ELEMENTA
Science of the Anthropocene

Kivlin, SN, et al. 2021. Arbuscular mycorrhizal fungal response to fire and urbanization in the Great Smoky Mountains National Park. *Elem Sci Anth*, 9: 1. DOI: https://doi.org/10.1525/elementa.2021.00037

RESEARCH ARTICLE

Arbuscular mycorrhizal fungal response to fire and urbanization in the Great Smoky Mountains National Park

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Wildfires are increasing in frequency and intensity as drier and warmer climates increase plant detrital fuel loads. At the same time, increases in urbanization position 9% of fire-prone land within the United States at the wildland–urban interface. While rarely studied, the compounded effects of urbanization and wildfires may have unknown synergistically negative effects on ecosystems. Previous studies at the wildland–urban interface often focus on aboveground plant communities, but belowground ecosystems may also be affected by this double disturbance. In particular, it is unclear how much fire and urbanization independently or interactively affect nutritional symbioses such as those between arbuscular mycorrhizal (AM) fungi and the majority of terrestrial plants. In November 2016, extreme drought conditions and long-term fire suppression combined to create a wildfire within the Great Smoky Mountains National Park and the neighboring exurban city of Gatlinburg, TN. To understand how the double disturbance of urbanization and fire affected AM fungal communities, we collected fine roots from the 5 dominant understory species in September 2018 at each of 18 sites spanning 3 burn severities in both exurban and natural sites. Despite large variation in burn severity, plant species identity had the largest influence on AM fungi. AM fungal colonization, richness, and composition all varied most among plant species. Fire and urbanization did influence some AM fungal metrics; colonization was lower in burned sites and composition was more variable among exurban locations. There were no interactions among burn severity and urbanization on AM fungi. Our results point to the large influence of plant species identity structuring this obligate nutritional symbiosis regardless of disturbance regime. Therefore, the majority of AM fungal taxa may be buffered from fire-induced ecosystem changes if plant community composition largely remains intact, plant species life history traits allow for AM fungal persistence after fire disturbance, and/or nearby undisturbed habitat can act as an inoculum source for recolonization following fires. Thus, it is critical to maintain natural, undisturbed habitats interspersed within the wildland–urban interface.

Keywords: Wildfire, Soil biogeochemistry, Plant-fungal symbiosis, Burn severity, Mycorrhizal fungi

Introduction

Wildfires are increasing in frequency and intensity as drier climates increase plant detrital fuel loads (McKenzie et al., 2004; Flannigan et al., 2006; Williamson et al., 2009; Knorr et al., 2016). This trend is occurring in the southeast United States, where higher temperatures and changing precipitation patterns are expected to increase the annual burned area by 34% by 2060 (Prestemon et al., 2016). Wildfires were once common in the southeastern United States, creating mosaics of forests interspersed with recently burned grassland patches (Waldrop et al., 1992). However, historical wildfire suppression over the past century in the southeastern United States has created novel ecosystems that have become increasingly dominated by fire-sensitive plant taxa (Nowaki and Abrams, 2008). Belowground, important symbiont fungal communities also lack a recent history of fire or fire adaptation in many southeastern U.S. ecosystems (Brown et al., 2013). Instead, most knowledge on belowground ecosystem recovery from wildfires has been derived from fire-prone ecosystems in the western United States and other arid areas around the globe where long fire legacies and frequent

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fires have created fire-tolerant plant (Keeley et al., 2011) and fungal communities (Glassman et al., 2016). Thus, it remains unknown how key plants and fungi in southeastern U.S. forests will respond to current and predicted fires after over a 100 years of fire absence.

Increases in urbanization positions 9% of fire-prone land within the United States at the wildland–urban interface (Radeloff et al., 2005), with expected increases of 25% per decade (Zhang et al., 2008). Land-use change due to urbanization may also affect ecosystems if disturbance leads to decreased habitat quality or biotic homogenization (Pointing et al., 2016). The effects of urbanization on belowground ecosystems have received less attention compared to other global change drivers, but fungal composition may be particularly sensitive (Moora et al., 2014; Reese et al., 2016). For example, a study of 5 urbanized areas demonstrated mycorrhizal fungi were the most vulnerable community to urbanization with both decreased abundance and diversity (Schmidt et al., 2017). Moreover, the environmental drivers of belowground communities in urban environments may be different than those in natural settings. For example, in a survey of 12 urban green roofs across Chicago, arbuscular mycorrhizal (AM) fungal composition largely varied with ecosystem age (Chaudhary et al., 2019), and not soil chemistry or plant communities as in natural systems (Kivlin et al., 2011). Finally, the combined disturbance of urbanization and wildfires may not be additive and instead may have unknown synergistically negative effects on belowground ecosystems (Costanza et al., 2015).

AM fungi create the foundation of nutritional symbioses in southeastern U.S. hardwood forests where over 70% of overstory and understory taxa rely on these belowground associations (Soudzilovskaia et al., 2020). AM fungi provide up to 50% of plant nitrogen and 80% of plant phosphorus in exchange for plant photosynthate (Smith et al., 2003; Parniske, 2008). In many areas of the southeastern United States, both fire and urbanization pressures have the potential to simultaneously affect AM fungi belowground. However, it is unclear how much either of these disturbance pressures independently or interactively affect AM fungi. In fire-prone ecosystems, belowground fungi may be adapted to cycles of disturbance, but when fire is suppressed, as is the case in many eastern North American forests, the fire cycle is broken and fire-adapted soil fungi may decline in soils and seek refugia in other habitats (e.g., bryophytes; Raudabaucgh et al., 2020; plant roots, Hughes et al., 2020a). Previous studies of AM fungi in more frequently burned habitats suggest that root-associated AM fungal communities recover quickly from fire (Treseder et al., 2004). However, it is unknown whether all AM fungal taxa recover equally or whether some AM fungi are more fire-resistant than others (sensu Hart and Reader,2004), especially when roots are not affected by low severity fires.

AM fungi may also track indirect effects of wildfire and urbanization. For example, shifts in plant host identity or function following fire or urbanization may indirectly affect obligate AM fungal symbionts by altering symbiosis outcomes. In global analyses, AM fungal abundance, diversity, and composition all vary among plant hosts species (Kokkoris et al., 2020) and plant functional groups (Davison et al., 2020). Moreover, AM fungal communities are also sensitive to plant host age (Husband et al., 2002), photosynthetic rates (Konvalinkova et al., 2015), and stress (Kazenel et al., 2019). Finally, AM fungi respond to shifts in soil nutrients (Hoeksema et al., 2010). Thus, wildfire-induced increases in soil phosphorus in particular (Butler et al., 2018) may have detrimental effects on AM fungal symbionts, which are often sanctioned by host plants under nutrient replete conditions (Johnson et al., 2015).

In November 2016, extreme drought conditions and long-term fire suppression combined to create ideal conditions for wildfire within the Great Smoky Mountains National Park (GSMNP). The Chimney Tops 2 wildfire started in November 2016 and burned to the end of 2016, with flare-ups until April 2017. In September 2018, we collected AM fungi from roots of the 5 most dominant understory plant species at each of 18 sites spanning burn severities along the wildland–urban interface that included the GSMNP. We chose sites within the burn matrix that varied from unburned to medium/low burn and high burn severity in both natural areas within the park and in exurban areas of Gatlinburg, TN. We expected that both fire severity and urbanization would decrease the abundance, diversity, and composition of AM fungi in roots of understory plants. We also expected variation in AM fungal diversity and composition among plant species. Finally, we expected fire and urbanization to homogenize AM fungal communities such that there would be higher heterogeneity in AM fungal composition among unburned and natural park samples compared to burned and exurban samples.

