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The impact of ice storms on mycorrhizal fungi varies by season and mycorrhizal type in a hardwood forest

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Abstract

Extreme weather events, such as ice storms, are increasing and have potentially large impacts on forests, including belowground structures such as fine roots and mycorrhizal fungi. Many forest trees rely on the mutualistic relationship between mycorrhizal fungi and plants; a relationship that when disrupted can negatively impact tree net primary productivity. We took advantage of a large-scale ice storm manipulation in the northeastern United States to test the hypothesis that increasing ice storm intensity and frequency would reduce ectomycorrhizal fungal root tips per unit root length and arbuscular mycorrhizal fungal structures per unit root length, hereafter colonization. We found that ice storm intensity reduced spring ectomycorrhizal fungal and arbuscular mycorrhizal fungal colonization. However, these patterns changed in the fall where ice storm intensity still reduced ectomycorrhizal fungal root tips, but arbuscular mycorrhizal fungal colonization was higher in ice storm treatments than controls. The amount of ectomycorrhizal fungal root tips and arbuscular mycorrhizal fungal colonization differed seasonally: ectomycorrhizal fungal root tips were 1.7× higher in the spring than in the fall, while arbuscular mycorrhizal fungal colonization was 3× higher in the fall than in the spring. Our results indicate that mycorrhizal fungal colonization responses to ice storm severity vary temporally and by mycorrhizal fungal type. Further, arbuscular mycorrhizal fungi may recover from ice storms relatively quickly, potentially aiding forests in their recovery, whereas ice storms may have a longer lasting impact on ectomycorrhizal fungi.

Introduction

Periodic, extreme weather events such as ice storms are expected to increase in frequency and severity in the northeastern United States as the climate changes (Hayhoe et al. 2007, Cheng et

al. 2011, Swaminathan et al. 2018). Such high impact events may have a greater influence on forest ecosystem structure and function than the gradual changes in temperature and precipitation associated with climate change (Jentsch et al. 2007, Arnone et al. 2011). Although relatively rare on an annual time scale, over 1300 ice storms occurred in the northeastern US between 2000 and 2018 (NCEI 2019). These storms can substantially damage forest canopies (Rhoads et al. 2002, Weeks et al. 2009, Rustad and Campbell 2012, Fahey et al. 2020), but belowground impacts are largely unquantified.

Mycorrhizal fungal associations play a large role in maintaining forest structure and function, increasing plant water and nutrient uptake (van der Heijden et al. 1998, Smith and Read 2008) in exchange for carbon provided by host plants. Yet, mycorrhizal fungi species and groups vary widely in traits, including root colonization strategy, competitive ability (e.g., Allen et al. 2003, Lilleskov and Bruns 2003, Talbot and Treseder 2010), hyphal density, length, and turnover, and nutrient uptake mechanisms and rates (Agerer 2001, Johnson and Gehring 2007, Peay et al. 2011, Chagnon et al. 2013, Averill 2016). This trait variation mediates mycorrhizal ecosystem impacts and responses to disturbances (van der Heyde et al. 2017, Bennett and Classen 2020, Rodriguez-Ramos et al. 2021), but little is known about how they will respond to ice storms (Gellesch et al. 2013).

Arbuscular mycorrhizal fungi and ectomycorrhizal fungi are two types of mycorrhizal fungi that often co-occur (Bennett and Classen 2020), but come from different ancestral fungal species, use different physiological structures to obtain soil nutrients (Tinker et al. 1992), and diverge in the benefits they provide to plants (Smith and Read 2008). Arbuscular mycorrhizal fungi can obtain inorganic nitrogen and amino acids (Govindarajulu et al. 2005, Whiteside et al. 2012) and high levels of arbuscular mycorrhizal fungal colonization correlate with high

inorganic nutrient availability and fast nitrogen and carbon cycling rates (Phillips et al. 2013). In contrast, ectomycorrhizal fungi can produce extracellular enzymes to mine soil organic matter for nitrogen (Chalot and Brun 1998, Courty et al. 2010) and phosphorous, so they have greater access to organic nutrients than arbuscular mycorrhizal fungi (Turner 2008, Averill et al. 2014). They can also release chelators to weather minerals and mobilize phosphorus and calcium to maintain tree health (van Breemen et al. 2000). Because the different roles played by arbuscular mycorrhizal fungal and ectomycorrhizal fungal impact ecosystem level nutrient and carbon dynamics (Phillips et al. 2013, Averill et al. 2014, Averill 2016), if ice storms have different effects on these mycorrhizal fungal types, the biogeochemical and forest production effects could be large over time.

Ice storms damage forest canopies and trees by reducing leaf area and bud formation and breaking twigs, branches, and trunks (Rhoads et al. 2002, Weeks et al. 2009, Fahey et al. 2020). Ice storm damage can therefore change the quantity, quality, and timing of litter deposited on the forest floor, shift the soil microclimate, and reduce growing season photosynthesis pending canopy recovery. For example, canopy gaps formed by the 1998 ice storm at the Hubbard Brook Experimental Forest in the northeastern US resulted in increased soil temperatures (Likens et al. 2004) and reduced leaf area (Rhoads et al. 2002) for 3 years following the storm. A pilot ice storm simulation at the Hubbard Brook Experimental Forest in 2011 produced a year's worth of fine litter and dramatically increased coarse litter deposition (Rustad and Campbell 2012). This same pilot study found that as ice storm intensity increased, so did the damage inflicted on trees (Rustad and Campbell 2012).

