Title: Invader disruption of belowground plant mutualisms reduces carbon acquisition and alters allocation patterns in a native forest herb

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Summary (200 word limit)

- Invasive plants impose novel selection pressures on naïve mutualistic interactions between native plants and their partners. Since most plants critically rely on root fungal symbionts (RFS) for soil resources, invaders that disrupt plant-RFS mutualisms can significantly depress native plant fitness. Here, we investigate the consequences of RFS mutualism disruption on native plant fitness in a greenhouse experiment with a forest invader that produces known anti-fungal allelochemicals.
- Over five-months, we regularly applied 1) green leaves of the allelopathic invader *Alliaria petiolata,* 2) a non-systemic fungicide to simulate *Alliaria*'s effects, or 3) green leaves of non-allelopathic *Hesperis matronalis* (control) to pots containing the native *Maianthemum racemosum* and its RFS. We repeatedly measured *Maianthemum* physiology and harvested plants periodically to assess carbon allocation.
- *Alliaria* and fungicide treatment effects are indistinguishable: we observe significant inhibition of the RFS soil hyphal network and significant reductions in *Maianthemum* physiology (photosynthesis, transpiration, conductance) and allocation (carbon storage, root biomass, asexual reproduction) in both treatments relative to the control.
- Our findings suggest a general mechanistic hypothesis for local extinction of native species in ecosystems challenged by allelopathic invaders: RFS mutualism disruption drives carbon stress, subsequent declines in native plant vigor, and if chronic, declines in RFS-dependent species abundance.

Key Words: mutualism disruption, invasion, *Alliaria petiolata,* allelopathy, root fungal symbionts, physiology, carbon allocation

Introduction

Mutualisms intimately link the success of partner species together. Consequently, declines in one partner can profoundly affect the fitness of the other. A critical mutualism for most terrestrial plants involves root fungal symbionts (RFS), such as mycorrhizae and dark septate endophytes. Plants provide their symbionts with carbon, while RFS enhance the plant resource status (Parniske, 2008; Jumpponen, 2001; Newsham, 2011). For plants, the resources received are nontrivial – arbuscular mycorrhizal fungi (AMF) can alleviate plant water stress (Augé, 2001) and provide up to 80% of plant phosphorus and 25% of plant nitrogen (Marschner & Dell, 1994). Similarly, dark septate endophytes can increase shoot phosphorus and nitrogen up

to 100% relative to controls (Newsham, 2011). Because plant physiological activity is intimately tied to resource status (Wright *et al.*, 2004), even short-term disruption of RFS function can have dramatic impacts on plant growth (e.g. Stinson *et al.*, 2006) and photosynthesis (e.g. Hale *et al.*, 2011).

The rapid pace of global change (Millenium Ecosystem Assessment, 2005) creates growing concern for the stability of plant mutualisms (Staddon *et al.*, 2003; Memmot *et al.*, 2007; Tylianakis *et al.*, 2008; Kiers *et al.*, 2010; Aslan *et al.*, 2013). Specifically, exotic plant invasion is an important disrupter of pollination, seed dispersal, and microbial mutualisms (reviewed by Traveset & Richardson, 2014). Several invasive plants produce novel biochemical weapons (i.e. allelopathic compounds; Callaway & Ridenour, 2004) that are toxic to RFS (see Cipollini *et al.*, 2012; e.g. *Tamarix* sp., Meinhardt & Gehring, 2012; *Sisymbrium loeselii*, Bainard *et al.*, 2009; *Alliaria petiolata*, Roberts & Anderson, 2001; Stinson *et al.*, 2006; Callaway *et al.*, 2008; Cantor *et al.*, 2011). To explore RFS mutualism disruption, we focus on one of these allelopathic invaders, *Alliaria petiolata* (Brassicaceae, garlic mustard), a prominent invader of forest understories throughout northeastern North America (reviewed in Rodgers *et al.*, 2008). *Alliaria* produces glucosinolate-derived compounds that are bioactive and exhibit anti-fungal properties (e.g. allyl isothiocyanate derived from sinigrin; Vaughn & Berhow, 1999).

Prior work has established the potency of *Alliaria*'s allelochemicals: Field concentrations inhibit mycorrhizal spore germination and the growth of fungal hyphae in soil (Cantor *et al.*, 2011) while single applications of fresh *Alliaria* tissue depressed soil respiration rates and the physiology of an RFS-dependent native herb in the field (Hale *et al.*, 2011). Declines in native perennial herb (Rodgers, 2008) and tree seedling abundance (Stinson *et al.*, 2007) have been documented in *Alliaria* invaded forests. Further, at the community level, native plant biodiversity is negatively correlated with *Alliaria* density (Stinson *et al.*, 2007). These results are consistent with mutualism disruption as the majority of forest trees, shrubs, and herbs form associations with RFS (Brundrett & Kendrick, 1988; Whigham, 2004). Thus, the success of this invader has in large part been attributed to its allelopathic effects on RFS (Roberts & Anderson, 2001; Stinson *et al.*, 2006; Callaway *et al.*, 2008; Lankau *et al.*, 2009, Lankau, 2010, Cantor *et al.*, 2011). Data from these studies assume a causal chain between *Alliaria* mutualism disruption and native plant population declines. However, our mechanistic understanding of the intermediate links in this causal chain - if and how mutualism disruption affects native plant physiological

processes and carbon balance over the long-term and the ultimate outcome of sustained mutualism disruption for individual plants or populations - remains rudimentary.

