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Benefit or Liability? The Ectomycorrhizal Association May Undermine Tree Adaptations to Fire After Long-term Fire Exclusion

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ABSTRACT

Long-term fire exclusion may weaken ecosystem resistance to the return of fire. We investigated how a surface wildfire that occurred after several decades of fire exclusion affected a southern Appalachian forest transitioning from a fire-adapted to a fire-intolerant state. Tree traits associated with fire adaptation often co-occur with traits for nutrient conservation, including the ectomycorrhizal (ECM) association. In the absence of fire, the ECM association may facilitate the accumulation of organic matter, which becomes colonized by fine roots that then become vulnerable to consumption or damage by fire. Therefore, a deeper organic horizon might make stands of fire-adapted, ECM trees less resistant to a surface wildfire than stands of arbuscular mycorrhizal (AM), fire-intolerant trees. To test this hypothesis, we established plots in stands that fall along a gradient of mycorrhizal tree relative abundance both inside and outside the perimeter of the 2016 Rock Mountain wildfire. With increasing relative abundance of ECM trees, we found increasing organic horizon depth and mass and slower rates of decay, even for litter of ECM tree species. We calculated a major (73–83%) reduction in fine root biomass and length in the organic horizon following the wildfire. Over three years post-fire, we observed a higher probability of crown decline, basal sprouting and aboveground biomass mortality with increasing abundance of ECM trees. We propose that the biogeochemistry of mycorrhizal associations can help explain why fire exclusion makes stands of fire-adapted trees less resistant to a surface wildfire than those with fire-intolerant trees.

Key words: Wildfire; Disturbance; Reintroduction; Ecosystem resilience; Biogeochemistry; Mycorrhizal fungi; Plant functional traits.

HIGHLIGHTS

- Dominance of ectomycorrhizal trees was associated with a deeper organic horizon.

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Author Contributions NW, JJO and JKH conceived of the idea. All authors contributed to the design of the study. DOC and MKT performed the research. DOC and NW analyzed data. DOC and NW wrote the paper, with contributions from all authors.

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- A wildfire consumed fine roots in the organic horizon, relating to tree decline.
- The ectomycorrhizal association may reduce fire resistance after long-term exclusion

INTRODUCTION

Human activities are inducing novel changes to the structure and function of terrestrial ecosystems (Williams and Jackson 2007; Williams and others 2007; Hobbs and others 2014). One such human activity is the alteration of disturbance regimes, which can weaken the resilience of ecosystems to future disturbance (Varner and others 2005; O'Brien and others 2010; Johnstone and others 2016; Dudney and others 2018). An example of this problem is the exclusion of fire from fire-adapted forests, as it selects for fire-intolerant species (Abrams 1992; Frost 1998; Scott and others 2012) that further suppress fire (Nowacki and Abrams 2008), eventually causing the ecosystem to transition to a fire-intolerant state (O'Brien and others 2008; Scott and others 2012; Kreye and others 2013). However, state changes can take decades or centuries to fully manifest over the course of forest succession, resulting in a contemporary forest landscape composed of both fire-adapted and fire-intolerant stands (Nowacki and Abrams 2015). This leads to a critical question for ecologists and land managers alike: How do contemporary forest ecosystems undergoing a state transition respond to the reoccurrence of fire after long-term fire exclusion?

A consideration of fire and the evolution of plant traits is central to this question. Throughout evolutionary history, plants evolved traits to tolerate, recover from and even promote fire (Bond and others 2004; Kane and others 2008; Lamont and He 2017). However, fire-dependent ecosystems also tend to be nutrient poor (Boerner 1982), since nutrient losses are triggered by the consumption of vegetation and organic matter (Boring and others 2004; Lavoie and others 2010). As a result, functional traits relating to fire adaptation tend to co-occur with those that facilitate nutrient conservation (Mooney and Dunn 1970; Orians and Milewski 2007). In the absence of fire, however, traits for nutrient conservation may supersede those of fire adaptation, resulting in ecosystem properties that are novel relative to the conditions under which the traits evolved.

One possible outcome of long-term fire exclusion is that it leads to abiotic and biotic properties that weaken the resistance (that is, the capacity to withstand the impact of a disturbance; sensu Hodgson and others 2015) of the ecosystem to fire. When leaf litter is long unburned, it can accumulate in organic soil horizons (Varner and others 2005) that become colonized by fine roots (Schenk and Jackson 2002). Fine roots contained in organic materials are vulnerable to fire consumption or damage (Ryan and Frandsen 1991; Swezy and Agee 1991; Smirnova and others 2008; O'Brien and others 2010). Although fire-adapted tree species have specialized traits that facilitate their direct resistance to fire (that is, bark thickness) (Abrams 1992; Varner and others 2016), all trees—regardless of their aboveground adaptations to fire—are likely to experience physiological stress when fire triggers a major loss of fine root biomass (Varner and others 2005; O'Brien and others 2010; Bär and others 2019). In some cases, fine root consumption leads to delayed tree mortality (that is, 3+ years post-fire) when the fine root system is inadequate to support nutrient and water acquisition causing slow, but imminent death (Swezy and Agee 1991; Varner and others 2007; Bär and others 2019). In contrast, under a frequent fire regime, the organic horizon is primarily composed of leaf litter as the frequent fires consume the majority of litter production and limit the formation of humus (Loucks and others 2008; Stambaugh and others 2015). As a result, fine roots predominantly colonize mineral soil horizons that are thermally protected from fire (McLean 1969; Brown and Smith 2000).

Over periods of long-term fire exclusion, fire-adapted tree species may increase the vulnerability of fine roots to fire damage due to the coordination of fire adaptive and nutrient conservative traits. Fire-adapted tree species tend to have leaf litter that is more flammable (Dell and others 2017; Pausas and others 2017) and holds less moisture (Kreye and others 2013, 2018), but is also more nutrient-poor and resistant to decay (Alexander and Arthur 2014) compared to litter from fire-intolerant species. Further, fire-adapted tree species tend to associate with ectomycorrhizal (ECM) fungi, which independently evolved from arbuscular mycorrhizal (AM)-associating ancestors over multiple plant lineages (for example, Pinaceae, Fagaeae and Eucalypteae; (Brundrett and Tedersoo 2018)). These mycorrhizal associations employ contrasting strategies for nutrient acquisition, where AM fungi scavenge mineral nutrients, while ECM fungi mine nutrients from organic matter, albeit at a higher carbon (C) cost to the plant (Lu and Hedin 2019). In temperate forests, dominance by either type represents contrasting stable states (Lu and Hedin 2019), such that ECM dominance leads to slower rates of nutrient cycling and larger stores of nutrients in organic matter relative to those dominated by AM-associated species (Phillips and others 2013; Lin and others 2016). Important in the context of fire, the ECM association appears to suppress the activity of saprotrophs and slow the rate of decay, through direct antagonistic effects of ECM fungi (Averill and Hawkes 2016) and/or indirectly through the low nutrient content of leaf litter their host plant produces (Taylor and others 2016). Thus, the formation of deep organic horizons in forests dominated by fire-adapted trees may be due to slower rates of decomposition associated with ECM dominance. As a result, long-term fire exclusion can make fire-adapted, ECM-dominated forests less fire resistant than fire-intolerant, AM-dominated forests, because fine roots in the organic horizon are vulnerable to consumption or damage by fire, increasing the probability of tree decline and mortality (O'Brien and others 2010).