Given that ice storms significantly impact forest canopies and soils, mycorrhizal fungal colonization may be positively or negatively impacted for a variety of reasons. First, lower

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photosynthesis following ice storms reduces carbon availability and thus may reduce

mycorrhizal fungi colonization and development (Bücking and Heyser 2003, Johnson and

Gehring 2007, van der Heyde et al. 2017). Alternatively, the pulse of carbon inputs to soil

resulting from canopy damage may immobilize soil nutrients (e.g., within 1 yr of wood addition

in Homyak et al. 2008, Piatek 2011, Lajtha 2020), causing trees to allocate more carbon

belowground to access scare nutrients, thereby increasing support for mycorrhizal fungal

associations (Allen et al. 2003, Treseder 2004, Kivlin et al. 2013). Finally, warmer soils under

canopy gaps may increase mycorrhizal fungal colonization (Kivlin et al. 2013).

We studied mycorrhizal fungal colonization responses to ice storms in the large-scale Ice

Storm Simulation Experiment at the Hubbard Brook Experimental Forest in the northeastern US.

This large-scale ecosystem experiment simulated four ice storm intensity levels and one level of

increased frequency to better understand the impacts of these extreme events on Eastern North

American deciduous forests (Rustad and Campbell 2012). Given the potential for ice storms to

cause above-ground damage that reduces photosynthesis and thus the availability of carbon for

mycorrhizal fungal associations, we hypothesized that increased ice storm intensity would

decrease ectomycorrhizal fungal root tips and arbuscular mycorrhizal fungal root colonization.

Understanding the effects of ice storms on plant-fungal mutualisms will enhance our ability to

predict the consequences of these extreme events for plant productivity and forest nutrient

cycles.

Methods

Study Site and Experimental Design

We conducted this study in the Hubbard Brook Experimental Forest located in North Woodstock NH, US (43° 56' N, 71° 44' W, 500 m elevation). The climate is cool, humid, and continental with monthly mean air temperature ranging from -9 °C in January to 18 °C in July. Average annual precipitation is 1400 mm, with roughly one third falling as snow. Hubbard Brook lies within the ice belt of the US. Since the 1800s, Hubbard Brook has experienced a documented 26 major ice storm events and suffered major ecological setbacks due to a 1998 ice storm, which resulted in ice thicknesses ranging from 6-14 mm (Rhoads et al. 2002, Rustad and Campbell 2012).

To investigate the impact of ice storms on forest structure and function, ten experimental plots (20 × 30 m each) were created in the Hubbard Brook Experimental Forest near the main branch of the Hubbard Brook in a tract composed of 70-100 year old northern hardwood tree species. Dominant tree species include sugar maple (*Acer saccharum* Marsh.), red maple (*Acer rubrum* L.), and yellow birch (*Betula alleghaniensis* Ehrh.) with American beech (*Fagus grandifolia* Britt.) predominant in the understory (Rustad and Campbell 2012). Common understory species include Hobblebrush (*Viburnum alnifolium*), *Lycopodium* spp., *Dryopteris intermedia*, and *Maianthemum canadense*. Ice storms were simulated by spraying stream water, pumped from the main branch of Hubbard Brook, over the canopy on below freezing winter nights so that falling water would freeze on contact with vegetation, closely approximating natural conditions leading to ice accretion (see Rustad and Campbell 2012, Campbell et al. 2020). Ice accretion was measured using the caliper method on four passive ice collectors that were located within in each plot. At the time of application, surface air temperatures ranged from -13 to -4 °C. Ten plots, 20 × 30 m, with a 5 m buffer between plots, were assigned one of five ice severity treatments (n = 2). In most parts of the US, ice thicknesses between 6-19 mm are

considered mild to moderate (return interval of 2-5 yr); thicknesses between 19-32 mm are considered severe (return intervals of 35-85 yr; Jones and Mulherin 1998, Irland 2000, Changnon 2003). Following this classification, ice severity treatments included no ice (control), low ice with a target treatment of 6.4 mm in thickness (6.3 ±0.3 mm measured), moderate ice with a target of 12.7 mm (8.4 ±1.1 mm measured), extreme ice with a target treatment of 19 (13.3 ±1.2 mm measured), or moderate ice for two consecutive years with a target of 12.7 mm per year (moderate ×2; 11.0 ±1.6 mm measured in year 1 and 11.3 ±0.2 mm measured in year 2)(Campbell et al. 2020). During the winter of 2016, all treatments received at least one ice event (January 18-19 or February 10-11). During the winter of 2017, the moderate ×2 treatment received an additional ice treatment (January 9-13). Within each plot, three 5 × 5 m subplots were established for destructive soil sampling. Thus, the ecosystem scale nature of this experiment, combined with limits placed on destructive sampling (due to a long-term and interdisciplinary sampling plan), limited treatment and sampling replication (more detail below). However, the insights provided by such large-scale, interdisciplinary experiments remain valuable, and some recent work suggests that a gradient approach such as ours can be as effective as extensive replication, particularly for the detection of nonlinear effects (Kreyling et al. 2018, Hanson and Walker 2020).

Root Sampling

We collected 3 soil cores (0-10 cm with diameter of 7.62 cm) within each of the 10 plots on May 12, 2017, and on November 7, 2017, for a total of 6 soil cores per treatment per sampling date (30 cores per sampling; 60 cores total). Our sampling occurred 17 months after the initial ice storm treatments, meaning that mycorrhizal fungal colonization observed in the light, moderate, and extreme treatments had one full growing season in between the treatment and our

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measurements, and moderate ×2 was measured after the second ice event. Our sampling on May 12 occurred as trees were beginning to leaf out (stage 2 of four stages of leaf out) whereas our November 7 sampling occurred post-leaf fall.

We manually extracted all roots from soil cores to quantify both ectomycorrhizal fungal tips and arbuscular mycorrhizal fungal colonization according to root morphology (note that we did not identify root species). We removed all roots that were less than 0.5 mm from each soil core and washed the extracted roots three times in DI water. We analyzed roots where the mantle was intact and that had the characteristic forked appearance for ectomycorrhizal fungal colonization, and roots without forking for arbuscular mycorrhizal fungal colonization. Using a dissecting microscope at 12× magnification, we quantified ectomycorrhizal fungal infection of root tips on ~20 cm of fine roots from each soil core (Celestron Professional Stereo Zoom Microscope, Torrance, CA). Following microscopic inspection, we scanned roots using an Epson perfection V39 scanner (model J371A, Seiko Epson Corp, Indonesia) and measured the length using the measurement tool in ImageJ (Schnieder et. al, 2012). We used exact root length to calculate the number of ectomycorrhizal tips per cm of root length.