Methods

Site selection

Wildfires have been suppressed in GSMNP since 1934 following a national policy of fire suppression within national parks and, within recent decades, concern about the increasing wildland–urban interface. During late November and early December 2016, the Chimney Tops 2 wildfire burned approximately 44.4 km^2 within the GSMNP. On November 28, the fire moved into the city of Gatlinburg, TN, and burned a further 28.2 km^2. This wildfire was extremely heterogeneous, with unburned sites adjacent to severely burned sites, often within meters of each other. From within the burn matrix, we randomly selected 3 sites from "high burn" areas, 3 "low/medium burn" sites, and 3 "no burn" sites both within natural areas of the GSMNP and the exurban area of Gatlinburg, TN (Supplemental Figure 1). Burn levels were determined using a Burned Area Reflectance Classification (BARC) map generated by U.S. Forest Service Remote Sensing Application Center from 2015 and 2016 EO ALI and Landsat satellite images. In brief, the map was generated by calculating a normalized burn ratio from 2 spectral bands, near infrared and shortwave infrared, pre- and postfire, and represents the soil burn severity. Burned categories typically fall within 3 classes of fire severity, with Unburned/Very Low $\leq$ 75; Medium = 76–187; and High

$\geq$ 187. Satellite imagery data were collected from the Forest Service Remote Sensing Applications Center and the U.S. Geological Survey Center for Earth Resources Observation and Science. While BARC classes matched visual estimates of burn severity at our sites (e.g., amount of bark scorch, plant mortality, soil organic matter, and litter layer accumulation; Hughes et al., 2020a), satellite data can only resolve burn severity at coarser scales (30 $\times$ 30 m) than our 1 $\times$ 1 m sampling plots. Therefore, these estimates should only be interpreted as approximations of burn severity. More details on soil responses to fire are presented below.

Overstory plant community at all sites is primarily mixed deciduous hardwood with oak species dominant. Elevation ranges from 407 to 630 m above sea level. Precipitation varies between mountains and valleys with an annual mean between 1,397 and 2,159 mm, and mean daily temperatures range from 10°C in January to 31°C in July at low elevations and 1°C–18°C at high elevations (Shanks, 1954).

Sampling

In September 2018, we collected fine roots from 1 individual of each of the 5 dominant understory species co-occurring in established two 1 $\times$ 1 m plots at each of 18 sites. All plant species were growing with active AM fungi within plant roots. We only sampled plant host species that were present in at least 2 burn severity categories and both exurban and natural settings to minimize the confounding effects of plant host and these other parameters (Supplemental Table 1). Species included *Acer saccharum*, *Amphicarpaea bracteata*, *Kalmia latifolia*, *Lactuca canadensis*, *Lysimachia quadrifolia*, *Panicum milliaceum*, *Parthenocissus quinquefolia*, *Phytolacca americana*, *Smilax glauca*, and *Viola palmata*. Species ranged from annual (*Amphicarpaea*, *Lactuca*, *Panicum*) to perennial (*Acer*, *Kalmia*, *Lysimachia*, *Parthenocissus*, *Phytolacca*, *Smilax*, *Viola*) life cycles with woody tree and shrub saplings (*Acer*, *Kalmia*), woody vines (*Parthenocissus*, *Smilax*), herbaceous forbs (*Amphicarpaea*, *Lactuca*, *Lysimachia*, *Phytolacca*, *Viola*), and grasses (*Panicum*).

In the field, fine root samples (<2 mm in diameter) were immediately placed in sterile whirlpak bags and placed on ice until transport to the laboratory (within 24 h). Once in the laboratory, roots were surface-sterilized with 70% EtOH and 10% NaClO, followed by a sterile water rinse and stored frozen at –80°C.

In October 2018, we also collected bulk soils from all 18 sites. At each site, we collected the organic and mineral horizons from ten 5 $\times$ 10 cm cores. Cores were homogenized into 1 aggregate sample for each horizon at each of the 18 sites. Following fire, only mineral horizon soils remained in high burn sites. From these homogenized samples, we measured soil organic matter content via mass loss on ignition at 360°C for 3 h (Davies, 1974) and soil nutrients following extraction in 0.5M K_2SO_4 (ammonium and nitrate) or 0.5M HCl (phosphate). All soil nutrients were quantified colorimetrically (see Methods section in D'Angelo et al., 2001; Doane and Horwath, 2003).

Microscopy

Plant roots from each sample were cleared in 10% KOH and stained with 0.01% acid fuchsin. Roots were scored for percent colonization of AM fungal hyphae, arbuscules, and vesicles with the gridline intercept method (McGonigle et al., 1990) on a Laxco 3000 microscope at 200$\times$ magnification.

DNA extraction and amplification

Roots were ground to a fine powder using a sterile mortar and pestle with liquid nitrogen. DNA was then extracted from approximately 250 mg of roots using the Qiagen DNeasy Plant mini kit (Qiagen, Germantown, MD, USA). DNA was quantified with the Qubit high sensitivity kit (Qubit Fluorometer, Life Technologies, Carlsbad, CA, USA) and diluted to approximately 10 ng/µl in sterile water. Due to limited AM fungal DNA, we then performed a nested PCR reaction. The first reaction amplified approximately an 800 b region of AM fungi and plants in the 18S region using the NS1–NS4 primers (White et al., 1990), the preferred marker gene for AM fungi (Lekberg et al., 2018). The nested reaction amplified approximately a 400 b region of 18S AM fungal DNA with barcoded Illumina TruSeq V3 indices (Illumina, San Diego, CA, USA) linked to the NS31–AML2 primers (Morgan and Egerton-Warburton, 2017). Each reaction contained: 21.5 µl of Platinum PCR Supermix (Invitrogen, Carlsbad, CA, USA), 1.25 µl of each primer (10 µM), 0.5 µl of BSA (20 mg/ml), and 2 µl (approximately 20 ng) of DNA. The first reaction ran at 94°C for 3 min, followed by 30 cycles of 94°C for 30 s, 40°C for 1 min, and 72°C for 1 min, and the nested reaction at 94°C for 5 min, followed by 40 cycles of 94°C for 45 s, 63.1°C for 1 min, and 72°C for 1.5 min. Triplicate nested reactions were combined, cleaned with Agencourt AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA), and quantitated fluorometrically (Qubit Fluorometer, Life Technologies, Carlsbad CA, USA). Samples were pooled into equal amounts and run on an Illumina MiSeq v3 sequencer in a 2 $\times$ 275 b run at the University of Tennessee Center for Environmental Biotechnology Core Facility.

Bioinformatics

AM fungal sequences were processed in the DADA2 pipeline in R (Callahan et al., 2016). First, primers were trimmed from all sequences and sequence error rates were calculated. Sequences were then merged into unique amplicon sequence variants (ASVs). Finally, chimeras were removed using a denovo chimera checker. Because the NS31–AML2 primers may amplify some non-AM fungal fungi, we then BLASTed representative sequence reads from each ASV against the MaarjAM database (Opik et al., 2010) and only retained reads that matched a known AM fungal virtual taxonomic unit by at least 97%. Sequences are deposited in the NCBI Sequence Read Archive (BioProject ID: PRJNA771625). All other data are available via the Environmental Data Initiative (doi:10.6073/pasta/1cac7b2ccd2262773f92600205f1d812).