Here, we evaluate how the return of wildfire after long-term fire exclusion affected the response of southern Appalachian forests undergoing transition from a fire-adapted to a fire-intolerant state (Harrod and others 2000; Brose and others 2001). Prior to European settlement, this region was dominated by fire-adapted tree species, including *Castanea dentata* (Marshall) Borkh., *Pinus* spp. and *Quercus* spp. and burned frequently (for example, every 2–4 years) due to lighting-ignited wildfire and management fires set by indigenous peoples (Delcourt and Delcourt 1997, 1998; Nowacki and Abrams 2008; Flatley and others 2013). Fire exclusion since the turn of the twentieth century and a suite of other factors (for example, climate, loss of *C. dentata*, nitrogen deposition (Elliott and Swank 2008; Pederson and others 2015; Jo and others 2019)) have led to a mosaic of fire-adapted and fire-intolerant stands in southern Appalachia, where the encroachment of fire-intolerant species is widespread and inter-mixed with fire-tolerant species, but also dependent on topographic position (Elliott and Swank 2008). Given that historical fire frequency was high over the majority of the southern Appalachians (Lafon and others 2017), we can infer that a deep layer of humus was largely absent during that time. Indeed, in one of the few studies where prescribed fires have been repeatedly applied in this forest type, the depth of the humus layer diminished by one-quarter after 3 fires in 15 years (Waldrop and others 2016). These observations provide evidence that the deep organic horizons common in southern Appalachian forests today (Knoepp and others 2000; Wurzburger and Hendrick 2007) are likely the product of fire exclusion.

Numerous wildfires burned across the southern Appalachians following a severe drought in the fall of 2016. This provided an opportunity to evaluate the mechanisms of long-term fire exclusion that weaken ecosystem resistance to wildfire, and at a landscape scale larger than that usually afforded by prescribed fires. The Rock Mountain wildfire was ignited in northern Georgia and moved north into western North Carolina, burning over 9000 ha before it was extinguished. The fire was slow-moving with persistent smoldering that consumed much of the organic horizon. We tested the idea that long-term fire exclusion would make stands composed of fire-adapted species less resistant to wildfire than those with fire-intolerant species. Such a response could manifest from differences in the accumulation of organic matter and resulting fine root distributions due to the coordination of fire-adaptive traits with nutrient conservative traits, including the ECM association. Specifically, we hypothesized that with increasing abundance of ECM trees: (1) the organic soil horizon depth and stock would increase, (2) fine root biomass and length in the organic soil horizon would increase and (3) leaf-litter decay rates would decline. As a result, we hypothesized that the wildfire would (4) increase the loss of organic soil horizon and fine root biomass and length and (5) increase crown decline and stem mortality with increasing abundance of ECM trees.

METHODS

The Rock Mountain wildfire (centroid − 83.52, 34.987) was ignited by an arsonist in November 2016 and burned 9824 ha in the Chattahoochee and Nantahala National Forests of northeast Georgia and southwest North Carolina over 25 days. The wildfire ignited during the driest fall in the 84-year climate record at the Coweeta Hydrologic Laboratory, which is adjacent to the Rock Mountain wildfire area, and where the combined precipitation for September and October was only 2.4 cm (Miniat and others 2018). This seasonal drought allowed much of the organic soil horizon and downed woody materials to be completely consumed by the fire, which is rare in the southern Appalachian region (van Lear and Waldrop 1989). The surface fire, however, burned under what would be considered low intensity (Appendix S1: Figure S1), and there was limited damage to aboveground vegetation. Despite widespread consumption of the soil organic horizon throughout the burn perimeter, only 14.5 hectares (0.01%) of the burned area was immediately classified as high or moderate severity (Ellsworth and others 2016), using methods standardized by the USDA Forest Service (Parsons and others 2010). These protocols were developed to characterize immediate impacts of a fire that might require rapid mitigation and not to predict delayed mortality or long-term impacts. Therefore, this short-term assessment of low severity using standard methods may not reflect long-term ecological effects of organic matter consumption. Prior to the Rock Mountain wildfire, we find no record that a fire occurred in our study area for at least the last four decades (USGS Federal Wildland Fire Occurrence Database) and it is likely that fire has not been a factor in the area since the turn of the twentieth century (Fowler and Konopik 2007; Lafon and others 2017).

The vegetation in the area of the Rock Mountain wildfire is a mixed hardwood forest, dominated by oak (*Quercus* spp.) and hickory (*Carya* spp.) in xeric areas transitioning to maple (*Acer* spp.) and tulip poplar (*Liriodendron tulipifera* L.) dominance in mesic areas. The hemlock woolly adelgid (*Adelges tsugae* (Annand).) created areas of standing dead hemlocks (*Tsuga canadensis* (L.) Carrière) in riparian areas. At the Coweeta Hydrologic Laboratory, the mean annual precipitation is 190 cm and the mean monthly temperatures range between 20°C in June through August and 5°C in December and January (Knoepp and others 2008). The soils across the area are Inceptisols or Ultisols weathered from igneous or metamorphic parent material. Most soils of the area are Typic or Umbric Dystrudepts, Typic Humudepts, Typic Hapludults or Typic Kanhapludults with soil textures characterized variously as loamy, fine-loamy, coarse-loamy, clayey and loamy-skeletal (Anon 2019).

In the summer of 2017, we established four spatial blocks distributed across the fire perimeter. Block locations were identified with GIS and stratified across the burned area to capture differences in elevation and the progression of the fire. Each block contained eight plots, where four were inside and four were outside the burned area (that is, burned or unburned condition), and plots within each block were separated by a maximum distance of 2 km. Each set of four plots (within each block and burned/unburned condition) was distributed along a topographic gradient to capture four positions: low elevation, two mid-slopes (one north-facing and one south-facing) and ridge. As a result, our 32 plots captured a range of elevations (low elevation: 762–895 m, midslopes 805–927 m and ridgetops: 957–1035 m). This design allowed us to partition variance associated with the spatial variability of the burn, topographic variation in tree species composition, soils, and capture a wide variation in the abundance of ECM-associated and fire-adapted tree species across plots.

Each plot was 12 m in radius (452.4 m^2), and we tagged and identified all living trees or shrubs with a stem diameter at breast height (dbh) greater than 10 cm in the summer of 2017 and monitored them for the following two summers. At this initial sampling, none of the trees greater than 10 cm dbh in our plots appeared to have died as a result of the wildfire. Trees were categorized by their mycorrhizal association type observed in field conditions (Brundrett and Tedersoo 2018) and whether they were fire-adapted (Brose and Van Lear 1999; Varner and others 2016). We calculated the relative abundance, by percent basal area, of ECM and fire-adapted trees separately, hereafter referred to as abundance of ECM trees and abundance of fire-adapted trees. Trees or large shrubs with the ericoid mycorrhizal (ERM) association, including *Oxydendrum arboreum* (L.) DC. and *Rhododendron maximum* L., were grouped with ECM trees due to ERM fungal traits being more similar to those of ECM fungi than AM fungi (Read and Perez-Moreno 2003) and ERM species having similar effects on organic matter accumulation (Wurzburger and Hendrick 2007, 2009) as do ECM tree species.