We quantified arbuscular mycorrhizal fungal colonization using a modified protocol following Brundrett (1991). To lighten root pigments, we bleached fine roots (10 cm length) in 10% KOH for 48 hours and then washed them in DI water for 5 minutes. Next, we acidified roots in a 2% HCl solution for 30 minutes prior to staining them with 0.05 % Trypan Blue solution overnight. We stored the stained roots in a 1:1 acidified glycerol solution in the refrigerator to allow remaining trypan blue stain to diffuse from the roots. After 2 days, we washed the root samples with DI water, and mounted the stained roots on microscope slides using polyvinyl-lacto-glycerol glue (INVAM 2017) and counted the fungal structures

(arbuscules, soils, vesicles) at 200× with a Nikon Eclipse E600 (Nikon Instruments, Melville, New York, USA) using the intersect method (McGonigle et al. 1990). The arbuscular mycorrhizal fungal "score" was calculated as the number of arbuscular mycorrhizal fungal structures (arbuscules, vesicles, and hyphae) observed per cm root length. These counts were summed into one "score number" for arbuscular mycorrhizal fungal structures expressed as arbuscular mycorrhizal fungal structures per cm of root length (Brundrett 1991, Claassen and Zasoski 1992, Vierheilig et al. 2005).

Statistical Analysis

We used linear mixed effects models with subplot nested within plot as a random effect to determine statistical significance of experimental ice storm treatment and season on ectomycorrhizal fungal and arbuscular mycorrhizal fungal colonization (nlme package, Pinheiro et al. 2021). Models were inspected for normality and homogeneity of variance by visual inspection of residual and QQ plots. We used a constant variance structure (varIdent) to account for heterogeneity in variance associated with treatment (nlme package, Pinheiro et al. 2021). We log transformed arbuscular mycorrhizal fungal data to meet normality assumptions. Type 3 Analysis of variance (ANOVA, car package, Fox and Weisberg 2011) was completed on the models to determine statistical significance and we calculated marginal and conditional R^2 values using the *piecewiseSEM* package (Lefcheck 2015) to determine model fit. Given that the basal area of arbuscular mycorrhizal and ectomycorrhizal trees species varied across our ten plots, and that the abundance of these trees types impacts the presence and abundance of arbuscular mycorrhizal and ectomycorrhizal fungal colonization and association (respectively; e.g., Rosling et al. 2016, Grünfeld et al. 2020, Eagar et al. 2022), we ran a second analysis, using the statistical model described above, where we corrected for these differences by normalizing the arbuscular

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mycorrhizal fungal structure and ectomycorrhizal fungal tip data by the basal area of trees in 2017 (measured as in Rustad et al. 2020) associated with arbuscular or ectomycorrhizal fungi (respectively) in each plot (trees were associated with mycorrhizae type based on Chaudhary et al. 2016). We conducted all analyses in R version 3.4.4 (R Core Team 2016).

Results

Ectomycorrhizal fungal root tips

Ice storm treatments reduced ectomycorrhizal root tips per unit root length relative to control plots, but this effect was larger in the spring than in the fall (significant ice treatment by season interaction, $P < 0.05$; Table 1; Fig. 1a and c). These results were sustained even when data were normalized by the basal area of ectomycorrhizal trees that occurred within each plot (Fig. 1a and c; Table 1). In the spring, there were the most ectomycorrhizal fungal root tips in the control plots (1.8 ± 0.09, mean ± SE; Fig. 1a). On average, the low (1.0 ±0.07) and moderate ×2 (1.1 ±0.07) ice treatments decreased ectomycorrhizal fungal root tips by 48% and 40%, respectively, while the moderate (1.4 ±0.02) and extreme (1.4 ±0.08) ice treatments only decreased root tips by approximately 24% relative to controls. There were more ectomycorrhizal fungal root tips, on average, in the spring than in the fall, except for in the moderate ×2 treatment where ectomycorrhizal root tips were similar in the spring and the fall (1.0 ±0.06 in the fall; Fig. 1a). In the fall, the control (1.1 ±0.13) and moderate ×2 treatments had similar ectomycorrhizal root tips per root length, which were up to 37% higher than ectomycorrhizal root tips measured in the low, moderate, and extreme ice treatments (0.7 ±0.09, 0.8 ±0.05, and 0.7 ±0.05, respectively; Fig. 1a). The statistical model explained 67% of the variation in ectomycorrhizal fungal root tips across our treatments (Table 1).

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Arbuscular Mycorrhizal Fungal Colonization

Similar to our ectomycorrhizal results, we found a strong season by treatment interaction with arbuscular mycorrhizal fungal colonization (P < 0.05; Table 1), but colonization was greater in the fall than in the spring (Fig. 1b and d). Again, these results were similar whether data were normalized by the basal area of arbuscular mycorrhizal trees in each plot or not (Fig. 1b and d; Table 1). Arbuscular mycorrhizal fungal colonization decreased in the spring with increasing ice storm treatment severity and frequency, such that the control (8.9 ± 0.88), and the low (8.3 ± 0.42) ice treatments were comparable and 30-40% higher than in the moderate treatment (6.3 ± 0.25), which was 50-60% higher than the extreme (3.9 ± 0.28) and moderate ×2 treatments (4.2 ± 0.45; Fig. 1b). While arbuscular mycorrhizal fungal colonization decreased with ice storm severity and frequency in the spring, it increased with severity and frequency in the fall (Fig. 1b). Control and low ice treatments had similar colonization (15.9 ± 0.65 and 13.9 ± 1.43, respectively), followed by higher colonization in the moderate (20.9 ± 0.44) and extreme (19.0 ± 2.7) ice treatments and finally the highest and most variable colonization in the moderate ×2 treatment (24.4 ± 2.81; Fig. 1b). The statistical model explained 84% of the variation in arbuscular mycorrhizal fungi colonization across all the treatments (Table 1).

