



Tree seedling responses to multiple environmental stresses: Interactive effects of soil warming, nitrogen fertilization, and plant invasion



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ABSTRACT

Temperate deciduous forest ecosystems in northeastern North America are under increasing biotic and abiotic stresses that can have interactive effects on understory vegetation, and thus impact the next generation of forest canopy trees. We examined seedling responses of the common and increasingly dominant species *Acer rubrum* (red maple) to the combined effects of soil warming (+5 °C), chronic nitrogen fertilization (+50 kg N ha⁻¹ yr⁻¹), and invasion by the phytotoxic plant *Alliaria petiolata* (garlic mustard), and their interactions. We planted 296 first-year *A. rubrum* seedlings in a multifactorial field experiment to examine the effects of all combinations of the experimental treatments. Second-year *Acer rubrum* seedlings demonstrated higher aboveground growth under soil warming conditions. Further, soil warming positively influenced plant-soil feedbacks through higher arbuscular mycorrhizal colonization in fine roots. However, the positive growth responses and mycorrhizal colonization observed under soil warming were moderated by both N fertilization and *A. petiolata* invasion. Our results highlight the importance of developing management plans that consider how multiple environmental change factors affect tree seedling performance, particularly via the plant-soil interface.

1. Introduction

Temperate forest ecosystems in northeastern North America are undergoing modification via multiple anthropogenically-driven environmental change factors, thus presenting challenges for adaptive forest management (Chmura et al., 2011). Ongoing abiotic changes include both warming temperatures and atmospheric nitrogen (N) deposition, with long-term models for the Northeast predicting air temperature increases up to 5.3 °C and patterns of regionally high reactive N deposition (Galloway et al., 2014; Hayhoe et al., 2007; IPCC, 2013). Atmospheric and concomitant soil warming can increase growth in some forest species, alter mycorrhizal community composition, and stimulate carbon (C) loss from soil (e.g. Butler et al., 2012; Fernandez et al., 2017; Melillo et al., 2011). Chronic soil N enrichment via atmospheric N deposition can enhance tree growth and suppress microbial activity and soil organic matter decomposition, leading to soil C accumulation and fundamental shifts in the fungal community (e.g. Butler et al., 2012; Frey et al., 2014; Morrison et al., 2016). Further, soil warming can lead to N enrichment through increases in net N mineralization and nitrification (Butler et al., 2012; Melillo et al., 2011). Biotic changes include invasions by non-native organisms that can alter

the structure, diversity, and function of native forest communities. Invasive species often exploit abiotic disturbances (MacDougall and Turkington, 2005), but abiotic conditions can also mediate the impacts and dynamics of biological invasions (Eisenhauer et al., 2014). Thus, temperate forests are in a state of flux due to ongoing perturbations to climate, biophysical factors, and the biotic environment: these biotic and abiotic stressors can then interact to have complex effects on individual plant species and communities (Tylianakis et al., 2008). Understanding these interacting stressors is thus critical for predicting how key forest species will perform under future, changing climates and how land managers can best respond with appropriate management practices.

The impacts of plant invasions on recipient ecosystems have been widely documented (e.g. reviewed by Levine et al., 2003; Vitousek et al., 1997) and include changes to native plant performance via competition, allelopathy, and alteration of plant-microbial interactions (e.g. Schmidt et al., 2008; Stinson et al., 2006; Yuan et al., 2013). A growing body of literature also suggests that these impacts may intensify as changes in the abiotic environment facilitate both the performance and spread of invasive organisms (e.g. Dukes et al., 2009; MacDougall and Turkington, 2005; Valliere and Allen, 2016). However,

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we still know very little about the interactive effects of invasion and ongoing abiotic stresses on the growth, survival, and plant-soil interactions of native plants.

Acer rubrum L. (red maple) is one of the most widely distributed eastern temperate tree species (Chapman and Bolen, 2015). Across much of its range, this species is increasingly dominating forests, mainly in response to anthropogenic changes such as fire suppression and land use changes, and by selective inhibition of competitors by deer browsing, defoliating insects and pathogens (Abrams, 1998). Seedlings are strongly responsive to temperature, with leaf phenology, photosynthetic rates, respiration and growth all acclimating rapidly to atmospheric as well as direct soil warming (Kaye and Wagner, 2014; Lee et al., 2005; Sendall et al., 2015; Wheeler et al., 2016). However, recent research has highlighted the need to consider the aggregate effects of multiple environmental drivers on tree seedlings and forest understory species (e.g. Fisichelli et al., 2014; Petrie et al., 2016), including *A. rubrum*. For example, *A. rubrum* and *A. saccharum* saplings demonstrate increased growth under climate warming, but deer browsing inhibits this positive growth response (Fisichelli et al., 2012), and disturbance to understory vegetation communities by invasive earthworms is mediated by environmental factors like soil pH and precipitation (Fisichelli et al., 2013). This highlights the need to consider both abiotic conditions and biological stressors acting in conjunction to influence species and community responses.