To understand how fire and the relative abundance of ECM trees affected O horizon depth and stocks, we conducted a series of measurements in the summer of 2017. First, we sampled the combined depth of the Oe and Oa horizon (that is, the humus or duff layer which is the intermediately to highly decomposed organic horizon; hereafter referred to as Oea). We removed partially decomposed litter (Oi horizon) and measured the combined depth of the Oea to the nearest 0.25 cm using a steel probe (2 cm diameter). We collected five samples in each cardinal direction at 2-m increments (2, 4, 6, 8, and 10 m) away from plot center for a total of 20 samples per plot. We then sampled the entire mass of the O horizon from four locations in each plot. First, we determined sampling location by haphazardly tossing a 0.04-m^2 quadrat from plot center toward each cardinal direction. We collected the entire O horizon within the quadrat using a serrated knife, and the material was dried at 60°C for more than 48 h. We sorted the dried organic horizon matter into two categories—Oi and Oea. To separate the Oea horizon from roots and adhering mineral soil, we used a 2-mm sieve, and when necessary we submerged samples in DI water over night to allow mineral soil to sink. The following day we decanted the floating organic matter into pre-weighed Whatman 41 filter paper placed inside funnels and drained completely. The filter paper and organic matter were subsequently dried at 60°C for more than 48 h. Total organic matter mass was calculated for each quadrat on a g m^{-2} basis. From each quadrat, Oi and Oea samples were homogenized individually before grinding to a fine powder and weighing into tin capsules to be analyzed for total C and nitrogen (N) by Micro-Dumas combustion. Total C and N were expressed on a g m^{-2} basis.

To understand how fire and ECM abundance affected fine root biomass and length in the organic horizon, we collected four, randomly located soil cores from each of the 32 plots during the summer of 2017 using a 4-cm-diameter, PVC cylinder with a beveled edge and a rubber mallet. We separated the soil core into depth increments—O horizon, 0–10 cm mineral soil and 10–20 cm mineral soil. All cores were frozen until processed. At the time of processing, we randomly selected three of the four cores to be fully processed, and we only processed the O horizon depth of the fourth core. We increased the sampling of O horizon depth because of the greater variability in root mass and length among cores from the same plot. Cores were thawed for 48 h before roots were hand-separated from soil as best as possible using forceps for no longer than 10 min per sample. Fine roots from the organic horizon were often entangled in organic matter at this stage. Roots with organic matter were transferred to a large plexiglass tray with 1 × 1 cm grids, illuminated on a light board, and were submerged with tap water. Using digital calipers, roots were placed into one of three diameter categories: smaller than 1 mm, 1–2 mm and larger than 2 mm. For each category, we quantified root length using the line-intersect technique modified from Hendrick and Pregitzer (1993) and Wurzburger and Hendrick (2007). Roots in the larger than 2 mm and 1-2 mm size classes were then cleaned with a paintbrush, dried and weighed. Because roots in the smaller than 1 mm class were often entangled in organic matter and fungal hyphae, we collected a representative subsample of cleaned root length to quantify specific root length (SRL; cm g^{-1}), which was applied to root length data to estimate biomass. All cleaned root samples were placed in a drying oven at 60°C for more than 48 h before weighing. For each depth and size class, root data were expressed as cm m^{-2} and g m^{-2}.

We quantified leaf-litter decay in a subset of unburned plots to verify that leaf-litter decay was influenced by the abundance of ECM trees. We collected leaf litter from elevated litter traps in the fall of 2017 in our study area and created two litter groupings: AM litter and ECM litter. The AM litter was a mixture of *Acer* species and *Liriodendron tulipifera* combined at a ratio of 1.8:1, while the ECM litter was a mixture of *Quercus* species and *Carya* species litter combined at a ratio of 1.8:1. These ratios reflected the relative contribution of these species to forest basal area in our plots. Each bag contained 3.5 g of litter inside a 2-mm mesh. For each litter type, we installed three replicate bags in the winter of 2017 across nine unburned plots on the surface of the Oea horizon (if it was present) or on the surface of the mineral soil (if the Oea was absent). After an average of 280 days (range 266–302 days), we collected bags, soaked litter for 12 min and washed it over a 2-mm sieve to remove adhering soil. Litter was then dried in an oven at 60°C for more than 72 h before weighing. Litter data were expressed as percent of mass loss day^{-1}.

To understand how the fire and ECM abundance affected tree response post-fire, we surveyed all trees (355 in burned and 427 in unburned plots) during the summers of 2018 and 2019 for signs of tree stress or mortality. For each tree, we recorded basal sprouts and epicormic sprouts as a count per tree, and the crown class was categorized as either 0 (no crown), 1 (1–25% crown), 2 (26–50% crown), 3 (51–75% crown), or 4 (76–100% crown). We identified two, non-mutually exclusive classes of mortality: ''complete'' where there were no leaves in the canopy or sprouts of any kind, and ''aboveground'' where canopy had no leaves or epicormic sprouts, but basal sprouts could be present. We also noted whether trees had been windthrown or damaged by other fallen trees.

Statistical Analyses

We sought to test whether the species composition of stands affected its resistance to wildfire after long-term exclusion. Our hypothesis was based on the idea that most fire-adapted tree species in temperate forests associate with ECM fungi, and ECM-dominated stands tend to have more organic matter accumulation and slower decomposition relative to AM-dominated stands, at least in the absence of fire. Thus, the co-occurring trait of mycorrhizal association and its effect on biogeochemical cycles, not fire tolerance *per se*, were the hypothesized driver of organic matter accumulation and fine root loss following wildfire. To test this assumption about the coordination of traits, we categorized the 35 tree species found in our 32 study plots by mycorrhizal association type and whether they were fire adapted, where we considered bark thickness and flammable foliage as traits associated with fire adaptation (Brose and Van Lear 1999; Varner and others 2016) (Appendix S1: Table S1). We found a strong correlation between the relative abundance of ECM tree species and fire-adapted tree species at the plot level (r^2=0.90, $p < 0.0001$) (Appendix S1: Figure S2). Underscoring this result, only one AM tree species in this study, *Robinia pseudoacacia* L., is considered fire-adapted owing to its thick bark, and only a fraction of ECM tree species are considered fire-intolerant, including *Betula alleghaniensis* Britt., *B. lenta* L., *Fagus grandifolia* Ehrh., *Tilia americana* L., and *Tsuga canadensis* (Appendix S1: Table S1). These non-fire-adapted ECM species accounted for about 14% of the ECM species, but less than 6% of the stems in our study. Thus, fire adaptation was correlated with the ECM association across our study plots, and all of our statistical analyses test the importance ECM abundance as a potential contributor of organic matter accumulation, fine root loss and tree stress following fire.

Organic Horizon

To determine the probability of Oea presence across the gradient of ECM abundance and presence or absence of fire, we constructed a generalized linear mixed model with a binomial distribution, where fire, ECM basal area and their interaction were fixed effects and topographic position and block were random effects, using glmer (*lme4*; (Bates and others 2015). For all of our mixed-effects models, topographic position and block were assigned as random effects. This allowed us to isolate ECM effects above those of topography, which are well known to influence forest community composition, soil properties and climate. Further, using spatial blocks allowed us to explain variance due to day of the wildfire. If random effects accounted for no variance in the model, we ran a logistic regression model with no random effects.