Statistics

All statistics were performed in R v. 3.5.1 (R Core Team, 2009). Soil nutrients ($n = 26$) were analyzed using a general linear model with the fixed effects of soil horizon, burn severity, and urbanization status (i.e., exurban sites in Gatlinburg or natural sites from GSMNP), and their interactions, and the random effect of site using the lmer function in the lme4 package. When soil horizons differed in nutrient concentrations (NH_4^+ and NO_3^-), we also performed an ANOVA on each soil horizon separately with the fixed effects of burn severity, and urbanization and their interaction using the aov function in base R.

AM fungal colonization rates ($n = 100$) were logit-transformed to improve normality before analysis. We used general linear mixed effects models to test how AM fungal colonization rates (hyphae, arbuscules, and vesicles) and α diversity (richness, Shannon's Diversity, Simpson's Diversity) varied with the fixed effects of plant host identity, burn severity, and urbanization status with the random effect of site using the lmer function in the lme4 package (Bates et al., 2015). We tested for all pairwise interactions but did not have enough statistical power to test for the 3-way interaction of plant host identity × burn severity × urbanization status. When significant, we tested for post hoc differences within fixed effects while correcting for multiple comparisons with the false discovery rate of $\alpha = .05$ in the lsmeans package (Lenth, 2016).

AM fungal composition ($n = 118$) was visualized in nondimensional multivariate space using the Vegan package (stress = 0.14; Oksanen et al., 2009). Variation in AM fungal composition measured as beta-diversity (turnover among individual sites) was partitioned into fractions owing to plant host identity, burn severity, space, and urbanization using the VarPart function in Vegan (Oksanen et al., 2009). Since urbanization status did not explain any of the variation in AM fungal composition, it was dropped from the analysis. An overlap in AM fungal taxa among burn severity classes and in exurban versus natural samples was visualized using Venn diagrams with the VennDiagram package in R (Chen and Boutros, 2011). Finally, heterogeneity in AM fungal composition was tested for each factor (i.e., burn severity, urbanization status, and plant host identity) with the betadispr function in Vegan (Oksanen et al., 2009).

Results

Soil nutrients

Soil nutrients (ammonium, nitrate, and phosphate) mostly differed by soil horizon with approximately 3 times higher ammonium and nitrate concentrations in organic horizon compared to mineral horizon soils ($\chi^2 = 15.088$, $df = 1$, $P < 0.001$; Supplemental Figure 2C and E). Within horizons, ammonium concentrations were highest in natural compared to exurban sites in the organic horizon ($F = 12.098$, $df = 1$, $P = 0.010$). Ammonium concentrations were also higher in the mineral horizon of unburned soils compared to moderate or highly burned sites ($F = 4.857$, $df = 2$, $P = 0.031$). There were no significant trends in carbon or nutrient concentrations and no interactions among burn severity levels and exurban versus natural sites ($P > 0.050$).

AM fungal colonization

Colonization of AM fungal hyphae varied among plant host species ($\chi^2 = 68.304$, $df = 9$, $P < 0.001$), and burn severities ($\chi^2 = 13.877$, $df = 2$, $P < 0.001$; **Figure 1A** and **B**), but not their interaction ($P > 0.050$). *Amphicarpaea bracteata* and *P. americana* roots contained the most AM fungal hyphae while *P. milliaceum* roots contained the fewest. Plant roots from unburned sites had approximately 2 times higher AM fungal hyphal colonization compared to both moderate and high burn severity sites. However, AM fungal hyphal colonization did not differ between natural and exurban sites ($P > 0.050$). Colonization of arbuscules (sites of nutrient exchange) only varied among plant host species ($\chi^2 = 32.582$, $df = 9$, $P < 0.001$; **Figure 1D**), with the highest colonization in *L. canadensis*, *L. quadrifolia*, and *S. glauca* roots. Arbuscule colonization did not respond to fire or urbanization disturbance ($P > 0.050$) or their interactions with plant hosts or each other ($P > 0.050$). Finally, vesicle colonization was higher in natural compared to exurban sites ($\chi^2 = 6.353$, $df = 1$, $P < 0.001$; **Figure 1I**) but did not vary among plant host species or with fire severity or their interactions ($P > 0.050$).

AM fungal diversity

Richness of AM fungi varied among plant host species ($\chi^2 = 39.811$, $df = 9$, $P < 0.001$; **Figure 2A**) and was highest in *V. palmata* roots and lowest in *A. saccharum* roots. Richness was also approximately 2 times higher in in unburned versus burned sites ($\chi^2 = 19.322$, $df = 1$, $P < 0.001$; **Figure 3B**). Similarly, Shannon's diversity of AM fungi was 1.5× higher in unburned compared to burned sites ($\chi^2 = 7.7083$, $df = 2$, $P = 0.021$; **Figure 3E**) but did not vary among plant host species. Simpson's diversity metric did not vary with any factors ($P > 0.050$). There was also no variation in any diversity metric in exurban versus natural locations ($P > 0.050$) and no significant interactions among plant host species, burn severity, or exurban versus natural location ($P > 0.050$) for any diversity metric.

AM fungal composition

Variance partitioning of AM fungal composition revealed shifts among plant host species (adj $R^2 = 0.083$, $df = 9$, $P = 0.002$) but composition was not significantly affected by burn severity (adj $R^2 = 0.018$, $df = 2$, $P > 0.050$), exurban versus natural locations (adj $R^2 = 0.001$, $df = 1$, $P > 0$ .050), geographic location (adj $R^2 = 0.011$, $df = 17$, $P > 0.050$) or any interactions among these factors ($P > 0.050$; **Figure 2**). Heterogeneity of AM fungal communities varied among plant species ($F = 2.925$, $df = 9$, $P = 0.007$) but differences were minor. Only AM fungal communities on *S. glauca* were more homogenous than *L. canadensis*–associated AM fungal communities. AM fungal communities were also more homogenous among natural sites compared to exurban sites ($F = 7.381$, $df = 1$, $P = 0.009$).