Alliaria petiolata (M. Bieb.) Cavers and Grande, family Brassicaceae, is a Eurasian, non-mycorrhizal herb that invades both disturbed and pristine hardwood forest understories in the United States and Canada (Nuzzo, 1999). Though widely studied for its ecology and impacts on native organisms (reviewed in Rogers et al. 2008), how this invasive plant interacts with abiotic stressors to affect native forest species remains largely unexplored. *Alliaria petiolata* inhibits mycorrhizal colonization in tree seedling roots of *A. rubrum* and other forest species via the release of antifungal glucosinolate phytochemicals through root exudates and decaying litter (Barto et al., 2011; Stinson et al., 2006). Despite the short half-life of these compounds in soils, the legacy of mycorrhizal community disruptions from *A. petiolata* invasions have been shown to persist in forest soils for years, even after eradication (Lankau et al., 2014). These critical soil fungi are also affected by abiotic changes such as soil warming (e.g. Mohan et al., 2014) and N deposition (e.g. Egerton-Warburton and Allen, 2000; Lilleskov et al., 2001; Morrison et al., 2016; van Diepen et al., 2011). However, despite extensive research on the impact of this common invasive on plant and soil communities, it remains poorly understood how *A. petiolata* may interact with abiotic environmental changes to impact plant-fungal interactions and subsequent tree seedling growth.

Our objectives in this study were to determine how soil warming, chronic N fertilization, and invasion by *A. petiolata* interact to affect the survival, phenology, growth, and AMF colonization of *A. rubrum* seedlings. As part of an ongoing long term global change experiment (Contosta et al., 2011), we use soil warming as a treatment, as opposed to elevated air temperature. Soil temperature may be more important at the tree seedling stage, since seedlings have low growth forms that may be more closely ecologically linked to ground-level microclimatic conditions (Körner, 2003). Further, previous work has shown *A. rubrum* first-year seedling growth responds strongly to soil warming (Wheeler et al., 2016). Although other studies have examined the effects of concurrent environmental changes on temperate forest species (e.g. Maes et al., 2014), to our knowledge this is the first study to examine the interaction between invasion and concurrent global abiotic stresses, and as such, provides new data for categorizing and planning for forest responses to ongoing environmental change.

2. Methods

2.1. Field site description

We established our experiment within a long-running global change manipulation: the Soil Warming and Nitrogen Addition (SWaN) Study at the Harvard Forest Long-Term Ecological Research (LTER) site in Petersham, Massachusetts, USA. The experimental site is situated in a mixed, even-aged hardwood forest stand characterized by red and striped maple (*Acer rubrum*, *A. pensylvanicum*), red and black oak (*Quercus rubra*, *Q. velutina*), American beech (*Fagus grandifolia*), white birch (*Betula papyrifera*), and American chestnut (*Castanea dentata*). Air temperatures at the experimental site range from -25 to 30 °C through the year, with an annual mean of 7 °C; total annual precipitation is approximately 1100 mm, including the water equivalent of winter snowfall (Contosta et al., 2015). *Alliaria petiolata* is present in nearby tracts of contiguous forest of the same composition, but has no history of invasion at this experimental site prior to our study.

2.2. Field experiment

The SWaN Study, established in 2006, is a fully factorial field experiment, with $24 \times 3 \times 3$ m plots randomly assigned one of four treatments with six replicates per treatment: control, N addition, warming, or warming \times N addition. Soils in all heated plots are continuously warmed to 5 °C above ambient using buried electrical cables. Nitrogen fertilization is applied to all N treated plots in equal monthly doses between May–October as an aqueous solution of NH_4NO_3 at a rate of $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. Due to mechanical failure in one set of buried heating cables, we used a subset of 20 plots (5 replicates per treatment) for our study. Further details on the SWaN experimental setup can be found in Contosta et al. (2011). The N fertilization rate simulates the rate of atmospheric N deposition currently found in parts of Europe and Asia (Vet et al., 2014) and predicted for many parts of the world by 2050 (Galloway et al., 2008). The soil warming level reflects the upper end of predicted changes in temperature in the Northeast in the next century (IPCC, 2013).