We analyzed Oea depth, C and N stocks and the C:N with a linear mixed-effects model using lmer (*lme4*; Bates and others 2015) where fire, ECM basal area and their interaction were fixed effects and topographic position and block were random effects. We conducted F tests using Kenward-Roger approximated degrees of freedom (*afex*; Singmann and others 2019). For all linear mixed-effect models, we determined the marginal and conditional R^2 values using the function rsquared. This calculates the variance associated with the fixed effects and both the fixed and random effects, respectively (*piecewiseSEM*; Lefcheck 2016). Thus, the difference between marginal and conditional R^2 values provides the proportion of variance explained by topography and block.

Root Biomass and Length

To determine how ECM abundance, fire, and their interaction affected root biomass and length in the different horizons, we fit linear mixed-effect models (as above) where fire, ECM basal area, soil depth increment and all possible interactions were fixed effects, while topographic position and block were random effects. We excluded nonsignificant interactions from the final models. We constructed these models for each of the three root diameter size classes. We used emmeans (*emmeans*; Lenth 2019) to evaluate significant differences between the root biomass or length in a specific depth of the burned or unburned plots, and to assess differences in biomass or length at different depths.

Leaf-Litter Decay

We analyzed percent mass loss per day as a function of litter type (AM or ECM leaf-litter) and with ECM relative abundance as a continuous variable using linear regression. Because a subset of plots was selected for this experiment, we did not have enough replication across blocks and topographic positions to include them as random effects.

Tree Stress

We used an ordinal logistic regression approach to determine how the probability of tree stress (basal sprouts and crown class) changed with ECM abundance, fire, the mycorrhizal association type of the tree and all possible interactions. We first used cumulative link mixed models (*ordinal;* (Christensen 2019) where ECM basal area, mycorrhizal type, fire and their interactions were fixed effects and topographic position, block and species were random effects. To graph predictions, we used polr (*MASS*; (Venables and Ripley 2002), where all model terms were fixed effects and we assigned levels of topographic position and block that generated the smallest random effects. We separately analyzed variables from 2018 and 2019.

To determine how the probability of tree mortality and aboveground biomass mortality varied with plot-level ECM abundance, fire, the mycorrhizal association type of the individual tree and their interactions, we utilized a generalized linear mixed model with a binomial distribution (as above) where ECM abundance, fire, the mycorrhizal association type of the tree and all possible interactions were fixed effects. If random effects accounted for no variance in the model, we ran a logistic regression model with no random effects. For all analyses, when necessary we square-root or ln-transformed (after adding 1 to each value if there were 0 values) to resolve issues with non-normal error distributions. All analyses were conducted in R (version 3.6.1, (Core Team 2014)).

RESULTS

Organic Horizon

We found that the probability of a plot containing an Oea horizon increased with increasing ECM abundance (z=2.57, p=0.010) (Figure 1A) and declined following fire (z=2.05, p=0.040) (Figure 1B). Similarly, the depth of the Oea horizon increased with increasing ECM abundance ($F_{1,15}$=7.96, p=0.010) (Figure 1C) and declined with fire ($F_{1,23}$=35.60, $p<0.0001$) (Figure 1D). For neither the presence nor the depth of the Oea horizon did we detect a significant interaction between ECM abundance and fire, suggesting that a similar proportion of O horizon was lost to fire regardless of ECM tree abundance. After accounting for topographic effects, our model predicted that the depth of the Oea horizon increased fivefold (from 0.47–2.36 cm) from 0 to 100% ECM abundance in the unburned plots. On average, the wildfire consumed 1 cm of the Oea horizon. The fixed effects of fire and ECM abundance accounted for a larger proportion of variance than did our random effects of

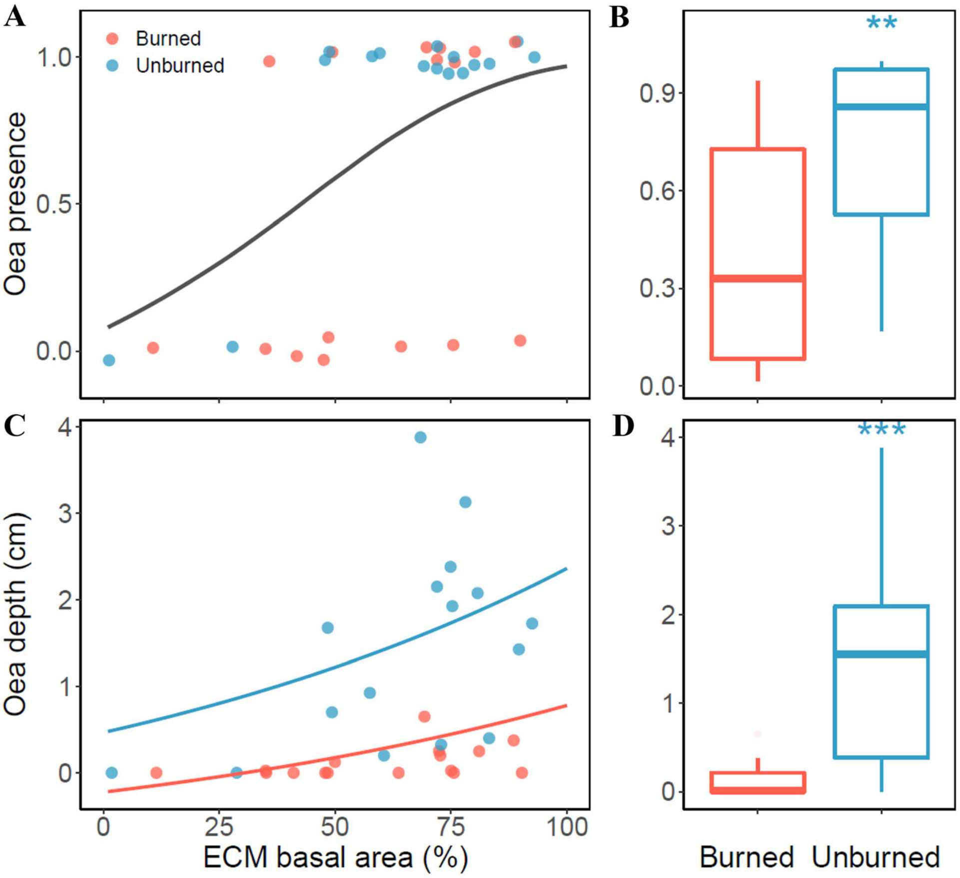

Figure 1. Presence and depth of the Oea horizon in the area of the Rock Mountain wildfire, about 7 months after the wildfire. The presence of the Oea horizon **A** with increasing abundance of ECM trees, and **B** in burned or unburned plots. The depth of the Oea horizon **C** with increasing abundance of ECM trees and **D** in burned or unburned plots. Values in (A) are jittered along the y axis for better visualization, and values in (**B**) and (**D**) are presented as boxplots with the median value and upper and lower quartiles. Curves are (**A**) probability of Oea horizon presence with increasing ECM abundance (p=0.01) and (**C**) smoothed, back-transformed predictions of Oea depth with increasing ECM abundance (p=0.01), holding random effects at their minimum value. Differences between burned and unburned plots are denoted by the following: ($p<0.001$ = ***; $p<0.05$ = **).

topographic position and block (R^2_m = 0.57, R^2_c=0.66).