Seasonal Comparison of Ectomycorrhizal and Arbuscular Mycorrhizal Fungal Colonization

Across all treatments, ectomycorrhizal fungal and arbuscular mycorrhizal fungal had different root colonization patterns in the fall and the spring. Arbuscular mycorrhizal fungal colonization was 3× higher in the fall than in the spring. In contrast, ectomycorrhizal fungal colonization was 1.7× higher in the spring than in the fall. Among all the ice storm treatments and across both

types of mycorrhizae, only ectomycorrhizal fungal root tips in the moderate ×2 treatment were similar across seasons (Fig. 1). In all other treatments, ectomycorrhizal fungal root tips and arbuscular mycorrhizal fungal colonization varied with season (Table 1).

Discussion

Ice storms are extreme events that damage forest canopies and alter forest inputs and microclimates – changes that may impact the belowground mycorrhizal system. Using a novel, large-scale ice-storm experiment that manipulated ice storm intensity and frequency in a mixed deciduous forest, we found that the impact ice storms have on mycorrhizae was different when observing colonization in the fall and in the spring. Ice storm damage decreased both ectomycorrhizal fungal root tips and arbuscular mycorrhizal fungal colonization in the spring, but this pattern only held for ectomycorrhizal fungal root tips in the fall, for all treatments except the moderate ×2 treatment. Arbuscular mycorrhizal fungal colonization, on the other hand, increased with ice storm severity and frequency in the fall. We also found large differences in mycorrhizal root tips and colonization in the fall and the spring. Ectomycorrhizal fungal root tips were, on average, 1.7× greater in the spring than in the fall, whereas arbuscular mycorrhizal fungal colonization was around 3× greater in the fall than in the spring.

Both ectomycorrhizal fungal and arbuscular mycorrhizal fungal colonization decreased with ice storm frequency and severity in the spring. The low ice treatment reduced ectomycorrhizal fungal colonization by 52% relative to control. The greatest reduction in arbuscular mycorrhizal fungal colonization was in the extreme ice treatment with an average decrease of 42% (vs. the control). This reduced colonization could indicate that, due to aboveground damage sustained from ice events, plants reduced belowground allocation of non-

structural carbohydrates, which would also reduce carbon availability for mycorrhizal fungal symbionts. That this trend was detectable in the second spring following ice storm damage suggests that the effects of this punctuated storm may be relatively long-lasting. Further, the mycorrhizal responses to the more frequent, moderate storm were not substantially different from this longer-term response (Fig. 1). Indeed, previous research found that leaf area was not restored to pre-storm values until three years following a moderate to severe ice storm event (Rhoads et al. 2002). Such a post-storm lowered photosynthetic potential throughout the growing season could reduce non-structural carbohydrate stores with effects across years pending leaf area recovery. This idea is supported by evidence from the ice storm experiment that tree wound closure, which also relies on non-structural carbohydrates, was reduced with increased crown damage in the ice storm treatment plots (P. Schaberg, *personal communication*). While speculative, the detrimental impact of ice storm frequency and intensity may have been amplified during our spring sampling because it was conducted prior to full leaf out of deciduous trees. At this time, tree reserves of non-structural carbohydrates are typically at their lowest point, since reserves are built up during the growing season and depleted throughout the dormant season to meet metabolic demands (Furze et al. 2019). Exploring the plant physiological drivers for our observed patterns would be an exciting area for further ice storm research.

The amount of mycorrhizal fungal root tips and colonization, as well as responses to treatments, varied by mycorrhizal fungi type and season. Ectomycorrhizal fungal root tips per cm in the spring and in the fall were lower than the control in all ice treatments except the moderate ×2 treatment in the fall, which were not different from the controls. These results suggest that ice storm severity could depress ectomycorrhizal fungi throughout the year, but that an increase in ice storm frequency may result in the rapid recovery of ectomycorrhizal fungi. Overall, the

moderate ×2 treatment maintained a relatively constant number of ectomycorrhizal fungal root tips per cm throughout the growing season, perhaps because annual ice storm disturbances caused this community of ectomycorrhizal fungal to grow more slowly. Unfortunately, the large-scale nature of our experiment limited our ability to further disentangle relationships among ice storm frequency and intensity.

In contrast to the relatively consistent response of ectomycorrhizal fungi to treatments throughout the year, the response of arbuscular mycorrhizal fungal colonization to ice storms was different in the fall than in the spring. In the spring, arbuscular mycorrhizal fungal colonization decreased with increasing ice storm severity and frequency, but in the fall, arbuscular mycorrhizal fungal colonization increased with increasing ice storm severity and frequency. This suggests that, while reduced availability of belowground plant carbon for arbuscular mycorrhizae in the spring may have exacerbated ice storm impacts on arbuscular mycorrhizal fungal colonization, later in the growing season these same plants may have increased photosynthate allocation belowground and to arbuscular mycorrhizal fungal symbionts with increased ice storm severity and frequency, perhaps due to nutrient limitation resulting from the addition of ice storm-produced woody debris (Johnson and Gehring 2007, Homyak et al. 2008, Piatek 2011, Lajtha 2020). The positive response of arbuscular mycorrhizal fungal colonization to ice storm severity and frequency in the fall could also be a response to enhanced root growth (Giovannetti et al. 1993, Rhoads et al. 2002) but, to date, there have been no significant differences in root growth among the ice storm treatments (T.J. Fahey, *personal communication*).