In order to simulate forest invasion by *A. petiolata* and to test the effect of secondary phytochemicals, we established pairs of invaded and uninvaded 1×1 m subplots within each of the abiotic treatment plots at the SWaN experiment. At each experimental plot, we transplanted 32 overwintered *A. petiolata* rosettes from a nearby forest (< 5 km) into one randomly selected subplot (invaded subplot) in April 2015. Since *A. petiolata* is a biennial, we added first year *A. petiolata* seedlings periodically throughout the growing season and into autumn, to simulate typical invasion demography and maintain similar experimental densities. With a final planting of first-year rosettes in December, we maintained the *A. petiolata* invasion overwinter until May 2016. To avoid accidental invasion to non-experimental areas, plants were removed prior to reproduction in May 2016. In addition to live plants, we added *A. petiolata* leaf litter periodically to all invaded plots through the summer to ensure consistent inputs of glucosinolates. Thus, the total experimental invasion treatment occurred over a period of twelve months, simulating the effects of a recent invasion followed by eradication. Uninvaded plots were disturbed with trowels to simulate the disturbance that occurred in the invaded plots, but these plots were not experimentally invaded with *A. petiolata* plants nor was litter added.

In October 2015, we transplanted 7–8 first-year *A. rubrum* seedlings into each uninvaded and invaded subplot within each abiotic (warmed or N amended) treatment for a total of $N = 296$ seedlings. Seeds were sourced from local Massachusetts genotypes and grown for one growing season by a local nursery (New England Wetland Plants, Amherst, MA). Prior to transplanting in October, we tagged plants by treatment and measured initial stem height (from root collar to tallest terminal bud) to ensure that initial seedling height was similar across all treatments. We also trimmed roots for each seedling down to a 7-cm diameter root ball

Table 1 Spring budburst phenology, vegetative growth, arbuscular mycorrhizal (AMF) colonization and survival in *Acer rubrum* (red maple; N = 296) seedlings under soil warming (+5 °C), nitrogen addition (5 g N m⁻² y⁻¹), *A. petiolata* invasion and their interactions during the 2016 growing season at the Harvard Forest in Petersham, MA, USA. The data are from linear and general linear mixed effects models (or a general linear model for AMF colonization), with t- or z-values and p-values reported (bolded when significant at p < 0.05).

Response	Soil warming	Nitrogen addition	GM invasion	Warming × nitrogen	Warming × GM invasion	Nitrogen × GM invasion	Warming × Nitrogen × GM invasion
Spring budburst onset	t = 1.88 p = 0.060	t = -0.02 p = 0.99	t = -1.39 p = 0.17	t = -0.62 p = 0.54	t = 0.86 p = 0.39	t = 1.23 p = 0.22	t = -0.59 p = 0.55
Max height during growing season	t = 2.27 p = 0.030	t = -0.59 p = 0.56	t = -0.09 p = 0.93	t = -2.37 p = 0.024	t = -1.38 p = 0.18	t = 0.85 p = 0.40	t = 0.67 p = 0.51
Max leaf area during growing season	t = 4.05 p < 0.001	t = -0.67 p = 0.51	t = -0.73 p = 0.47	t = -3.23 p = 0.0029	t = -2.71 p = 0.011	t = -0.68 p = 0.50	t = 2.03 p = 0.051
Final dry fine root mass post-harvest	t = 1.20 p = 0.24	t = -0.70 p = 0.49	t = -1.68 p = 0.11	t = -0.52 p = 0.61	t = -0.59 p = 0.56	t = 1.48 p = 0.15	t = -0.37 p = 0.72
Percent root AMF colonization	t = 2.78 p = 0.0071	t = 0.12 p = 0.91	t = -0.38 p = 0.70	t = -1.75 p = 0.085	t = -2.96 p = 0.0042	t = -0.71 p = 0.48	t = 1.47 p = 0.15
Final survival	z = 1.92 p = 0.055	z = 0.91 p = 0.36	z = -0.22 p = 0.82	z = -1.76 p = 0.079	z = -1.11 p = 0.27	z = 0.47 p = 0.64	z = 0.50 p = 0.61

to ensure similar initial root size across treatments and to minimize soil disturbance during planting. The *A. rubrum* seedlings were thus grown under eight different field experimental treatments: control, N addition, soil warming, soil warming × N addition, control × invasion, N addition × invasion, soil warming × invasion, soil warming × N addition × invasion, with N = 37 seedlings planted per treatment. The experimental design is presented in the Supplemental Information (Fig. S1).

2.3. Field data collection

Beginning in March 2016, we visited the site weekly to determine the approximate day of onset of spring leaf phenology, at which point we recorded the day of onset of budburst. From mid-May, continuing until late August, we measured stem height weekly (from the root collar to the highest point with green leaf tissue), and on alternate weeks we measured the largest leaf area (leaf length × width) using calipers to determine the largest leaf size produced during the growing season. *Acer rubrum* leaves are characterized by lobes and sinuses, so our individual leaf area is meant as an approximation. We continued to visit the experimental site weekly until October to record survival.

In October 2016, once senescence had been initiated for all seedlings, we harvested all surviving *A. rubrum* seedlings. Roots were carefully excavated to minimize loss of fine root tissue. Root tissues were washed, and all root tissues were then dried at 60 °C for 72 h. We separated coarse from fine roots (< 1 mm diameter) for each seedling and weighed the fine root fraction.