We next assessed the standing stocks of mass, total C and total N in the O horizon. The mass of the Oi and Oea horizons increased with increasing ECM abundance ($F_{1,15}$=8.74, p=0.004) (Figure 2A) and declined after the fire ($F_{1,55}$=45.65, $p<0.0001$; R^2_m = 0.70, R^2_c=0.73) (Figure 2b). The stock of C in the O horizon followed the same pattern (ECM abundance: $F_{1,57}$=9.71, p=0.003; fire: $F_{1,57}$=46.08, $p<0.001$; R^2_m = 0.68, R^2_c=0.71) (Appendix S1, Figure S3ab), as did the stock of N (ECM abundance: $F_{1,15}$=8.02, p=0.006; fire: $F_{1,57}$=43.42, $p<0.0001$; R^2_m = 0.75, R^2_c=0.77) (Appendix S1, Figure S3cd). Within the O horizon, the Oea horizon contained more mass than did the Oi horizon ($F_{1,54}$=104.00, $p<0.0001$) (Figure 2A), and the same was true for total C and N ($F_{1,57}$=87.65, $p<0.001$; $F_{1,57}$=150.86, $p<0.0001$ for C and N, respectively) (Appendix S1, Figure S3). Because the interaction of ECM abundance and fire was not significant in these models, it suggests that a similar proportion of C and N were volatilized in the wildfire across plots (Appendix S1: Table S2). To understand the effect of ECM tree abundance on C and N stocks in unburned plots, our model predicted 16–81 g·m^{-2} of C and 0.4–1.6 g·m^{-2} of N in the Oi horizon, and 114–585 g·m^{-2} of C and 5–20 g·m^{-2} of N in the Oea horizon across the gradient of 0–100% ECM abundance. We verified that total basal area was not correlated with ECM abundance across plots (r^2 = 0.02, $p>0.05$), excluding the possibility that total forest biomass, rather than mycorrhizal association, was responsible for the patterns in organic horizon depth and abundance.

Fine Root Biomass and Length

We found that the biomass of fine roots (<1 mm diameter) increased with increasing ECM abundance in the O horizon, but not in the mineral soil depths (Figure 3A; Appendix S1: Tables S3 and S4). Fine root biomass in the O horizon was lower in burned versus unburned plots, but not in the other soil depths (Figure 3b; Appendix S1: Tables S3 and S4). The difference between burned and unburned plots indicates that on average 100 g m^{-2}, or about 83%, of fine root biomass in the O horizon was lost due to the wildfire. The medium (1–2 mm diameter) and coarse (>2 mm) root size classes were consistently affected by depth, but of these only the medium roots were affected by ECM abundance. Both the medium and coarse roots were significantly affected by the interaction of depth and fire (Appendix S1: Tables S3 and S4).

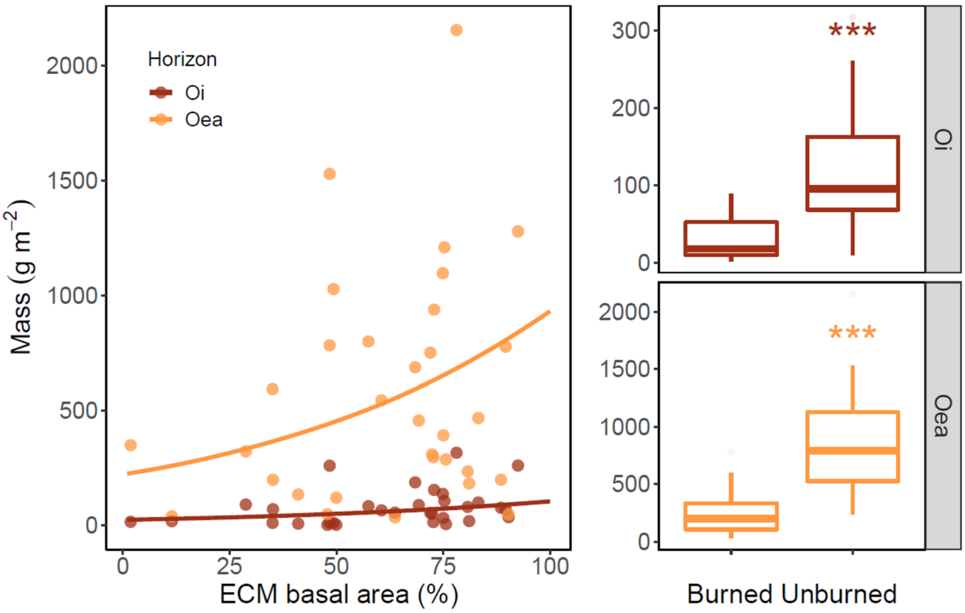

Figure 2. Organic horizon mass in the area of the Rock Mountain wildfire, about 7 months after the wildfire. O horizon mass **A** with increasing abundance of ECM trees and by Oi and Oea horizon; and **B** in burned or unburned plots by horizon. Curve in (**A**) is a smoothed, back-transformed prediction of mass in burned and unburned plots with increasing ECM abundance ($p<0.05$), holding random effects at their minimum value. Values in (**B**) are presented as boxplots with the median value and upper and lower quartiles. Differences between burned and unburned plots are denoted by the following: ($p<0.001$ = ***; $p<0.05$ = **).