Overall and across treatments, we found that ectomycorrhizal and arbuscular mycorrhizal fungi had opposite seasonal trends. There were more ectomycorrhizal fungal root tips in the

spring than in the fall, whereas arbuscular mycorrhizal fungal colonization was higher in the fall than in the spring. These results support prior work showing a higher percentage of root area colonized by ectomycorrhizal fungal in the early versus late growing season (Sung et al. 1995). A recent review found that ectomycorrhizal fungi were less sensitive than arbuscular mycorrhizal fungi to temperature (Soudzilovskaia et al. 2015) and ectomycorrhizal fungi may also be less sensitive than arbuscular mycorrhizal fungi to reductions in plant carbon allocation, since many ectomycorrhizal fungi can produce extracellular enzymes that degrade soil organic matter (Shah et al. 2016, Nicolás et al. 2019). Thus, ectomycorrhizal fungi may have been less sensitive than arbuscular mycorrhizal fungi to low early spring temperatures as well as to declines in plant belowground carbon allocation.

We observed higher arbuscular mycorrhizal fungal colonization in the fall than in the early spring, suggesting that more plant photosynthate may be allocated and available belowground for mycorrhizal fungal symbionts later in the growing season than in the winter and early spring when plant non-structural carbohydrates are depleted. Indeed, carbon allocation to fungal symbionts can increase later in the growing season based on host plant physiology (Brundrett 1991, Högberg et al. 2010). Further, arbuscular mycorrhizal fungal plant root colonization declines at low temperatures (Brundrett and Kendrick 1988, Soudzilovskaia et al. 2015). Thus, arbuscular mycorrhizal fungal temperature constraints and host physiology may interact to affect colonization, where host plants that produce roots during the late spring to mid fall when the soil is warm have more arbuscular mycorrhizal fungal colonization relative to plants that produce roots in the spring when soils are cold (Brundrett and Kendrick 1988, Santos-González et al. 2007). Finally, arbuscular mycorrhizal fungal hyphae can have relatively short turnover times (days vs. months to years for ectomycorrhizal hyphae; Frey 2019) and species,

diversity, and community dynamics can vary seasonally (Abbott and Robson 1991, Santos-González et al. 2007, Dumbrell et al. 2011). Thus, the high fall arbuscular mycorrhizal fungal colonization observed in this study may be a result of mycorrhizal succession with faster growing arbuscular mycorrhizal fungal species appearing later in the growing season, tolerant species resuming full growth (Klironomos et al. 2001), seasonally driven plant or arbuscular mycorrhizal fungal community growth, or species turnover due to changing environmental conditions over the season and in response to ice storm severity and frequency.

Overall, our results suggest that arbuscular mycorrhizal fungi may recover from ice storm events relatively quickly, potentially aiding forests in their recovery, while the impacts of ice storms on ectomycorrhizal fungi are longer lasting. While there is substantial trait variation among species of arbuscular and ectomycorrhizal fungi that may impact ecosystem properties and processes (e.g., Agerer 2001, Johnson and Gehring 2007, Chagnon et al. 2013), systems dominated by arbuscular mycorrhizal associations are often characterized by fast turnover of inorganic nutrients and carbon, as opposed to the slow cycling and carbon accumulation often prevalent in stands dominated by ectomycorrhizal associations (Phillips et al. 2013, Averill et al. 2014, Averill 2016, Jo et al. 2019). Thus, the faster recovery of arbuscular mycorrhizal fungi in these forests may result in relatively faster biogeochemical cycling relative to ectomycorrhizal dominated forests or forests undamaged by ice storms.

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Conflict of Interest Statement

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data (Yancey et al. 2023) are available from the Environmental Data Initiative Data Portal:

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References

Abbott, L. K., and A. D. Robson. 1991. Factors influencing the occurrence of vesicular-arbuscular mycorrhizas. Agriculture, Ecosystems & Environment 35:121-150.

Agerer, R. 2001. Exploration types of ectomycorrhizae. Mycorrhiza 11:107-114.

Allen, M. F., W. Swenson, J. I. Querejeta, L. M. Egerton-Warburton, and K. K. Treseder. 2003. Ecology of Mycorrhizae: A Conceptual Framework for Complex Interactions Among Plants and Fungi. Annual Review of Phytopathology 41:271-303.

Arnone, J. A., R. L. Jasoni, A. J. Lucchesi, J. D. Larsen, E. A. Leger, R. A. Sherry, Y. Luo, D. S. Schimel, and P. S. J. Verburg. 2011. A climatically extreme year has large impacts on C4 species in tallgrass prairie ecosystems but only minor effects on species richness and other plant functional groups. Journal of Ecology 99:678-688.

Averill, C. 2016. Slowed decomposition in ectomycorrhizal ecosystems is independent of plant chemistry. Soil Biology and Biochemistry 102:52-54.

Averill, C., B. L. Turner, and A. C. Finzi. 2014. Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. Nature 505:543-545.

Bennett, A. E., and A. T. Classen. 2020. Climate change influences mycorrhizal fungal–plant interactions, but conclusions are limited by geographical study bias. Ecology 101:e02978.

Brundrett, M. 1991. Mycorrhizas in Natural Ecosystems. Pages 171-313 *in* M. Begon, A. H. Fitter, and A. Macfadyen, editors. Advances in Ecological Research. Academic Press.

Brundrett, M. C., and B. Kendrick. 1988. The mycorrhizal status, root anatomy, and phenology of plants in a sugar maple forest. Canadian Journal of Botany 66:1153-1173.

Bücking, H., and W. Heyser. 2003. Uptake and transfer of nutrients in ectomycorrhizal associations: interactions between photosynthesis and phosphate nutrition. Mycorrhiza 13:59-68.

Campbell, J. L., L. E. Rustad, C. T. Driscoll, I. Halm, T. J. Fahey, H. Fakhraei, P. M. Groffman, G. J. Hawley, W. Leuenberger, and P. G. Schaberg. 2020. Simulating impacts of ice storms on forest ecosystems. JoVE:e61492.