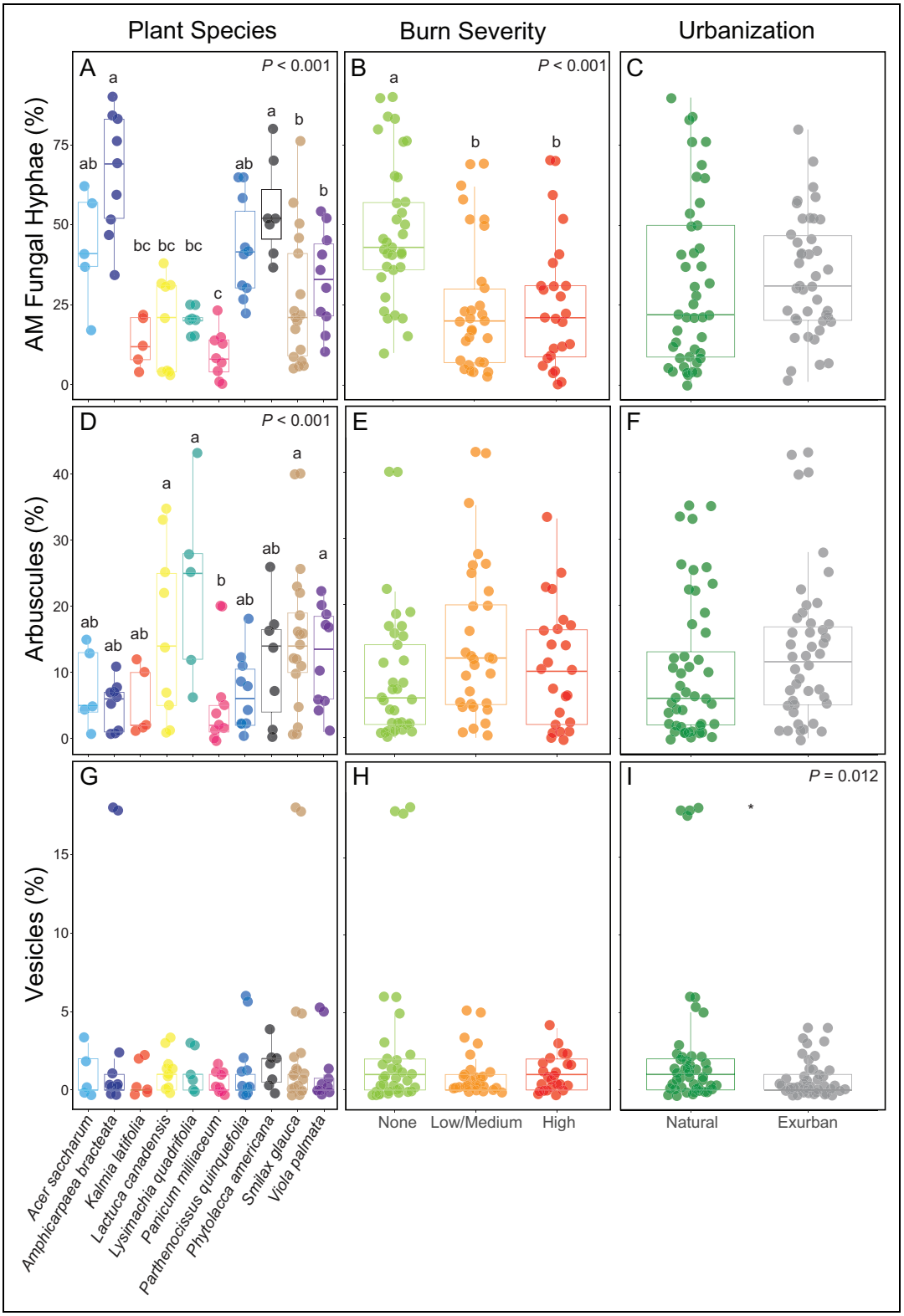

Figure 1. Colonization rates of AM fungal hyphae (A, B, C), arbuscules (D, E, F), and vesicles (G, H, I) across plant species (A, D, G), burn severities (B, E, H), and in exurban versus natural sites (C, F, I). Each point represents an individual plant. Boxplots represent medians (thick line), interquartile ranges (box), and 95% confidence intervals (error bars). All colonization data were logit-transformed for analysis and back-transformed for this figure. Significant differences in colonization among plant species, burn severities, or exurban versus natural sites were compared with lsmeans R function and adjusted for multiple comparisons with a false discovery rate of $\alpha = .05$.
DOI: https://doi.org/10.1525/elementa.2021.00037.f1

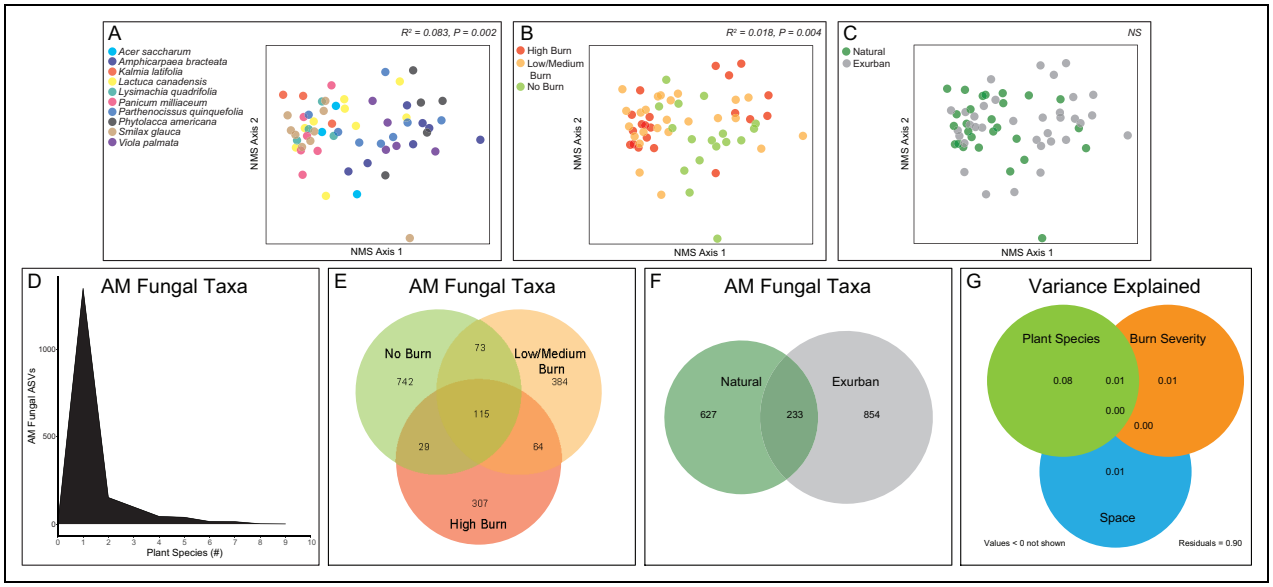

Figure 2. Community composition of AM fungi among plant species (A), burn severities (B), and in exurban versus natural sites (C). Overlap in AM fungi among host taxa (D), burn severity levels (E), and in natural versus exurban sites (F). The variance in composition explained by plant species, burn severity, exurban versus natural sites, space, and their interactions as determined by varpart R function (G). When a factor did not describe any variation (exurban vs. natural sites), it was excluded from the final variance partitioning model. DOI: https://doi.org/10.1525/elementa.2021.00037.f2

Discussion

Overall, AM fungi were more sensitive to plant host compared to fire or urbanization, but the extent to which each of these factors affected AM fungal communities varied among metrics. Alpha diversity and composition of AM fungal communities was more responsive to plant host identity than wildfire or urbanization disturbance. This effect was consistent and not driven by shifts in plant hosts within either disturbance regime, as the same plant hosts were sampled across multiple site categories. Yet wildfire decreased abundance, richness, and Shannon diversity of AM fungal communities regardless of burn severity. Contrary to our expectations, AM fungal composition homogeneity was highest in natural sites compared to exurban locations and differed slightly among plant hosts. However, variability of AM fungal composition did not vary among sites with different burn severities. Instead, 57% of AM fungal taxa were located in at least 1 burned site. In comparison, segregation of AM fungal communities among plant hosts led to less than 21% of AM fungi occurring in more than 1 plant species. Stochastic recovery of AM fungal communities is common following disturbance (Lekberg et al., 2012) and may have caused higher heterogeneity among AM fungal communities in exurban sites.