2.4. Quantifying root AMF colonization

We randomly selected a subsample of 10 individual seedlings per treatment to assess root AMF colonization. We clipped a sample of terminal fine root tips (~1 cm segments) which are the roots that actively form associations with AMF in *A. rubrum* (Lee et al., 2014). Root tips were rehydrated in tap water for 24 h and then cleared in 10% KOH solution in a water bath at 60 °C, with solutions changed every 90 min until the roots were clear. Cleared root tips were rinsed several times with tap water and then stained by boiling in a Shaeffer ink-acetic acid solution (5% ink in 95% acetic acid) for 5 min (Vierheilig et al., 1998). Roots were de-stained by rinsing in tap water acidified with a few drops of 5% acetic acid for 20 min. We selected a random subsample of root segments (10 per seedling) and mounted these segments onto glass microscope slides in tap water. We quantified AMF colonization using the magnified intersections method at 200× magnification (McGonigle et al., 1990), where arbuscules, vesicles and non-septate hyphae within roots were recorded as colonized, then calculated as the percent of total root segment scored.

2.5. Statistical analyses

We performed all analyses using packages *lme4* and *lmerTest* using R statistical software v. 3.4.0 (R Core Team, 2014). We used linear and general linear mixed effects models using soil warming, N addition, and invasion as the explanatory variables to determine the effects of each treatment on spring budburst phenology, maximum single leaf area, maximum seedling height, fine root mass, and survival. We included all two-way and three-way interactions between soil warming, N addition and invasion. To account for plot-level variation, we included plot as a random factor in these models. To account for survival differences across treatments at the end of the growing season, we weighted final harvest data (root mass) by plot-level survival prior to analysis. We used a general linear model with soil warming, N addition, and invasion as the main explanatory variables, as well as all two-way and three-way interactions, to assess the effects of each treatment on AMF colonization. Since we examined a randomly selected subsample (N = 10 per treatment) of surviving seedlings for AMF colonization, this resulted in

a harvest of 0–3 stems per plot, depending upon plot-level survival and the results of the randomization. Since plot was un-evenly represented in this sampling design, plot was not included as a random factor in the AMF colonization model. In all cases, outliers were removed when residuals fell outside the 95% range. We used a Gaussian error structure for leaf area, seedling height, and fine root mass, a binomial error structure for survival and percent AMF colonization, and Gamma error structure for day of onset of bud burst. Differences in stem and leaf sizes, and AMF colonization, were compared by applying Tukey's test post hoc for all pairwise comparisons in the models when treatment effects were significant in the main models. The threshold of significance was set at $p = 0.05$ for all models.

3. Results

3.1. Survival

Seedling survival over the course of the experiment was highly variable at the individual plot level and was not significantly influenced by any of the treatments (Table 1).

3.2. Spring leaf phenology

Average day of onset of bud burst began on April 17th 2016 (day of year = 108), with 75% of budburst occurring in the first 7 days (Fig. 1). Budburst was similar across all treatments, with no significant effect of soil warming, N addition or invasion (Table 1; Fig. 1).

3.3. Aboveground growth

Overall, we saw a positive effect of soil warming on aboveground growth, but the positive effect was less pronounced in the N addition and *A. petiolata* invasion treatments. Soil warming had a significant positive effect on maximum leaf area and stem height (Table 1; Fig. 2). There was a significant negative interaction between soil warming and N addition on leaf area and stem height (Table 1; Fig. 2), and a significant negative interaction between soil warming and invasion on leaf area (Table 1; Fig. 2). Post-hoc Tukey testing indicated larger maximum leaf area in seedlings growing in warmed plots relative to all other treatments (Fig. 2; Table S1) and higher maximum stem height in seedlings growing on soil warming plots relative to soil warming \times N addition, and soil warming \times N addition \times invaded plots (Fig. 2, Table S2). These results highlight that N addition, *A. petiolata* invasion, and both factors in conjunction offset the positive effect of soil warming on aboveground growth.

3.4. Root growth and AMF colonization

Similarly to aboveground growth, we saw a positive effect of soil warming belowground on AMF colonization, but again, the warming

effect disappeared when combined with the *A. petiolata* invasion treatment. While there was no treatment effect on fine root mass (Table 1; Fig. 3), soil warming had a significant positive influence on AMF colonization (Table 1; Fig. 3). Further, there was a negative interaction between soil warming and *A. petiolata* invasion on AMF colonization. Post-hoc Tukey testing showed significantly higher AMF colonization in the soil warming treatment relative to control \times invaded, N addition \times invaded, soil warming \times invaded, and soil warming \times N addition \times invaded treatments (Fig. 3, Table S3). As with aboveground growth, these results highlight that *A. petiolata* invasion reduces the positive effects of warming on AMF colonization in fine roots.