Chagnon, P.-L., R. L. Bradley, H. Maherali, and J. N. Klironomos. 2013. A trait-based framework to understand life history of mycorrhizal fungi. Trends in Plant Science 18:484-491.

Chalot, M., and A. Brun. 1998. Physiology of organic nitrogen acquisition by ectomycorrhizal fungi and ectomycorrhizas. FEMS Microbiology Reviews 22:21-44.

Changnon, S. A. 2003. Characteristics of Ice Storms in the United States. Journal of Applied Meteorology 42:630-639.

Chaudhary, V. B., M. A. Rúa, A. Antoninka, J. D. Bever, J. Cannon, A. Craig, J. Duchicela, A. Frame, M. Gardes, C. Gehring, M. Ha, et al. 2016. MycoDB, a global database of plant response to mycorrhizal fungi. Scientific Data 3:160028.

Cheng, C. S., G. Li, and H. Auld. 2011. Possible impacts of climate change on freezing rain using sownscaled future climate scenarios: updated for eastern Canada. Atmosphere-Ocean 49:8-21.

Claassen, V. P., and R. J. Zasoski. 1992. A containerized staining system for mycorrhizal roots. New Phytologist 121:49-51.

Courty, P.-E., M. Buée, A. G. Diedhiou, P. Frey-Klett, F. Le Tacon, F. Rineau, M.-P. Turpault, S. Uroz, and J. Garbaye. 2010. The role of ectomycorrhizal communities in forest

ecosystem processes: New perspectives and emerging concepts. Soil Biology and Biochemistry 42:679-698.

Dumbrell, A. J., P. D. Ashton, N. Aziz, G. Feng, M. Nelson, C. Dytham, A. H. Fitter, and T. Helgason. 2011. Distinct seasonal assemblages of arbuscular mycorrhizal fungi revealed by massively parallel pyrosequencing. New Phytologist 190:794-804.

Eagar, A. C., R. M. Mushinski, A. L. Horning, K. A. Smemo, R. P. Phillips, and C. B. Blackwood. 2022. Arbuscular Mycorrhizal Tree Communities Have Greater Soil Fungal Diversity and Relative Abundances of Saprotrophs and Pathogens than Ectomycorrhizal Tree Communities. Applied and Environmental Microbiology 88:e01782-01721.

Fahey, R. T., J. W. Atkins, J. L. Campbell, L. E. Rustad, M. Duffy, C. T. Driscoll, T. J. Fahey, and P. G. Schaberg. 2020. Effects of an experimental ice storm on forest canopy structure. Canadian Journal of Forest Research 50:136-145.

Fox, J., and S. Weisberg. 2011. An R Companion to Applied Regression. Second edition. Sage, Thousand Oaks, CA.

Frey, S. D. 2019. Mycorrhizal Fungi as Mediators of Soil Organic Matter Dynamics. Annual Review of Ecology, Evolution, and Systematics 50:237-259.

Furze, M. E., B. A. Huggett, D. M. Aubrecht, C. D. Stolz, M. S. Carbone, and A. D. Richardson. 2019. Whole-tree nonstructural carbohydrate storage and seasonal dynamics in five temperate species. New Phytologist 221:1466-1477.

Gellesch, E., R. Hein, A. Jaeschke, C. Beierkuhnlein, and A. Jentsch. 2013. Biotic Interactions in the Face of Climate Change. Pages 321-349 *in* U. Lüttge, W. Beyschlag, D. Francis, and J. Cushman, editors. Progress in Botany: Vol. 74. Springer Berlin Heidelberg, Berlin, Heidelberg.

Giovannetti, M., C. Sbrana, L. Avio, A. S. Citernesi, and C. Logi. 1993. Differential hyphal morphogenesis in arbuscular mycorrhizal fungi during pre-infection stages. New Phytologist 125:587-593.

Govindarajulu, M., P. E. Pfeffer, H. Jin, J. Abubaker, D. D. Douds, J. W. Allen, H. Bücking, P. J. Lammers, and Y. Shachar-Hill. 2005. Nitrogen transfer in the arbuscular mycorrhizal symbiosis. Nature 435:819-823.

Grünfeld, L., M. Wulf, M. C. Rillig, A. Manntschke, and S. D. Veresoglou. 2020. Neighbours of arbuscular-mycorrhiza associating trees are colonized more extensively by arbuscular mycorrhizal fungi than their conspecifics in ectomycorrhiza dominated stands. New Phytologist 227:10-13.

Hanson, P. J., and A. P. Walker. 2020. Advancing global change biology through experimental manipulations: Where have we been and where might we go? Global Change Biology 26:287-299.

Hayhoe, K., C. P. Wake, T. G. Huntington, L. Luo, M. D. Schwartz, J. Sheffield, E. Wood, B. Anderson, J. Bradbury, A. DeGaetano, T. J. Troy, et al. 2007. Past and future changes in climate and hydrological indicators in the US Northeast. Climate Dynamics 28:381-407.

Högberg, M. N., M. J. I. Briones, S. G. Keel, D. B. Metcalfe, C. Campbell, A. J. Midwood, B. Thornton, V. Hurry, S. Linder, T. Näsholm, and P. Högberg. 2010. Quantification of effects of season and nitrogen supply on tree below-ground carbon transfer to ectomycorrhizal fungi and other soil organisms in a boreal pine forest. New Phytologist 187:485-493.

Homyak, P. M., R. D. Yanai, D. A. Burns, R. D. Briggs, and R. H. Germain. 2008. Nitrogen immobilization by wood-chip application: Protecting water quality in a northern hardwood forest. Forest Ecology and Management 255:2589-2601.

International culture collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM). 2017. Recipes for voucher preservation. Morgantown, West Virginia, USA, https://invam.ku.edu/recipes.

Irland, L. C. 2000. Ice storms and forest impacts. Science of The Total Environment 262:231-242.