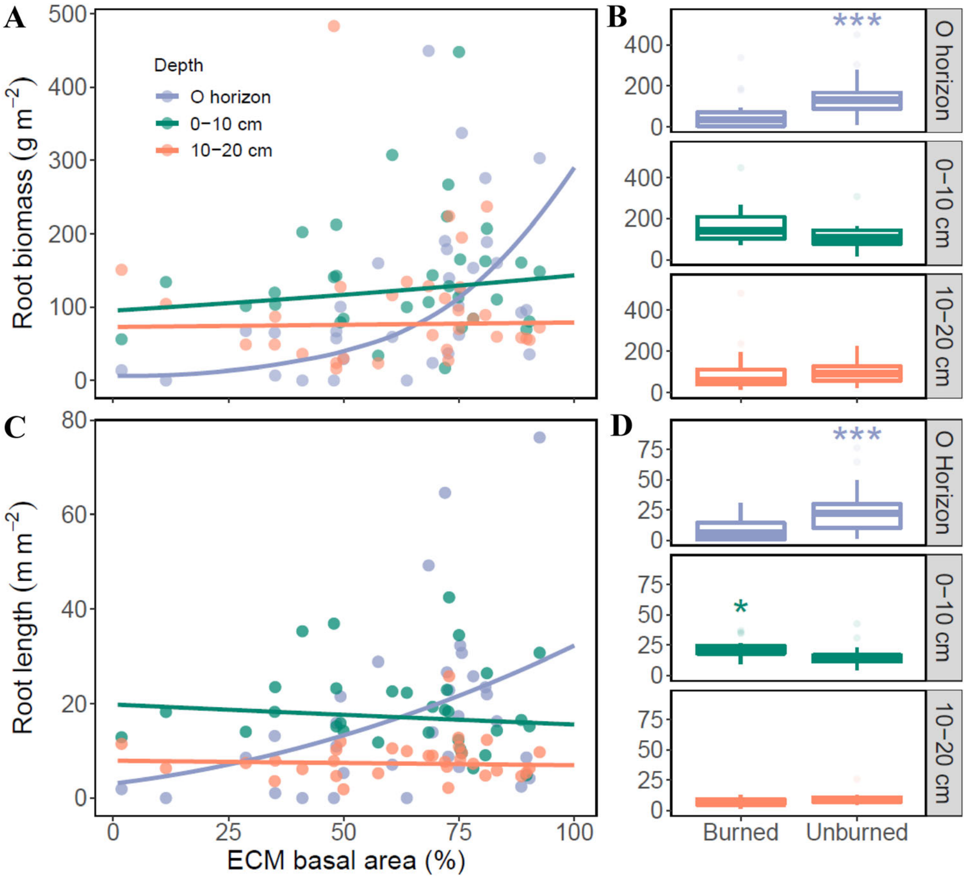

Figure 3. Fine root biomass **A** and length **C** with increasing abundance of ECM trees in three soil depths and fine root biomass **B** and length **D** in burned or unburned plots in the area of the Rock Mountain wildfire, about 7 months after the wildfire. Curves in (**A**) and (**C**) are smoothed, back-transformed predictions in burned and unburned plots with increasing ECM abundance, holding random effects at their minimum value. Values in (**B**) and (**D**) presented as boxplots with the median value and upper and lower quartiles. Biomass and length increased with ECM abundance in the O horizon but not in the other soil depths (depth by ECM interaction: p=0.0003, p=0.0005, respectively), and differences between burned and unburned plots are denoted by the following: ($p < 0.001$ = ***).

Fine root length followed a similar pattern to that of biomass and increased with increasing ECM abundance in the O horizon, but not in the mineral soil depths (Figure 3C, Appendix S1: Tables S5 and S6). Fine root length in the O horizon was lower in burned versus unburned plots, but in the 0–10 cm depth biomass was higher in burned versus unburned plots (Figure 3D, Appendix S1: Tables S5 and S6). Both the medium and coarse root size classes were affected by the interaction of depth and fire (Appendix S1: Tables S5 and S6). The difference between burned and unburned plots indicates that on average 1632 m m^{-2}, or about 73%, of fine root length in the O horizon was consumed or damaged by the wildfire.

Leaf-Litter Decay

The rate of litter decay was faster for AM versus ECM leaf litter ($F_{1,51}$ = 18.8, p < 0.0001) and declined with increasing ECM abundance ($F_{1,51}$=20.1, $p < 0.001$; Figure 4). This supports the idea that increasing ECM tree abundance slows decomposition, beyond the effects of leaf-litter quality.

Tree Stress

In the third summer post-fire, we surveyed live and dead trees and found a twofold increase in the probability of complete tree mortality (z=2.11, p=0.03) (Appendix S1: Fig S3), where 4.2% and 1.7% of trees had died in the burned vs. unburned plots, respectively. We found that wind and other tree damage were not related to fire, ECM abundance, nor their interaction ($p > 0.1$). As a result,

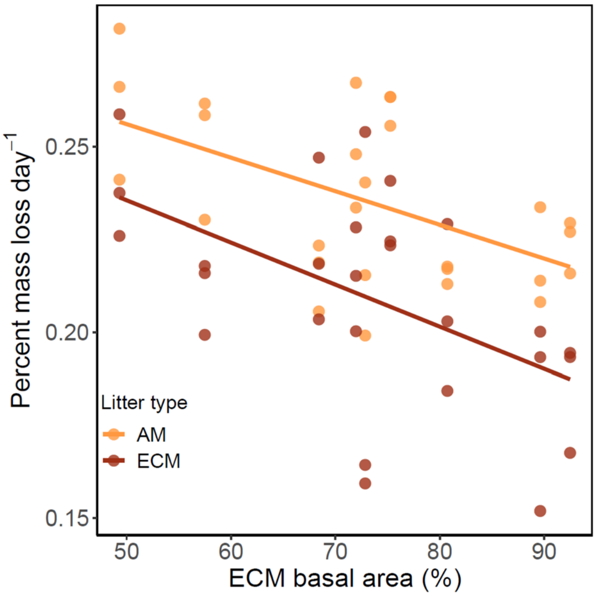

Figure 4. Leaf-litter decay in unburned plots near the site of the Rock Mountain wildfire. Decay rates were determined over an average of 280 days (range 266–302 days), in a subset of unburned plots. Rates were faster for AM versus ECM leaf litter ($p < 0.0001$), but both declined with increasing abundance of ECM trees ($p < 0.001$).

we excluded wind-killed or damaged trees from the analysis in order to examine metrics of tree health (that is, crown class and basal sprouting) on standing trees as a function of ECM abundance and in response to wildfire.

In 2018, the second summer post-fire, the probability of crown decline increased with ECM abundance ($z = - 2.477$, $p = 0.01$) and was modestly related to fire ($z = 1.676$, $p = 0.09$) (Figure 5A). In 2019, the probability of tree crown decline depended on the interaction of ECM abundance and fire ($z = 4.32$, $p < 0.0001$), where the probability of a tree having a full crown declined with increasing ECM basal area in burned stands (Figure 5B). This finding supports the idea that increasing fine root consumption or damage with increasing ECM abundance led to a reduction in crown class over the three years post-fire independent of tree mortality. Further, the relationship between crown decline and ECM abundance emerged beyond the effect of topographic position and day of fire, which were random effects in our model.

When analyzing the abundance of basal sprouts as an indicator or tree stress, both 2018 and 2019 data showed an interaction of ECM abundance and fire, where the probability of trees having basal sprouts increased with ECM abundance in the burned plots. This interaction, however, weakened between year 1 and year 2 of the study (2018: $z = - 3.17$, $p = 0.002$; 2019: $z = - 2.27$, $p = 0.02$) (Figure 6).

Because most southern Appalachian trees have the capacity to sprout from the base following top-kill, we categorized trees as either retaining or losing their pre-burn aboveground biomass as (that is, aboveground mortality = no crown, no trunk sprouts, but may possess basal sprouts). We found an increasing probability of aboveground mortality with increasing ECM abundance in the burned plots (fire by ECM abundance interaction; ($z = - 2.90$, $p = 0.004$) (Appendix S1: Fig S4), where about 16% of trees lost aboveground biomass in stands dominated by ECM trees following fire. In unburned plots, the highest probability of aboveground mortality was in AM-dominated plots (~8%).

Discussion

Our work provides evidence that fire exclusion can make stands of fire-adapted tree species less resistant to a surface wildfire than stands of fire-intolerant tree species. Our findings provide support for the idea that long-term fire exclusion may weaken the resistance (that is, the capacity to withstand the impact of a disturbance; sensu Hodgson and others 2015) of fire-adapted ecosystems (Larson and others 2013). This problem is typically examined in the context of high-intensity crown fires that result in direct tree mortality. However, low-intensity fires that consume the organic horizon and fine roots can also result in tree mortality, but the effect is often delayed (O'Brien and others 2010). We propose that the coordination of fire-adaptive traits with the ECM association can help explain a deeper soil organic horizon in stands of fire-adapted trees after long-term fire exclusion. These fuels, and the roots within them, represent a resilience debt (sensu Johnstone and others 2016) that is only paid after fire finally returns.