4. Discussion

4.1. Overview

Our findings highlight the importance of considering multiple abiotic and biotic stresses, which can act in conjunction to influence forest plant species. While invasion ecology continues to advance our understanding of interactions between recipient communities and introduced species (Mack et al., 2000), we know of no other studies that have experimentally tested the effects of simulated biological invasion within the context of other ongoing stresses on native forest species. While soil warming on its own augmented aboveground growth and increased AMF colonization in terminal fine roots, these effects were mitigated by chronic N fertilization and a year-long *A. petiolata* invasion. Our results have important implications for performance, potential recruitment and management of *A. rubrum*, an increasingly important species in the temperate deciduous forests of the Northeast.

4.2. Positive growth responses to soil warming

Increased growth early in the life history can be important for long term persistence and recruitment of tree seedlings under increasing environmental stress, particularly for *A. rubrum*. Wyckoff and Clark (2002) demonstrated that low growth rates are linked to mortality in *A. rubrum* and several co-occurring tree species. As in an earlier study examining first year *A. rubrum* seedlings (Wheeler et al., 2016), we found a uniformly positive aboveground growth response to soil warming. This study extends the work of Wheeler et al. (2016), which examined the effects of warming alone on red maple germinants, with data on second-year seedlings as well as their responses to warming in the context of multiple, interacting factors. In the literature, *A. rubrum* growth responses to air and soil warming are relatively mixed: no effect or reduced growth in *A. rubrum* saplings under warming conditions (Farnsworth et al., 1995; Norby and Luo, 2004), positive initial growth responses to warming, followed by higher fine root turnover and mortality (Wan et al., 2004), and increased growth in older trees (Butler et al., 2012). Although not observed in this study, others have

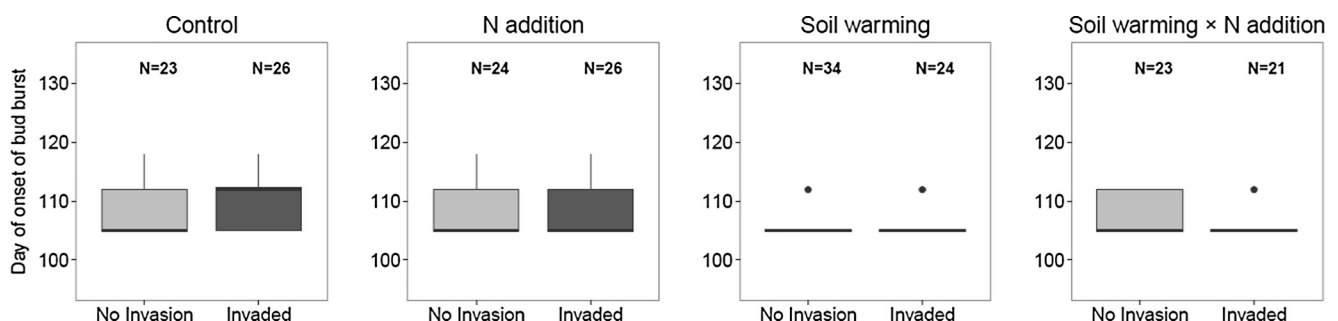


Fig. 1. *Acer rubrum* (red maple) day of onset of bud burst under no invasion (light gray boxes) and under invasion by *A. petiolata* (dark gray boxes) in control (ambient environment), N addition ($50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), soil warming ($+5 \text{ }^\circ\text{C}$ above ambient) and soil warming \times N addition at the Harvard Forest in Petersham, MA, USA. *N* indicates sample size. Post-hoc tests for differences in timing of budburst were not performed as there were no significant treatment effects in the main model.