AM fungi may be fire-tolerant or recover quickly from fire in a variety of ecosystems. In grasslands, AM fungal abundance in roots and soil was unaffected by fire (Eom et al., 1999). In forests, initial AM fungal biomass may decrease after fire, but AM fungi recovery is fast (Rashid et al., 1997; Xiang et al., 2015); AM fungi recovered from fire 15 years before ectomycorrhizal fungal communities (Treseder et al., 2004). However, many facets must be considered when classifying AM fungal responses to fire. First, AM fungal and plant communities may vary across studies, obscuring our ability to determine generalizable trends in AM fungal response to fire disturbance. Second, fires may not be the same severity across sites and ecosystems. It is still unclear how fire severity differentially affects AM fungal recovery. In our study, all responses were similar among fire severity classifications, but fire severity was not as severe as in other wildfires, which may have dampened the AM fungal responses. In lower severity fires where some live plants remain, AM fungi may be buffered from fire if they persist within plant roots. This mechanism may be likely as other pyrophilus fungi that recovered quickly following the GSMNP fire persisted by colonizing plant leaf and root tissues (Hughes et al., 2020a), as did ectomycorrhizal fungi following other burns (Hewitt et al., 2016). However, some of the plants we sampled were annuals that must have resprouted from seed or coppiced from remaining roots after the GSMNP 2016 fire. AM fungi in these plant taxa may have survived in roots or colonized from a surrounding common mycorrhizal network or from fire-tolerant spores. While fire tolerance of ectomycorrhizal spores is common (Baar et al., 1999; Glassman et al., 2016), this trait has not been examined in AM fungi specifically. However, there is some preliminary evidence that AM fungal spores do not shift in abundance or composition following fire even when surrounding AM fungal hyphae are consumed by fire (Longo et al., 2014). AM fungi may have also dispersed from unburned habitats to burned patches in the 20 months following the fire. The Chimney Tops 2 fire was heterogenous with unburned patches meters away from burned patches within the fire matrix, maximizing

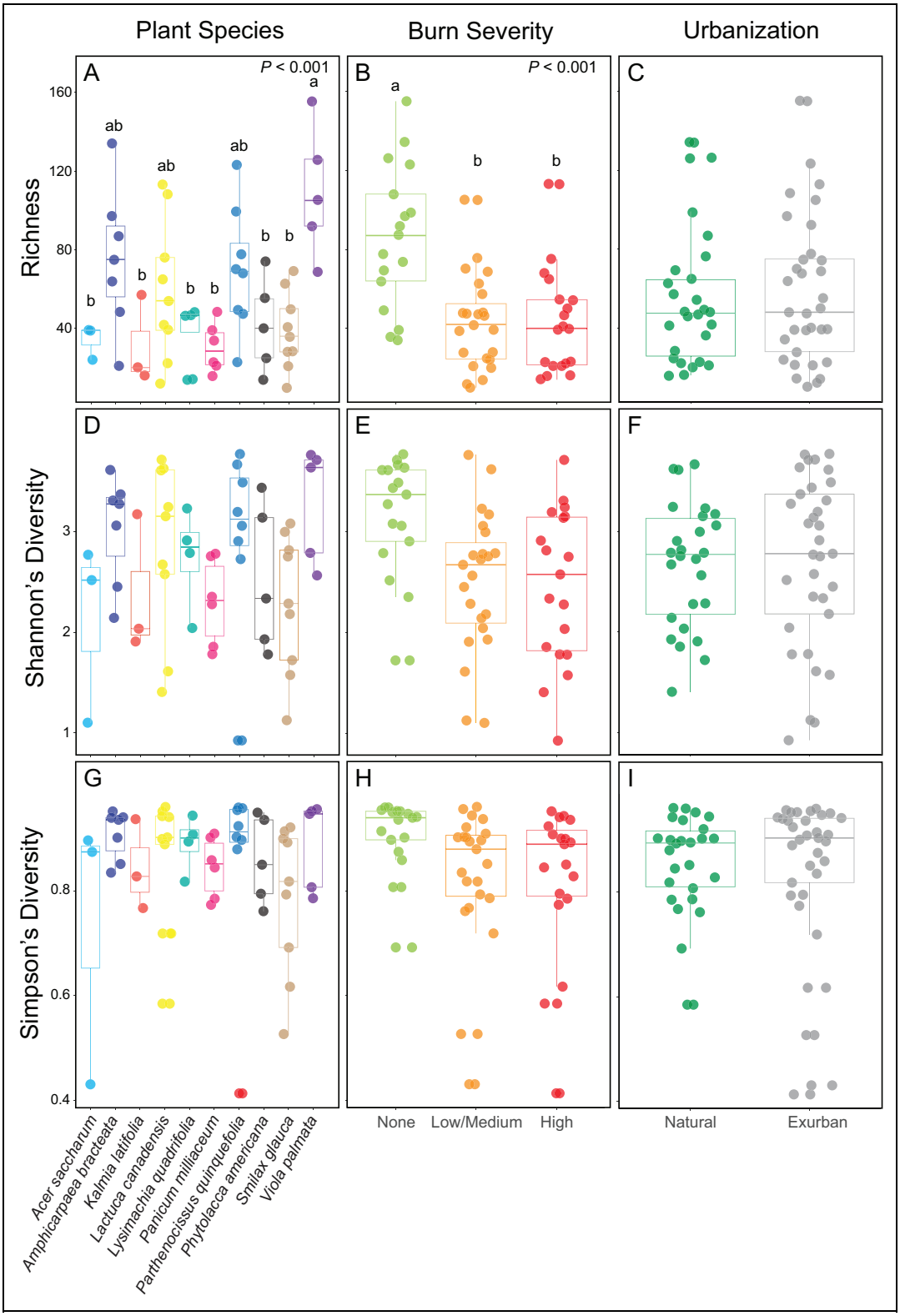

Figure 3. Diversity metrics for AM fungi (richness (A, B, C), Shannon's Diversity Index (D, E, F), and Simpson's Diversity Index (G, H, I)) across plant species (A, D, G), burn severities (B, E, H), and in exurban versus natural sites (C, F, I). Each point represents an individual plant. Boxplots represent medians (thick line), interquartile ranges (box), and 95% confidence intervals (error bars). Significant differences in diversity among plant species, burn severities, or exurban versus natural sites were compared with lsmeans R function and adjusted for multiple comparisons with a false discovery rate of $\alpha = .05$. DOI: https://doi.org/10.1525/elementa.2021.00037.f3

the potential for an undisturbed AM fungal spore bank. Recent evidence also suggests that airborne dispersal of AM fungal spores is much higher than previously recorded (Chaudhary et al., 2020), providing the potential for these rescue effects.

In contrast to our findings for AM fungi, bulk soil fungal and bacterial communities as a whole shifted in both diversity and composition following the Chimney Tops 2 fire (same fire, different sites; Brown et al., 2019). Similarly, pyrophilus fungal taxa (e.g., *Sphaerosporella* spp.) appeared within 6 months following the GSMNP fire and persisted for at least the next 2 years (Hughes et al., 2020a, 2020b). Shifts in microbial composition belowground can have functional consequences and even feedback to future fires, when decomposition slows (Hopkins et al., 2020). Moreover, shifts in decomposer fungi and bacteria may change soil resources pools and therefore affect AM fungal communities on longer time scales following a wildfire.