Jentsch, A., J. Kreyling, and C. Beierkuhnlein. 2007. A new generation of climate-change experiments: events, not trends. Frontiers in Ecology and the Environment 5:365-374.

Jo, I., S. Fei, C. M. Oswalt, G. M. Domke, and R. P. Phillips. 2019. Shifts in dominant tree mycorrhizal associations in response to anthropogenic impacts. Science Advances 5:eaav6358.

Johnson, N. C., and C. A. Gehring. 2007. CHAPTER 4 - Mycorrhizas: Symbiotic Mediators of Rhizosphere and Ecosystem Processes. Pages 73-100 *in* Z. G. Cardon and J. L. Whitbeck, editors. The Rhizosphere. Academic Press, Burlington.

Jones, K. F., and N. D. Mulherin. 1998. An Evaluation of the Severity of the January 1998 Ice Storm in Northern New England.

Kivlin, S. N., S. M. Emery, and J. A. Rudgers. 2013. Fungal symbionts alter plant responses to global change. American Journal of Botany 100:1445-1457.

Klironomos, J. N., M. M. Hart, J. E. Gurney, and P. Moutoglis. 2001. Interspecific differences in the tolerance of arbuscular mycorrhizal fungi to freezing and drying. Canadian Journal of Botany 79:1161-1166.

Kreyling, J., A. H. Schweiger, M. Bahn, P. Ineson, M. Migliavacca, T. Morel-Journel, J. R. Christiansen, N. Schtickzelle, and K. S. Larsen. 2018. To replicate, or not to replicate – that is the question: how to tackle nonlinear responses in ecological experiments. Ecology Letters 21:1629-1638.

Lajtha, K. 2020. Nutrient retention and loss during ecosystem succession: revisiting a classic model. Ecology 101:e02896.

Lefcheck, J. S. 2015. piecewiseSEM: Piecewise structural equation modeling in R for ecology, evolution, and systematics. Methods in Ecology and Evolution 75:573-579.

Likens, G. E., B. K. Dresser, and D. C. Buso. 2004. Short-Term Temperature Response in Forest Floor and Soil to Ice Storm Disturbance in a Northern Hardwood Forest. Northern Journal of Applied Forestry 21:209-219.

Lilleskov, E. A., and T. D. Bruns. 2003. Root colonization dynamics of two ectomycorrhizal fungi of contrasting life history strategies are mediated by addition of organic nutrient patches. New Phytologist 159:141-151.

McGonigle, T. P., M. H. Miller, D. G. Evans, G. L. Fairchild, and J. A. Swan. 1990. A new method which gives an objective measure of colonization of roots by vesicular— arbuscular mycorrhizal fungi. New Phytologist 115:495-501.

National Centers for Environmental Information (NCEI). 2019. Storm Events Database, Version 3.0. National Oceanic and Atmosphere Administration (NOAA), https://www.ncdc.noaa.gov/stormevents/.

Nicolás, C., T. Martin-Bertelsen, D. Floudas, J. Bentzer, M. Smits, T. Johansson, C. Troein, P. Persson, and A. Tunlid. 2019. The soil organic matter decomposition mechanisms in

ectomycorrhizal fungi are tuned for liberating soil organic nitrogen. The ISME Journal 13:977-988.

Peay, K. G., P. G. Kennedy, and T. D. Bruns. 2011. Rethinking ectomycorrhizal succession: are root density and hyphal exploration types drivers of spatial and temporal zonation? Fungal Ecology 4:233-240.

Phillips, R. P., E. Brzostek, and M. G. Midgley. 2013. The mycorrhizal-associated nutrient economy: a new framework for predicting carbon–nutrient couplings in temperate forests. New Phytologist 199:41-51.

Piatek, K. B. 2011. The fate of nitrogen mineralized from leaf litter — Initial evidence from 15N-labeled litter.*in* S. Fei, J. M. Lhotka, J. W. Stringer, K. W. Gottschalk, and G. W. Miller, editors. Proceedings, 17th central hardwood forest conference; 2010 April 5-7; Lexington, KY; Gen. Tech. Rep. NRS-P-78. U.S. Department of Agriculture, Forest Service, Northern Research Station, Newtown Square, PA.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R. C. Team. 2021. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-152. URL: https://CRAN.R-project.org/package=nlme.

R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rhoads, A. G., S. P. Hamburg, T. J. Fahey, T. G. Siccama, E. N. Hane, J. Battles, C. Cogbill, J. Randall, and G. Wilson. 2002. Effects of an intense ice storm on the structure of a northern hardwood forest. Canadian Journal of Forest Research 32:1763-1775.

Rodriguez-Ramos, J. C., J. A. Cale, J. F. Cahill Jr, S. W. Simard, J. Karst, and N. Erbilgin. 2021. Changes in soil fungal community composition depend on functional group and forest disturbance type. New Phytologist 229:1105-1117.

Rosling, A., M. G. Midgley, T. Cheeke, H. Urbina, P. Fransson, and R. P. Phillips. 2016. Phosphorus cycling in deciduous forest soil differs between stands dominated by ecto- and arbuscular mycorrhizal trees. New Phytologist 209:1184-1195.

Rustad, L. E., and J. L. Campbell. 2012. A novel ice storm manipulation experiment in a northern hardwood forest. Canadian Journal of Forest Research 42:1810-1818.

Rustad, L. E., J. L. Campbell, C. T. Driscoll, T. J. Fahey, P. M. Groffman, P. G. Schaberg, G. J. Hawley, I. Halm, F. Bowles, W. Leuenberger, G. Schwaner, et al. 2020. Experimental approach and initial forest response to a simulated ice storm experiment in a northern hardwood forest. PLOS ONE 15:e0239619.

Santos-González, J. C., R. D. Finlay, and A. Tehler. 2007. Seasonal Dynamics of Arbuscular Mycorrhizal Fungal Communities in Roots in a Seminatural Grassland. Applied and Environmental Microbiology 73:5613-5623.

