014; Bödeker et al., 2014; Pellitier and Zak, 2018; Talbot et al., 2013).

Field studies have shown that EM can vary temporally, spatially, and along gradients of both species richness and enzymatic activity (Courty et al., 2010a,b; Peay et al., 2010; Taylor et al., 2014). Studies of EM fruiting bodies have shown declines in fungal diversity and abundance with increasing N_{avail} at regional scales (e.g. Lilleskov and Bruns, 2001). New molecular techniques can provide finer resolution of EM community structure, revealing highly diverse and patchy belowground distribution and poor associations between sporocarp dominance and EM dominance on roots (Gardes and Bruns, 1996; Horton and Bruns, 2001). These distribution patterns may have important implications for nutrient cycling as research has shown that shifts in fungal community structure can influence soil C sequestration via differences in melanin production and mycelial physiology (Clemmensen et al., 2015; Fernandez and Kennedy, 2015; Fernandez and Koide, 2014; Siletti et al., 2017).

Fungal saprotrophs, a third functional group, secrete enzymes including glucosidase, hemicellulases, and phosphatases that serve to break down SOM (Talbot et al., 2013). Decomposition of SOM by saprotrophs makes N available in soil and produces CO_2 (Högberg et al., 2003; Štursová et al., 2012). Fungal saprotrophs compete for N with plants and their mycorrhizal symbionts as they break down SOM (Averill et al., 2014; Cairney and Meharg, 2002; Högberg et al., 2003). EM fungi and their plant symbionts may inhibit saprotrophic decomposition rates by removing available N from soils as well as by forming thick hyphal mats that prevent saprotroph growth (Averill et al., 2014; Cairney and Meharg, 2002; Lindahl et al., 2013). This competition between EM and saprotrophic fungi for N may lead to increases in soil C stocks if saprotrophic decomposition is inhibited as a result.

Contrary to EM presence possibly resulting in competition with decomposers, AM fungi presence has the potential to increase decomposer activity in forest soils by increasing substrate availability (Averill et al., 2014). AM fungi may increase substrate availability to saprotrophs by facilitating access to SOM patches as they preferentially grow towards the N-rich substrates (Bonfante and Anca, 2009; Cheng et al., 2012). They can also prime saprotroph-mediated decomposition of SOM by releasing labile C (Carney et al., 2007; de Graaff et al., 2010; Phillips et al., 2011). These interactions can have implications for decomposers, which when released from competitive restraints with EM due to increased N_{avail}, can break down organic C stocks accumulated in soil since the last major disturbance.

Measuring the effects of plant community succession on saprobic fungi and mycorrhizal associations is difficult on short time scales (*i.e.*, 1–10 years) and is more often investigated using long-term monitoring or chronosequence studies (Gartner et al., 2012). The Forest Accelerated Succession Experiment (FASET, described in METHODS) provided us with an opportunity to study relationships between changes in plant physiological responses, interspecific competition for soil resources, and fungal community structure in a large-scale, experimental increase in the rate of forest community succession.

Here, we address this question concerning impacts of a non-stand replacing disturbance in a temperate forest ecosystem: How do root and fungal communities differ in a mid-successional mixed hardwood forest following an intermediate disturbance? We hypothesized that girdling and subsequent mortality of mid-successional forest tree species affect below ground plant and fungal community composition by removing two dominant mid-successional EM associated species. This hypothesis leads to the following predictions:

1. Given previously observed increases in maple leaf production and the removal of two EM associated tree species (aspen and birch), the relative abundance of maple roots will increase in girdled stands.
2. The proportion of EM in the fungal community will decrease in treatment plots two tree species with ectomycorrhizal symbionts removed via girdling.
3. The proportion of saprobic fungi will increase in the treatment plots due to less competition for N.
4. The proportion of AM will increase in treatment plots and under higher N_{avail} as an AM plant symbiont becomes a canopy dominant.

2. Methods

2.1. Study site

The study was conducted at the University of Michigan Biological Staion (UMBS) in northern Michigan, USA (45°35′N 84°43′W). Mean annual temperature is 5.5 °C and mean annual precipitation is 817 mm. The bounds of the overall study site (including treatment and reference footprints and their plots) is ~140 ha and lies on a high-level sandy outwash plain and an adjacent gently sloping moraine and includes 17 unique landscape ecosystem types (Lapin and Barnes, 1995; Pearsall, 1985). The landscape ecosystems are generally similar in vegetation (northern mixed forest) and soils (coarse-textured), but vary locally (1–10 ha) in their topography and parent material, soil subgroup, and dominant tree and understory taxa. Across 60–65% of the area, soils are excessively well-drained Entic Haplorthods of the Rubicon series (Soil Survey Staff, 1991). The typical morphology of this series consists of an Oi and Oe horizons 1–3 cm thick, a bioturbated A horizon 1–3 cm, an E horizon 10–15 cm thick, and Bs and BC horizons of sand with occasional gravel and cobble (Nave et al., 2014). Approximately 30% of the study area is on more productive landscape ecosystems, where Lamellic and Alfic Haplorthods of the Blue Lake and Cheboygan series predominate. These soils differ from the Rubicon chiefly in the presence of stratified gravel, clay or loamy sand E′ and Bt horizons. The remaining 5–10% is underlain by Alfic Haploquads of the Riggsville series which are located in lower landscape positions (specifically, surrounding Treatment Replicate Stand #2; Fig. 1) and as a result have a seasonal water table and generally higher soil moisture status than the well-drained Haplorthods. Across all of these soils, approximately half of the fine root biomass is located in the upper 20 cm of soil and the forest floor C mass is approximately 5–15 Mg C ha^{-1}.

The main FASET treatment area occupies 33 ha of forestland within an eddy-covariance tower footprint, located within a larger area of more or less homogenous aspen-dominated, mixed, mid-successional forest. The treatment involved stem girdling of all mature *Populus tremuloides, P. grandidentata*, and *Betula papyrifera* trees (~6700 stems) in 2008, followed by mortality of nearly all girdled stems during the ensuing 3 years (Nave et al., 2014). Girdled trees were the dominant species in this mid-successional forest and represented ~30% of pre-treatment foliar biomass. In addition to the main treatment area, 3 replicated 2-ha experimental units located on other, nearby landscape ecosystems were also subjected to aspen and birch girdling. These plots served as independent replicates of the girdling treatment on sites with different ecosystem properties.

2.2. Field sampling

We installed 12 plots, consisting of 6 paired plots, with of each pair being a plot (16 m radius) within the (girdled) treatment area and a nearby plot located ~50 m outside the treatment area (Fig. 1). The 6 paired locations were selected on the basis of landform-productivity relationships at UMBS (Nave et al., 2017), which likely (based on variation in productivity) occupied a N_{avail} gradient. This allowed for analyses of both treatment and nutrient gradient effects. Each plot contained at least 1 canopy and sapling tree of each of *Pinus strobus* (white pine), *Quercus rubra* (northern red oak), and *Acer rubrum* (red