Our results add to the growing number of cases of host specificity among AM fungal taxa (Kokkoris et al., 2020). Previous constraints on sequencing capacity limited the ability to sample AM fungi within the same plant hosts over multiple environments. However, when surveys are able to sample the same plant taxa across multiple environmental conditions, plant host identity often has the largest influence on AM fungal composition (Kokkoris et al., 2020). AM fungal differentiation among plant hosts may be driven by differential plant resources (Johnson, 2010), plant host/environmental adaptation (Rua et al., 2016), or plant-AM fungal covariation among larger plant functional groups (Davison et al., 2020). Here, our unequal sampling design did not allow us to test for differences among plant functional groups, but given that the highest diversity and colonization rates were consistently found in annual forbs and perennial vines, this suggests that plants that recover the quickest from fire are the best mycorrhizal fungal hosts.

In contrast to plant host identity and fire severity effects, urbanization had very minimal effects on AM fungal communities, only decreasing the number of recorded AM fungal vesicles within plant roots and increasing heterogeneity in AM fungal composition among urbanized sites. AM fungi use vesicles to store C (Hawkes et al., 2008). Thus, urbanization-induced disturbance such as tillage or trampling may have hindered vesicle formation if excess plant photosynthate was unavailable. AM fungal organs are rarely measured under disturbance contexts (van der Heyde et al., 2019), but there is growing evidence that AM fungal communities are mostly robust to shifts in land-use intensity and disturbance (Violi et al., 2008; Lekberg et al., 2012). In a comparison of plant host identity versus land-use intensity, AM fungal community composition was twice as sensitive to shifts in plant hosts as land use (Valyi et al., 2015). Similarly, AM fungal communities within plant roots were buffered from disturbance compared to AM fungi in soil across a land-use intensity gradient (Moora et al., 2014; Sepp et al., 2018).

In our study, there were also no interactive effects of urbanization and wildfire on any of the AM fungal metrics we surveyed. If AM fungi are largely tracking plant hosts, these results could be due to the fact that plant composition did not vary among our sites and we purposely sampled the same plant species across multiple sites. Combined disturbances large enough to shift plant composition may affect AM fungal communities in more synergistic ways and should be the focus of future inquiries of fungal responses at the wildland–urban interface. Fire in our study system was also extremely patchy, which may have influenced the severity of disturbance for any given sample. Alternatively, perhaps sampling nearer to the time of fire disturbance or over longer time scales that allow for ecosystem-level change (e.g., dead plant decomposition following fire) would detect environmental filtering of AM fungal communities and thus a more concerted signal of recovery of AM fungal communities following a fire or urbanization disturbance. Indeed, soil nutrients rarely responded to fire severity among sites and instead differed by horizon. Because AM fungi are nutritional mutualists, more severe disturbances that disrupt belowground biogeochemical cycling may have stronger or long-lasting effects on AM fungal composition (e.g., Averill et al., 2018). These sampling constraints may explain why even our best model only captured approximately 11% of the variance in AM fungal composition. Differences in AM composition over unmeasured spatial and temporal gradients, in response to unmeasured environmental variables, neutral ecological processes, or even neighboring unsampled plant communities, may have influenced the resulting AM fungal community and should be the focus of future work in AM fungal disturbance ecology.

Conclusion

The forecasted increase in wildfire frequency and urbanization in the southeastern United States will change many facets of southeastern mesic forests (Dale et al., 2001; Bonan, 2008). However, because in our study AM fungi responded the most to plant host identity, we anticipate the majority of AM fungal taxa may be buffered from fire-induced ecosystem changes if plant community composition largely remains intact, plant species life history traits allow for persistence after fire disturbance, and/or nearby undisturbed habitat can act as an inoculum source for recolonization following fire disturbance. Therefore, natural, undisturbed habitats may be critical refugia for mycorrhizal fungi within the wildland–urban interface.

Data accessibility statement

Sequence data is available in the NCBI Sequence Read Archive under BioProject ID PRJNA771625. All other data is available via the Environmental Data Initiative (EDI), doi:10.6073/pasta/1cac7b2ccd2262773f92600205f1d812.

Supplemental files

The supplemental files for this article can be found as follows:

Figure S1. PDF
Figure S2. PDF
Table S1. Csv

Acknowledgments

Samples were collected under U.S. Department of the Interior, National Park Service permit #GRSM-2020-SCI-2024 to Jennifer Schweitzer. SNK and MP thank University of Tennessee startup funds for funding for this project. VRH, MMH, and JHT were funded by Breedlove, Dennis awards from the University of Tennessee Herbarium (TENN).

Competing interests

SNK is an associate editor for Elementa's Ecology and Earth Systems domain but did not have any role in handling or reviewing this manuscript. The authors declare no other conflicts of interest.

Author contributions

Designed experiments: JAS, MP, SNK.

Collected data: VRH, JHT, JAMM, LCM, KKB, MMH.

Performed laboratory experiments: VRH, JHT, JAMM, LCM.

Analyzed data and wrote the first draft of the manuscript: SNK.

Contributed edits to the manuscript: All authors.

References

Averill, C, Dietze, MC, Bhatnagar, JM. 2018. Continental-scale nitrogen pollution is shifting forest mycorrhizal associations and soil carbon stocks. *Global Change Biology* **24**: 4544–4553.

Baar, J, Horton, TR, Kretzer, AM, Bruns, TD. 1999. Mycorrhizal colonization of *Pinus muricata* from resistant propagules after a stand-replacing wildfire. *New Phytologist* **143**: 409–418.

Bates, D, Mächler, M, Bolker, B, Walker, S. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**: 1–48.

Bonan, GB. 2008. Forests and climate change: Forcings, feedbacks, and the climate benefits of forests. *Science* **320**: 1444–1449.

Brown, SP, Callaham Jr MA, Oliver, AK, Jumpponen, A. 2013. Deep ion torrent sequencing identifies soil fungal community shifts after frequent prescribed fires in a southeastern US forest ecosystems. *FEMS Microbiology Ecology* **86**: 557–566.

Brown, SP, Veach, AM, Horton, JL, Ford, E, Jumpponen, A, Baird, R. 2019. Context dependent fungal and bacterial soil community shifts in response to recent wildfires in the Southern Appalachian Mountains. *Forest Ecology and Management* **451**: 117520.

Butler, OM, Elser, JJ, Lewis, T, Mackey, B, Chen, C. 2018. The phosphorus-rich signature of fire in the soil-plant system: A global meta-analysis. *Ecology Letters* **21**: 335–344.

Callahan, BJ, McMurdie, PJ, Rosen, MJ, Han, AW, Johnson, AJ, Holmes, SP. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* **13**: 581–583.

Chaudhary, VB, Nolimal, S, Sosa-Hernández, MA, Egan, C, Kastens, J. 2020. Trait-based dispersal of arbuscular mycorrhizal fungi. *New Phytologist* DOI: http://dx.doi.org/10.1111/nph.16667.

Chaudhary, VB, Sandall, EL, Lazarski, MV. 2019. Urban mycorrhizas: Predicting arbuscular mycorrhizal abundance in green roofs. *Fungal Ecology* **40**: 12–19.

Chen, H, Boutros, PC. 2011. VennDiagram: A package for the generation of highly-customizable Venn and Euler diagrams in R. *BMC Bioinformatics* **12**: 35.

Costanza, JK, Terando, AJ, McKerrow, AJ, Collazo, JA. 2015. Modeling climate change, urbanization, and fire effects on Pinus palustris ecosystems of the southeastern U.S. 2015. *Journal of Environmental Management* **151**: 186–199.