Shah, F., C. Nicolás, J. Bentzer, M. Ellström, M. Smits, F. Rineau, B. Canbäck, D. Floudas, R. Carleer, G. Lackner, J. Braesel, et al. 2016. Ectomycorrhizal fungi decompose soil organic matter using oxidative mechanisms adapted from saprotrophic ancestors. New Phytologist 209:1705-1719.

Smith, S. E., and D. Read. 2008. Mycorrhizal Symbiosis (Third Edition). Academic Press, London.

Soudzilovskaia, N. A., J. C. Douma, A. A. Akhmetzhanova, P. M. van Bodegom, W. K. Cornwell, E. J. Moens, K. K. Treseder, M. Tibbett, Y.-P. Wang, and J. H. C. Cornelissen.

2015. Global patterns of plant root colonization intensity by mycorrhizal fungi explained by climate and soil chemistry. Global Ecology and Biogeography 24:371-382.

Sung, S.-J. S., L. M. White, D. H. Marx, and W. J. Otrosina. 1995. Seasonal ectomycorrhizal fungal biomass development on loblolly pine (Pinus taeda L.) seedlings. Mycorrhiza 5:439-447.

Swaminathan, R., M. Sridharan, and K. Hayhoe. 2018. A computational framework for modelling and analyzing ice storms. arXiv e-prints https://ui.adsabs.harvard.edu/abs/2018arXiv180504907S.

Talbot, J. M., and K. K. Treseder. 2010. Controls over mycorrhizal uptake of organic nitrogen. Pedobiologia 53:169-179.

Tinker, P., M. B. Jones, and M. Durall. 1992. A functional copmarioson of ecto- and endomycorrhizas. Pages 303-310 *in* D. Read, D. Lewis, and A. Fitter, editors. Mycorrhizas in Ecosystems. Alexander, University of York, UK.

Treseder, K. K. 2004. A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO2 in field studies. New Phytologist 164:347-355.

Turner, B. L. 2008. Resource partitioning for soil phosphorus: a hypothesis. Journal of Ecology 96:698-702.

van Breemen, N., R. Finlay, U. Lundström, A. G. Jongmans, R. Giesler, and M. Olsson. 2000. Mycorrhizal weathering: A true case of mineral plant nutrition? Biogeochemistry 49:53-67.

van der Heijden, M. G. A., J. N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, and I. R. Sanders. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69-72.

van der Heyde, M., B. Ohsowski, L. K. Abbott, and M. Hart. 2017. Arbuscular mycorrhizal

    fungus responses to disturbance are context-dependent. Mycorrhiza 27:431-440.

Vierheilig, H., P. Schweiger, and M. Brundrett. 2005. An overview of methods for the detection

    and observation of arbuscular mycorrhizal fungi in roots†. Physiologia Plantarum

    125:393-404.

Weeks, B. C., S. P. Hamburg, and M. A. Vadeboncoeur. 2009. Ice storm effects on the canopy

    structure of a northern hardwood forest after 8 years. Canadian Journal of Forest

    Research 39:1475-1483.

Whiteside, M. D., M. O. Garcia, and K. K. Treseder. 2012. Amino Acid Uptake in Arbuscular

    Mycorrhizal Plants. PLOS ONE 7:e47643.

Yancey, C., S. Juice, and E. Adair. 2023. Ice Storm Mycorrhizal Fungi data for Yancey et al. in

    press ver 1. Environmental Data Initiative.

    https://doi.org/10.6073/pasta/0c2c3eae0f6ba1fceb7ff16913c83712

Tables

Table 1. Analysis of variance (ANOVA) results for ectomycorrhiza (ECM) tips and arbuscular mycorrhiza fungal (AMF) structures normalized by the basal area of ECM or AM trees in each plot or not normalized.

Mycorrhizal type	Effect	Not normalized				Normalized			
		R^2	df	X^2	P	R^2	df	X^2	P
ECM tips	Treatment		4	7.58	0.1082		4	4.81	0.3071
	Season		1	24.88	<0.0001		1	20.40	<0.0001
	Treatment*Season		4	22.26	0.0002		4	31.18	<0.0001
	Marginal R^2	69%				61%			
AMF structures	Treatment		4	6.87	0.1430		4	7.40	0.1160
	Season		1	29.62	<0.0001		1	27.99	<0.0001
	Treatment*Season		4	46.75	<0.0001		4	51.45	<0.0001
	Marginal R^2	84%				73%			

Figures

Figure 1. Ectomycorrhiza (ECM) tips and arbuscular mycorrhiza fungal (AMF) structures by season and across ice treatments (mm of ice; 12.7x2 is 12.7 mm of ice in two consecutive winters). Boxplots of (a) ECM tips per cm or (c) ECM tips per cm normalized (norm.) by the basal area of ECM trees in each plot for the fall and spring sampling periods. In both cases, there were more (and more variable) ECM tips in the spring than in the fall. Treatment impacts varied by season (significant interaction; Table 1). Boxplots of (b) AMF structures per cm or (d) AMF structures normalized by the basal area of AM trees in fall and spring. For both cases, fall colonization was higher and more variable than fall, and ice storm intensity interacted with season to affect colonization (significant interaction; Table 1). In the boxplots, the lower and upper hinges correspond to 25th and 75th percentiles, respectively. The upper whisker extends from the hinge to the largest value no further than 1.5 * the inter-quartile range (IQR); the lower extends from the hinge to the smallest value at most 1.5*IQR. Data beyond the whiskers are outlier points.

(a) ECM tips cm^{-1} vs Ice Treatment (mm of ice)

(b) AMF structures cm^{-1} vs Ice Treatment (mm of ice)

(c) Norm. ECM tips cm^{-1} vs Ice Treatment (mm of ice)

(d) Norm. AMF structures cm^{-1} vs Ice Treatment (mm of ice)

Season [ ] Fall [ ] Spring