Organic Horizon

The association of traits for nutrient conservation with those for fire adaptation appears to weaken the fire resistance of fire-adapted stands, because they promote a deeper organic horizon in the long-term absence of fire. We found that the probability of O horizon presence and O horizon depth (Figure 1) and mass (Figure 2) increased with the increasing abundance of ECM trees, whether or not the plot had burned in the Rock Mountain wildfire. This finding supports biogeochemical theory that ECM-dominated temperate forests cycle nutrients

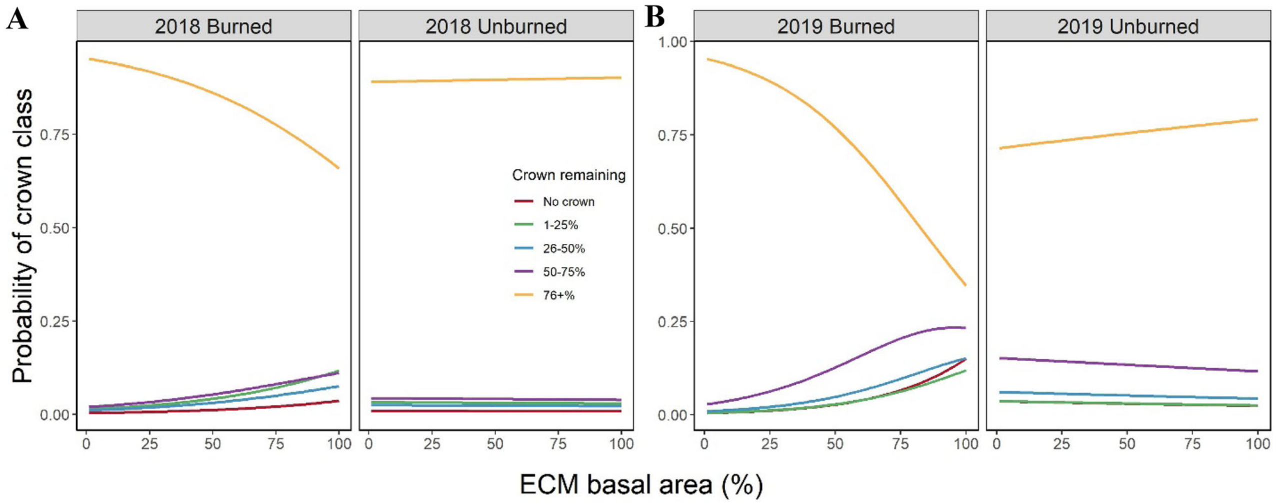

Figure 5. The probability of crown class in burned and unburned plots with increasing abundance of ECM trees for **A** 2018 and **B** 2019, which were the second and third summers, respectively, following the Rock Mountain wildfire. In 2018, the probability of a full crown declined with increasing ECM abundance (p=0.01) and was weakly related to fire (p=0.09), and in 2019, the probability of a full crown declined with increasing ECM abundance in burned plots (fire by ECM interaction: $p < 0.0001$).

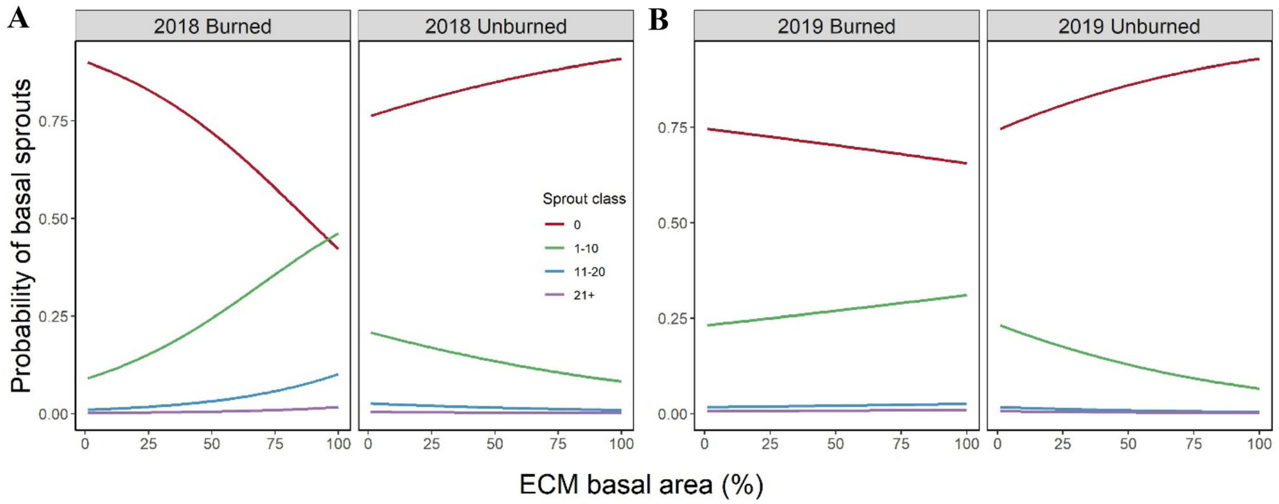

Figure 6. The probability of basal sprout class in burned and unburned plots with increasing abundance of ECM trees for **A** 2018 and **B** 2019, which were the second and third summers, respectively, following the Rock Mountain wildfire. The probability of basal sprouts increased with ECM abundance in burned plots (fire by ECM interaction: p=0.002, p=0.02 for 2018 and 2019, respectively).

at a slower rate than their AM-dominated counterparts (Lu and Hedin 2019). Leaf litter of ECM-associating trees is nutrient-poor (Averill and others 2019), and slow to decay (Keller and Phillips 2019), but litter quality may not be the only driver of this phenomenon. Slower rates of nutrient cycling in ECM-dominated versus AM-dominated ecosystems are also due to direct or indirect suppressive effects on decomposer organisms (Averill and Hawkes 2016; Wurzburger and Brookshire 2017). Indeed, we found evidence that decay rates, even for ECM leaf litter, declined moderately with increasing ECM abundance (Figure 4), suggesting the possible importance of mycorrhizal associations on nutrient cycling and decomposition rates. The coordination of fire-adaptive and nutrient-conservative traits likely maintained this ecosystem in an ECM-dominated state prior to European settlement. Frequent fire promoted by the flammable organic horizon directly suppressed the recruitment of fire-intolerant seedlings (Nowacki and Abrams 2008). Further, fire-adapted, ECM trees mined nutrients from organic matter and used them conservatively (Averill and others 2019), while limiting nutrient access to fire-intolerant, AM species by suppressing rates of decomposition and mineralization (Lu and Hedin 2019).

Roots

We found increasing biomass and the length of the fine roots contained in the O horizon with increasing ECM abundance (Figure 3), predisposing fine root loss by fire. In unburned plots, the O horizon accounted for nearly 40% of the total biomass and 50% of the total length of fine roots (< 1 mm) contained in the top 20 cm of soil (Appendix S1: Tables S3 and S5), indicating the potential for physiological stress following O horizon consumption by fire. Since we sampled roots about 7 months after the wildfire, we were unable to quantify the relative contribution of direct root consumption versus mortality of roots resulting from heat damage to higher-order roots (Bär and others 2019). Thus, our measures of fine root stocks represent the net effect of these potential loss pathways as well as new root production. Nonetheless, the loss of such a large portion of the fine root mass and length represents a significant physiological challenge driven by O horizon consumption, as has been demonstrated in other fire-adapted ecosystems that have been long-unburned (Varner and others 2009; O'Brien and others 2010).