Dale, VH, Joyce, LA, McNulty, S, Neilson, RP, Ayres, MP, Flannigan, MD, Hanson, PJ, Irland, LC, Lugo, AE, Peterson, CJ, Simberloff, D. 2001. Climate change and forest disturbances. *BioScience* **51**: 723–734.

D'Angelo, E, Crutchfield, J, Vandiviere, M. 2001. Rapid, sensitive, microscale determination of phosphate in water and soil. *Journal of Environmental Quality* **30**: 2206–2209.

Davies, BE. 1974. Loss-on-ignition as an estimate of soil organic matter. *Soil Science Society of America Journal* **38**: 150–151.

Davison, J, García de León, D, Zobel, M, Moora, M, Bueno, CG, Barceló, M, Gerz, M, León, D, Meng, Y, Pillar, VD, Sepp, SK. 2020. Plant functional groups associate with distinct arbuscular mycorrhizal fungal communities. *New Phytologist* **226**: 1117–1128.

Doane, TA, Horwath, WR. 2003. Spectrophotometric determination of nitrate with a single reagent. *Analytical Letters* **36**: 2713–2722.

Eom, AH, Hartnett, DC, Wilson, GW, Figge, DA. 1999. The effect of fire, mowing and fertilizer amendment on arbuscular mycorrhizas in tallgrass prairie. *The American Midland Naturalist* **142**: 55–70.

Flannigan, MD, Amiro, BD, Logan, KA, Stocks, BJ, Wotton, BM. 2006. Forest fires and climate change in the 21st century. *Mitigation and Adaptation Strategies for Global Change* **11**: 847–859.

Glassman, SI, Levine, CR, DiRocco, AM, Battles, JJ, Bruns, TD. 2016. Ectomycorrhizal fungal spore bank recovery after a severe forest fire: Some like it hot. *The ISME Journal* **10**: 1228–1239.

Hart, MM, Reader, RJ. 2004. Do arbuscular mycorrhizal fungi recover from soil disturbance differently? *Tropical Ecology* **45**: 97–112.

Hawkes, CV, Hartley, IP, Ineson, P, Fitter, AH. 2008. Soil temperature affects carbon allocation within arbuscular mycorrhizal networks and carbon transport from plant to fungus. *Global Change Biology* **14**: 1181–1190.

Hewitt, RE, Hollingsworth, TN, Chapin, FS III, Taylor, DL. 2016. Fire-severity effects on plant–fungal interactions after a novel tundra wildfire disturbance: Implications for arctic shrub and tree migration. *BMC Ecology* **16**(25). DOI: http://dx.doi.org/10.1186/s12898-016-0075-y.

Hoeksema, JD, Chaudhary, VB, Gehring, CA, Johnson, NC, Karst, J, Koide, RT, Pringle, A, Zabinski, C, Bever, JD, Moore, JC, Wilson, GW. 2010. A meta-analysis of context-dependency in plant responses to inoculation with mycorrhizal fungi. *Ecology Letters* **13**: 394–407.

Hopkins, JR, Huffman, JM, Platt, WJ, Sikes, BA. 2020. Frequent fire slows microbial decomposition of newly deposited fine fuels in a pyrophilic ecosystem. *Oecologia* **193**: 631–643.

Hughes, KW, Case, A, Matheny, PB, Kivlin, S, Petersen, RH, Miller, AN, Iturriaga, T. 2020a. Secret lifestyles of pyrophilous fungi in the genus *Sphaerosporella*. *American Journal of Botany* **107**: 876–885.

Hughes, KW, Matheny, PB, Miller, AN, Petersen, RH, Iturriaga, TM, Johnson, KD, Methven, AS, Raudabaugh, DB, Swenie, RA, Bruns, TD. 2020b. Pyrophilous fungi detected after wildfires in the Great Smoky Mountains National Park expand known species ranges and biodiversity estimates. *Mycologia* **112**: 677–698.

Husband, R, Herre, EA, Turner, SL, Gallery, R, Young, JP. 2002. Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest. *Molecular Ecology* **11**: 2669–2678.

Johnson, NC. 2010. Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. *New Phytologist* **185**: 631–647.

Johnson, NC, Wilson, GW, Wilson, JA, Miller, RM, Bowker, MA. 2015. Mycorrhizal phenotypes and the law of the minimum. *New Phytologist* **205**:1473–1484.

Kazenel, MR, Kivlin, SN, Taylor, DL, Lynn, JS, Rudgers, JA. 2019. Altitudinal gradients fail to predict fungal symbiont response to warming. *Ecology* **100**: e02740.

Keeley, JE, Pausas, JG, Rundel, PW, Bond, WJ, Bradstock, RA. 2011. Fire as an evolutionary pressure shaping plant traits. *Trends in Plant Science* **16**: 406–411.

Kivlin, SN, Hawkes, CV, Treseder, KK. 2011. Global diversity and distribution of arbuscular mycorrhizal fungi. *Soil Biology and Biochemistry* **43**: 2294–2303.

Knorr, W, Arneth, A, Jiang, L. 2016. Demographic controls of future global fire risk. *Nature Climate Change* **6**: 781–785.

Kokkoris, V, Lekberg, Y, Antunes, PM, Fahey, C, Fordyce, JA, Kivlin, SN, Hart, MM. 2020. Codependency between plant and arbuscular mycorrhizal fungal communities: What is the evidence? *New Phytologist* DOI: http://dx.doi.org/10.1111/nph.16676.

Konvalinkova, T, Puschel, D, Janouskova, M, Gryndler, M, Jansa, J. 2015. Duration and intensity of shade differentially affects mycorrhizal growth and phosphorus uptake response of *Medicago truncatula*. *Frontiers in Plant Science* DOI: http://dx.doi.org/10.3389/fpls.2015.00065.

Lekberg, Y, Schnoor, T, Kjøller, R, Gibbons, SM, Hansen, LH, Al-Soud, WA, Sørensen, SJ, Rosendahl, S. 2012. 454-sequencing reveals stochastic local reassembly and high disturbance tolerance within arbuscular mycorrhizal fungal communities. *Journal of Ecology* **100**: 151–160.

Lekberg, Y, Vasar, M, Bullington, LS, Sepp, S-K, Antunes, PM, Bunn, R, Larkin, BG, Opik, M. 2018. More bang for the buck? Can arbuscular mycorrhizal fungal communities be characterized adequately alongside other fungi using general fungal primers? *New Phytologist* **220**: 971–976.

Lenth, RV. 2016. Least-square means: The R package lsmeans. *Journal of Statistical Software* **69**: 1–33.

Longo, S, Nouhra, E, Goto, BT, Berbara, RL, Urcelay, C. 2014. Effects of fire on arbuscular mycorrhizal fungi in the Mountain Chaco Forest. *Forest Ecology and Management* **315**: 86–94.

McGonigle, TP, Miller, MH, Evans, DG, Fairchild, GL, Swan, JA. 1990. A new method which gives an objective measure of colonization of roots by vesicular arbuscular mycorrhizal fungi. *New Phytologist* **115**: 495–501.

McKenzie, D, Gedalof, ZE, Peterson, DL, Mote, P. 2004. Climatic change, wildfire, and conservation (Cambio Climatico, Incendios y Conservacion). *Conservation Biology* **18**: 890–902.