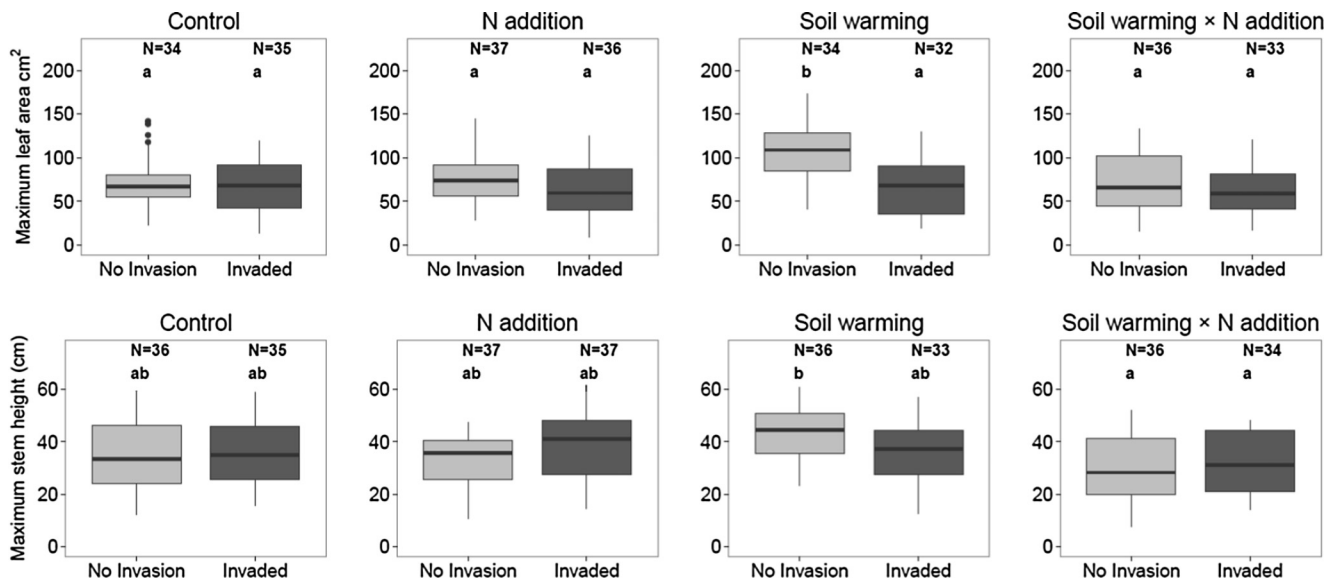


Fig. 2. *Acer rubrum* (red maple) largest average single leaf area (cm²) and maximum stem height (cm) during the growing season under no invasion (light gray boxes) and under invasion by *A. petiolata* (dark grey boxes) in control (ambient environment), N addition (50 kg N ha⁻¹ yr⁻¹), soil warming (+5 °C above ambient), and soil warming × N addition at the Harvard Forest in Petersham, MA, USA. N indicates sample size. Letters indicate significant differences in leaf area and stem height between treatments from post hoc Tukey test pairwise comparisons; treatments with at least one matching letter in the label did not significantly differ from one another, while treatments with non-matched letter labels are significantly different at the p = 0.05 level.

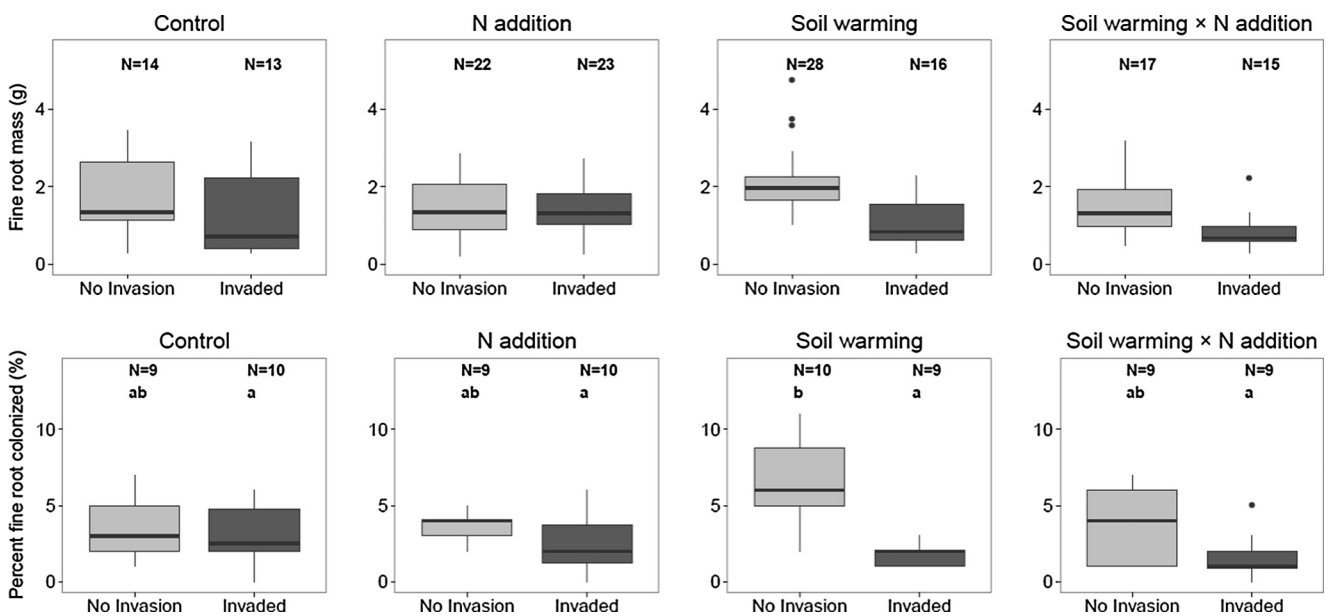


Fig. 3. *Acer rubrum* (red maple) dry fine root mass (g) and percent root colonization by AM fungi under no invasion (light gray boxes) and under invasion by *A. petiolata* (dark grey boxes) in control (ambient environment), N addition (50 kg N ha⁻¹ yr⁻¹), soil warming (+5 °C above ambient), and soil warming × N addition at the Harvard Forest in Petersham, MA, USA. N indicates sample size. Letters indicate significant differences in AMF colonization between treatments from post hoc Tukey test pairwise comparisons; treatments with at least one matching letter in the label did not significantly differ from one another, while treatments with non-matched letter labels are significantly different at the p = 0.05 level. Post-hoc tests for differences in fine root mass were not performed as there were no significant treatment effects in the main model.