We did not identify the fine roots colonizing the O horizon to tree species or mycorrhizal association; however, our prior work in the area demonstrates that both AM and ECM roots colonize the O horizon of soils (Wurzburger and Hendrick 2009). Consistent with this idea, we found no difference in tree stress and mortality between AM and ECM tree species regardless of the mycorrhizal dominance of the stand (see *Tree stress* below). Surprisingly, we observed greater fine root length in the 0–10 cm soil depth in burned vs. unburned plots (Figure 3), suggesting a redistribution of new fine roots into the mineral soil post-fire, which may have occurred in response to the losses of fine roots in the organic horizon. This is supported by evidence that fine root production can occur from spring to summer in southern Appalachian forests (Davis and others 2004). A downward migration of fine roots into mineral soil suggests that over the long term, ECM-dominated stands should become more resistant to future fire events.

Tree Stress

We found that after three summers following this large wildfire, the probability of tree stress and aboveground stem mortality increased with increasing abundance of fire-adapted, ECM-associated trees. Importantly, stands most negatively impacted by fire held the greatest relative abundance of fire-adapted tree species (that is, oak and hickory species), which are often promoted via prescribed fire to help maintain forest resilience to drought or fire. Specifically, we observed a decline in the crown class of trees, and an increase in basal sprouting and the mortality of aboveground biomass with increasing ECM abundance, likely due to the increased root consumption or damage by fire. We found that the mycorrhizal association of an individual tree did not help predict tree stress or aboveground mortality, suggesting that in ECM-dominated stands, AM- and ECM-associated trees are equally vulnerable to fine root loss and delayed decline.

We found that the decline in tree crown class increased with increasing ECM abundance following fire (Figure 5), suggesting that a reduction in canopy leaf area may have resulted from the consumption of the Oea horizon and loss of fine roots. After three summers post-fire, only ~30% of trees possessed a full crown in ECM-dominated stands, while about 75% maintained a full crown in our unburned plots (Figure 5). When under stress, many tree species produce basal sprouts (Meier and others 2012), which can replace the aboveground biomass that was lost to fire, drought or disease, functioning as an important trait for disturbance recovery (Clarke and others 2013). Accordingly, we found that the probability of basal sprouting increased with increasing ECM abundance following fire (Figure 6), but this relationship dampened between the first and second summers of our study, suggesting a loss of basal sprouts over time.

Unlike previous studies documenting high levels of fine root consumption (for example, O'Brien and others 2010), complete tree mortality 3 years post-fire was low in our study (Appendix S1: Figure S3) and was not affected by the abundance of ECM trees. Tree species of southern Appalachian forests might be more tolerant of fine root loss than longleaf pine (*Pinus palustris* Mill) due to their ability to reallocate resources to basal sprouting while shutting down the flow of C to stem growth and leaf production and maintenance (Clarke and others 2013). However, we found an increasing probability, albeit a modest one, of aboveground biomass mortality with increasing ECM abundance following fire (Appendix S1: Figure S4), where about 16% of trees lost their aboveground biomass in ECM-dominated stands compared to a maximum of about 8% in unburned stands.

Fire Effects

Our study provided the opportunity to quantify organic matter consumption from the Rock Mountain wildfire after several decades of fire exclusion (Lafon and others 2017). When scaled up, an average of 6537 kg ha^{-1} of O horizon mass, which represents 2906 kg ha^{-1} of C, and 98 kg ha^{-1} of N, were consumed in the wildfire. The greatest loss of C and N occurred in stands dominated by ECM trees, where our statistical models that predict 5360 kg ha^{-1} of C and 156 kg ha^{-1} of N were lost from the O horizon. These fire-induced N losses could be balanced by long-term inputs of N from atmospheric deposition. For example, the average losses of N could be balanced by 18 years of ammonium and nitrate wet deposition (average of 5.5 kg N ha^{-1} y^{-1} at the nearby Coweeta Hydrologic Laboratory since the 1970s; NADP), whereas the maximum N losses could be balanced by 28 years of deposition. Elevated atmospheric N deposition rates have been linked to changes in species and mycorrhizal composition (Bobbink and others 2010; McDonnell and others 2018; Jo and others 2019) and nutrient cycling (Knoepp and others 2008). Therefore, prescribed fire may be a strategy to remove this accumulated N, as has been demonstrated in longleaf pine ecosystems (Tierney and others 2019).

Methodological Considerations

We note two key challenges in capturing the effect of fire on ecosystem properties. Organic horizon mass and fine root biomass are highly variable at small spatial scales, and this spatial heterogeneity could be accentuated by fire. For example, the O horizon mass remaining post-fire depends on the starting mass and its flammability, but is also affected by small-scale differences in variables that influence combustion such as moisture, wind, and fire weather (Kreye and others 2014). A second consideration is the uncertainty introduced by inferring fire effects post hoc by comparing burned and unburned stands, as we lack information on the pre-fire condition of each of our plots. We sought to minimize this concern by establishing plots in unburned areas using the same criteria as those used within the fire perimeter (that is, plots located within spatial blocks and different topographic positions).

CONCLUSIONS

Human activities are changing disturbance regimes that are critical for maintaining ecosystem structure and function (Johnstone and others 2016). An increasingly important challenge is understanding how long-term fire exclusion affects the resistance of an ecosystem to the reintroduction of fire. In southern Appalachian forests, long-term fire exclusion has resulted in a mosaic of stands with varying abundances of fire-adapted and fire-intolerant species. Intuitively, we might expect the encroachment of fire-intolerant species to weaken the resistance of these forests to fire. Our study demonstrates the opposite phenomenon—stands dominated by fire-adapted, ECM trees were less resistant to a wildfire than stands dominated by fire-intolerant, AM trees. In this case, a slow-moving surface fire interacted with novel fuels (namely, a deeper organic horizon) that were formed by fire-adapted tree species in the absence of fire. Thus, our findings illustrate the concept of resilience debt (Johnstone and others 2016), explained by the coordination of fire-adaptive and nutrient-conservative traits among tree species. How well this framework might translate to other forest ecosystems is unclear and may depend on whether traits for fire adaptation align with the ECM association in those ecosystems. Other nutrient-conservative traits, such as the evergreen habit, may produce similar responses to the reintroduction of fire, especially when significant organic horizon is consumed.

In the long term, it is unclear how the return of fire might affect the composition of southern Appalachian forests. Initially, wildfires may have a greater negative effect on fire-adapted stands than fire-intolerant stands, which may further challenge management efforts. Although the initial assessment of low fire severity was based on what appeared to be modest loss of the organic horizon and little direct mortality, our study demonstrates delayed effects on tree crowns and aboveground mortality in the most desirable stands. These long-term effects add nuance to the ecological definition of fire severity (Keeley 2009) when the soil organic horizon is consumed. However, these negative effects could be minimized by applying prescribed fires under conditions that limit complete organic horizon consumption (for example, Waldrop and others 2016), applied at regular intervals and at a sufficient frequency to promote the downward growth of roots into mineral soils and to suppress the establishment of fire-intolerant seedlings. Perhaps most importantly, our work provides a cautionary tale to forest and fire managers, that the reintroduction of fire can have delayed negative effects due to the novel fuels caused by long-term fire exclusion.

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