Moora, M, Davison, J, Opik, M, Metsis, M, Saks, U, Jairus, T, Vasar, M, Zobel, M. 2014. Anthropogenic land use shapes the composition and phylogenetic structure of soil arbuscular mycorrhizal fungal communities. *FEMS Microbiology Ecology* **90**(3): 609–621. DOI: http://dx.doi.org/10.1111/1574-6941.12420.

Morgan, BST, Egerton-Warburton, LM. 2017. Barcoded NS31/AML2 primers for sequencing arbuscular mycorrhizal communities in environmental samples. *Applied Plant Sciences* **5**: 1700017.

Nowaki, GJ, Abrams, MD. 2008. The demise of fire and 'mesophication' of forests in the eastern United States. *Bioscience* **58**: 123–138.

Oksanen, J, Kindt, R, Legendre, P, O'Hara, B, Simpson, GL, Solymos, P, Solymos, P, Henry, M, Stevens, MH, Wagner, H. 2009. Vegan: Community ecology package. R package version 1.15-4. Available at https://cran.r-project.org/web/packages/vegan/vegan.pdf.

Opik, M, Vanatoa, A, Vanatoa, E, Moora, M, Davison, J, Kalwij, JM, Reier, U, Zobel, M. 2010. The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). *New Phytologist* **188**: 223–241.

Parniske, M. 2008. Arbuscular mycorrhiza: The mother of plant root endosymbioses. *Nature Reviews Microbiology* **6**: 763–775.

Pointing, SB, Fierer, N, Smith, GJD, Steinberg, PD, Wiedmann, M. 2016. Quantifying human impact on Earth's microbiome. *Nature Microbiology* **1**: 16145.

Prestemon, JP, Shankar, U, Xiu, A, Talgo, K, Yang, D, Dixon IV, E, McKenzie, D, Abt, KL. 2016.

Projecting wildfire area burned in the south-eastern United States 2011-60. *International Journal of Wildland Fire* **25**: 715–729.

R Development Core Team. 2009. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. Vienna, Austria. Available at http://www.R-project.org/.

Radeloff, VC, Hammer, RB, Stewart, SI, Fried, JS, Holcomb, SS, McKeefry, JF. 2005. The wildland-urban interface in the United States. *Ecological Applications* **15**: 799–805.

Rashid, A, Ahmed, T, Ayub, N, Khan, AG. 1997. Effect of forest fire on number, viability and post-fire re-establishment of arbuscular mycorrhizae. *Mycorrhiza* **7**: 217–220.

Raudabaugh, DB, Matheny, PB, Hughes, KW, Iturriaga, T, Sargent, M, Miller, AN. 2020. Where are they hiding? Testing the body snatchers hypothesis in pyrophilous fungi. *Fungal Ecology* **43**: 100870.

Reese, AT, Savage, A, Youngsteadt, E, McGuire, KL, Koling, A, Watkins, O, Frank, SD, Dunn, RR. 2016. Urban stress is associated with variation in microbial species composition – but not richness – in Manhattan. *The ISME Journal* **10**: 751–760.

Rúa, MA, Antoninka, A, Antunes, PM, Chaudhary, VB, Gehring, C, Lamit, LJ, Piculell, BJ, Bever, JD, Zabinski, C, Meadow, JF, Lajeunesse, MJ, Milligan, BG, Karst, J, Hoeksema, JD. 2016. Home-field advantage? Evidence of local adaptation among plants, soil, and arbuscular mycorrhizal fungi through meta-analysis. *BMC Evolutionary Biology* **16**(1): 122. DOI: http://dx.doi.org/10.1186/s12862-016-0698-9.

Schmidt, DJE, Pouyat, R, Szalazecz, K, Setala, J, Kotze, DJ, Yesilonis, I, Cilliers, S, Hornung, E, Dombos, M, Yarwood, SA. 2017. Urbanization erodes ectomycorrhizal fungal diversity and may cause microbial communities to converge. *Nature Ecology and Evolution* **1**: 0123.

Sepp, S-K, Jairus, T, Vasar, M, Zobel, M, Opik, M. 2018. Effects of land use on arbuscular mycorrhizal fungal communities in Estonia. *Mycorrhiza* **28**: 259–268.

Shanks, RE. 1954. Climates of the Great Smoky Mountains. *Ecology* 354–361.

Smith, SE, Smith, FA, Jakobsen, I. 2003. Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. *Plant Physiology* **133**: 16–20.

Soudzilovskaia, NA, Vaessen, S, Barcelo, M, He, J, Rahimlou, S, Abarenkov, K, Brundrett, MC, Gomes, SIF, Merckx, V, Tedersoo, L. 2020. FungalRoot: Global online database of plant mycorrhizal associations. *New Phytologist* **227**: 955–966.

Treseder, KK, Mack, MC, Cross, A. 2004. Relations among fires, fungi, and soil dynamics in Alaskan boreal forests. *Ecological Applications* **14**: 1826–1838.

Valyi, K, Rillig, MC, Hempel, S. 2015. Land-use intensity and host plant identity interactively shape communities of arbuscular mycorrhizal fungi in roots of grassland plants. *New Phytologist* **205**: 1577–1586.

van der Heyde, M, Abbott, LK, Gehring, C, Kokkoris, V, Hart, MM. 2019. Reconciling disparate responses to grazing in the arbuscular mycorrhizal symbiosis. *Rhizosphere* **11**: 100167.

Violi, HA, Barrientos-Priego, AF, Wright, SF, Escamilla-Prado, E, Morton, JB, Menge, JA, Lovatt, CJ. 2008. Disturbance changes arbuscular mycorrhizal fungal phenology and soil glomalin concentrations but not fungal spore composition in montane rainforests in Veracruz and Chiapas, Mexico. *Forest Ecology and Management* **254**: 276–290.

Waldrop, TA, White, DL, Jones, SM. 1992. Fire regimes for pine-grassland communities in the southeastern United States. *Forest Ecology and Management* **47**: 195–210.

White, TJ, Bruns, TD, Lee, SH, Taylor, JW. 1990. *PCR protocols: A guide to methods and application*. San Diego, CA: Academic Press.

Williamson, TB, Colombo, SJ, Duniker, PN, Gray, PA, Hennessey, RJ, Houle, D, Johnson, MH, Ogden, AE, Spittlehouse, DL. 2009. *Climate change and Canada's forests: From impacts to adaptation*. Edmonton, Canada: Canadian Forest Service.

Xiang, X, Gibbons, SM, Yang, J, Kong, J, Sun, R, Chu, H. 2015. Arbuscular mycorrhizal fungal communities show low resistance and high resilience to wildfire disturbance. *Plant and Soil* **397**: 347–356.

Zhang, YJ, He, HS, Yang, H. 2008. The wildland-urban interface dynamics in the southeastern US from 1990 to 2000. *Landscape and Urban Planning* **85**: 155–162.

How to cite this article: Kivlin, SN, Harpe, VR, Turner, JH, Moore, JAM, Moorhead, LC, Beals, KK, Hubert, MM, Papeş, M, Schweitzer, JA. 2021. Arbuscular mycorrhizal fungal response to fire and urbanization in the Great Smoky Mountains National Park. *Elementa: Science of the Anthropocene* 9(1). DOI: https://doi.org/10.1525/elementa.2021.00037

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Published: December 10, 2021 **Accepted:** October 22, 2021 **Submitted:** May 20, 2021

ELEMENTA Science of the Anthropocene

Elem Sci Anth is a peer-reviewed open access journal published by University of California Press.

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