demonstrated earlier leaf-out under both soil and air warming (Norby et al., 2003, Kaye and Wagner, 2014, Wheeler et al., 2016), suggesting an earlier start to the growing season as one potential mechanism driving increased growth with warming. Importantly, higher AMF colonization under soil warming may also have impacted growth both above and below-ground. In their review examining mycorrhizal responses to environmental change, Mohan et al. (2014) showed that most studies demonstrate increased mycorrhizal abundance with warming. Although to our knowledge the effect of soil warming on AMF colonization in other tree seedlings has not been examined, in grassland and weedy species both AMF hyphal production and root colonization

increase under warmer conditions, likely through increased photosynthate C allocation to AMF networks (Hawkes et al., 2008; Rillig et al., 2002; Staddon et al., 2004). A similar mechanism in *A. rubrum* seedlings grown in warmer soils, with increased C allocation to root AMF networks, may have led to the observed increased in colonization, leading to increased water and nutrient uptake and thus resulting in the higher overall growth. Forest managers may thus need to consider plant-soil feedbacks and the role of interacting factors, when developing adaptive management plans for tree seedling responses to environmental change. When compared to the literature, our results suggest potential ontogenetic responses to soil temperature in *A. rubrum*

growth, where seedlings may respond more positively to soil warming than saplings. Alternately, local conditions other than air or soil temperature may play a role in determining growth responses to warming. Indeed, as discussed in 4.3 and 4.4, our results also show that soil N availability and invasion by *A. petiolata* can reduce the positive effects of warming on *A. rubrum* seedlings.

4.3. Negative impacts of chronic N fertilization on growth and root colonization by AMF

Acer rubrum has relatively low nutrient requirements (Abrams, 1998), and at the local and landscape scale, individuals have been shown to cluster in forest sites with lower soil N concentrations (Peterjohn et al., 2015). In our study, it appears that N enrichment alone did not benefit *A. rubrum* growth, nor did it act as a stressor, but rather interacted with other factors to affect above-ground growth. Other studies (Canham et al., 1996; Klooster et al., 2012) have shown augmented growth and biomass production in *A. rubrum* seedlings under N enrichment, but this positive effect is influenced by water availability (Canham et al., 1996). Differences in soil water availability in our experimental site relative to other studies may explain why N enrichment alone did not benefit growth. Interestingly, we found that N enrichment in warmed soils, either alone or in conjunction with *A. petiolata* invasion, suppressed the positive responses of *A. rubrum* seedlings, as evidenced by reduced stem elongation and leaf size compared to the warming treatment alone. Since soil warming is known to increase soil N availability through enhanced rates of N mineralization and nitrification (Butler et al., 2012; Melillo et al., 2011), it is possible that in soil warming plots with addition N enrichment, N availability became detrimental to *A. rubrum* growth. Elsewhere, Van Diepen et al. (2007, 2010, 2011) showed multi-year chronic N fertilization reduced both root-level AMF colonization and proportional C allocation in *Acer saccharum* forests, through decreases in AMF abundance and alterations in soil AMF community structure. Likewise, Allen et al. (2016) showed declines in both root colonization and AMF abundance along increasing N gradients in Mediterranean shrub communities. Whether our observations of suppressed growth under warming and nitrogen addition were accompanied by suppression of soil fungi is not supported by our AMF colonization results, but the effects of our treatments on changes in overall soil fungal diversity and function is currently under investigation (Anthony et al. unpublished results).

4.4. Negative impacts of *A. petiolata* invasion on growth and AMF colonization

Suppression of growth and AMF colonization under *A. petiolata* invasion represents an important stress to *A. rubrum* and other northeastern forest seedlings (Barto et al., 2011; Stinson et al., 2006; Stinson et al., 2007). Here we found that invasion also interacts with soil warming to suppress the positive effects on growth and AMF colonization, suggesting that future performance of *Acer rubrum* depends on both the abiotic (warming) and biotic (invasion) environment. Recently, Hale et al. (2016) found invasion by *A. petiolata* reduced biomass in the AMF-dependent spring ephemeral, *Maianthemum racemosum*. They suggested two potential mechanisms driving this biomass reduction: first, that compromised root fungal networks reduced water uptake, and second, that high energy costs for re-establishing root fungal networks served to reduce the carbon pool available for plant growth. The reduced aboveground growth we observed in *A. rubrum* seedlings grown under *A. petiolata* invasion on the soil warming \times N addition treatments, and the overall lower AMF colonization under invasion, is consistent with this idea; i.e., suppression of mycorrhizae may have interacted with warmer temperatures to induce water stress, potentially reducing the carbon pool available for growth processes. Our findings suggest that suppression of AMF by *A. petiolata* invasion mitigates the growth enhancement in *A. rubrum* seedlings observed

under warming (Wheeler et al., 2016). Given that *A. petiolata* is also known to suppress arbuscular mycorrhizal colonization and growth in other forest species, our findings may also extend to seedlings of *A. saccharum*, *Fraxinus americana* (Stinson et al., 2006), or other understory plants including *M. racemosum* (Hale et al., 2016). The interactive effects of abiotic stresses and invasion by *A. petiolata* on ectomycorrhizal species, such as *Pinus strobus* and *Quercus rubra*, which are also known to be suppressed by *A. petiolata* (Wolfe et al., 2008), remains an open research area.

4.5. Interactions between abiotic stress and invasion may alter future forests

Understanding the interactive effects of biological invasions and concurrent abiotic stressors represents a critical gap in invasion ecology and adaptive ecosystem management. Invasion and shifts in abiotic conditions can impact growth and performance of native species, including forest tree seedlings, early in their life history (e.g. Petrie et al., 2016; Stinson et al., 2006; Wheeler et al., 2016). Abiotic stressors as a result of global change in the forests of northeastern North America, in conjunction with biological invasions, thus have the potential to alter seedling recruitment patterns at the local and landscape scale. Our results show that a decade of chronic N fertilization, and perhaps more importantly, even a relatively short and low-density *A. petiolata* invasion (1 year) can interact with local temperature conditions to significantly reduce growth in young *A. rubrum*, in addition to AMF colonization in fine roots. The fact that we detected differences in growth and AMF colonization within a single year, even after recent transplant and root pruning, points to the importance of invasion in conjunction with abiotic stressors in the early life stages of *A. rubrum*. Since the impacts of *A. petiolata* invasion on soil microbial communities can persist for years, even after the invasion has been eradicated (Lankau et al., 2014), our results highlight the importance of even short-term invasion dynamics, in conjunction with local abiotic conditions, as a driver of tree species performance in the early life stages, even as other microclimatic conditions change. In many cases, *A. petiolata* invasions persist unchecked for years in the forest understory, thus raising the need for similar studies with a sustained invasion treatment. As this study only covered a period of one year, we must extrapolate these responses to older seedlings and saplings with care: for example, it is possible that older individuals could acclimate to environmental conditions and stressors. However, it is also possible that differences in performance could be magnified as stress factors continued to reduce growth and competitive abilities, which could ultimately lead to enhanced mortality (Wyckoff and Clark, 2002). At minimum, we argue that soil warming, N enrichment and invasion can have strong impacts at the seedling stage for *A. rubrum*.

Further, while not examined directly in this study, N deposition and warming could also affect growth and secondary chemical production in *A. petiolata*. While there is some research examining how growth and secondary chemical production in *A. petiolata* respond to changing abiotic conditions like temperature, CO₂ and nutrient availability (Anderson and Cipollini, 2013; Hewins and Hyatt, 2010; Meekins and McCarthy, 2000), a clear understanding of how interacting abiotic factors influence growth and phytochemistry in this invasive species would help project how invasion dynamics in forest understories may shift under climate change. Inter-annual variation in other climatic factors, such as precipitation, can influence tree seedling growth, survival and mycorrhizal colonization (Petrie et al., 2016) and are not addressed in this single-year experiment. Interactions with other biological stressors, such as invasive earthworms or increasing deer densities (Davalos et al., 2015; Kalisz et al., 2014) could also affect our findings and warrant further study. In the long-term, canopy recruitment depends on species-level performance and biotic interactions during the early life history stages (Pacala et al., 1996). Overall, while 5 °C soil warming strongly benefits *A. rubrum* seedlings through augmented growth and increased mycorrhizal colonization, soil N

enrichment and the residual effects of an *A. petiolata* invasion significantly mediate this positive response. Our results thus provide insights into potential changes on forest tree seedling recruitment, with future warming potentially increasing early performance of *A. rubrum* in sites without *A. petiolata* invasion, as well as the potential for declines in forests affected by long-term N deposition. Such data for *A. rubrum* and other important trees of the Northeast are potentially useful for modeling future scenarios of forest cover (Thompson et al., 2014). We also suggest that additional research is needed on the combined effects of abiotic changes and biological invasion, broadly speaking, on key plant species in a broader range of forest types, and in other ecosystems.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foreco.2017.08.010>.

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