We developed a cost:benefit model for native plants associating with RFS in the presence or absence of an allelopathic invader (Fig. 1). We hypothesize that if allelochemicals inhibit the RFS soil hyphal network, plants will receive diminished resource benefits and experience physiological declines but pay the continuing costs of maintaining internal RFS structures. Root colonization rates of forest herbs are known to be unaffected by *Alliaria* invasion (Burke, 2008). Thus, high maintenance costs are expected for these plant species because their internal RFS structures have low turnover rates and persist for several months in their long-lived roots (Brundrett & Kendrick, 1990). Re-establishment of the hyphal network is expected to be a high priority in species obligately-dependent on RFS. Repeated carbon allocation to fungi for re-growth of the external hyphal network is anticipated to further raise carbon costs. The high cost of supplying already limited carbon to a malfunctioning mutualist is expected to negate the benefits received by a plant (*sensu* Johnson *et al.*, 2015) and could profoundly influence its carbon budget and allocation strategy. This model yields three nested predictions: 1. *Alliaria*'s allelochemicals inhibit the *external* hyphal network. 2. Loss of this network will translate into diminished plant physiological activity. 3. Long-term declines in physiological activity and high carbon demands to re-establish RFS function will reduce plant carbon allocation to fitness-related traits.

Here, we report novel results from a long-term mutualism disruption experiment using the native forest herb *Maianthemum racemosum* (hereafter *Maianthemum*). We exposed *Maianthemum* to one of three treatments: 1) *Alliaria* allelochemicals delivered through applications of fresh leaves onto the soil of potted plants; 2) application of a non-systemic fungicide with no known phytotoxic effects (Petit *et al.*, 2012) to simulate *Alliaria*'s chemical effect (positive control); 3) applications of fresh *Hesperis matronalis* (Brassicaceae) leaves (negative control). We selected *Hesperis* for our negative control because it is an invasive mustard and RFS have been detected in its roots (DeMars & Boerner, 1995) indicating a negligible allelopathic effect. We quantified soil RFS hyphal density, plant physiological activities, and plant carbon allocation in the three treatment groups.

Materials and Methods

Native Species

Maianthemum is a common perennial in *Alliaria*-invaded forests (e.g. Burke, 2008; Rodgers, 2008) with long-lived coarse roots (Brundrett & Kendrick, 1988) and high RFS dependence (colonization rates by AMF 76-94%; Brundrett & Kendrick, 1988; Burke, 2008; and dark septate endophytes; Hough, 2008). *Maianthemum* also exhibits no change in internal RFS colonization rates (Burke, 2008) but declines in flowering frequency in the presence of *Alliaria* (Brouwer *et al.*, 2015).

Experimental Design

Sixty-three dormant *Maianthemum* were procured (Source: Prairie Moon Nursery, Winona, MN; grown in raised beds with no pesticides), weighed and potted in a 3:1 mixture of autoclaved Fafard potting soil (Conrad Fafard Inc. Agawam, MA), Turface (Profile Products LLC, Buffalo Grove, IL) and 150 g of forest soil to ensure inoculation with RFS (pot volume: $3.5x10^{-3}m^3$). Plants were placed in a greenhouse with 2 layers of 65% shadecloth, where they experienced light levels approaching saturation for *Maianthemum* (~115-117 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at midday; Hale *et al.*, 2011) and were watered every 2-4 weeks, as needed (pH of water: 6.6).

Plants were randomly assigned to *Alliaria*, *Hesperis*, or non-systemic fungicide treatments. For the *Alliaria* and *Hesperis* treatments, we placed 25 g of fresh leaves from either species onto the soil of each pot on 11 June 2010. Leaves were re-applied every 2 weeks through 20 August. The mechanism of *Alliaria*'s allelochemical release into the soil is uncertain, but both root exudation and aboveground decomposition have been suggested (Stinson *et al.*, 2006). Our previous work suggests that plant decomposition may be a particularly important route for allelochemical delivery as our repeated assays for allelochemical in field soils across the growing season were only positive at the time of *Alliaria* leaf senescence (Cantor *et al.*, 2011). Therefore, our greenhouse treatment mimics a relevant biological process, with the ultimate goal of delivering biologically relevant concentrations of the allelochemicals into the soil of the pots. We verified that the *Alliaria* treatment effectively delivered allelochemicals by testing for the presence of sinigrin (the pre-cursor of *Alliaria*'s anti-fungal allelochemical allyl isothiocyanate, Vaughn & Berhow, 1999) using HPLC (see Supporting Information Methods). We found that the *Alliaria* treatment delivered sinigrin (Fig. S1) at concentrations consistent with those detected in the field (Cantor *et al.*, 2011). Fungicide-treated plant received monthly applications of either

Chipco 26019 (~50% (w/w) iprodione) or OHP 26 GT-0 (~23.3% (w/w) iprodione) at a rate of ~0.1 g active ingredient/plant.

Physiology

Baseline physiological rates were measured prior to treatments and used as covariates in all analyses. Post-treatment assessments were made one week after the first treatment and repeated weekly for five weeks. This timeframe is appropriate because the bulk of annual carbon acquisition of forest herbs is completed within ≤6 weeks (Neufeld & Young, 2014).

Single leaf gas exchange measures were made using a LI-COR 6400 infrared gas analyzer (IRGA; LI-COR Inc., Lincoln, NE, USA) at 25°C, 40-50% relative humidity, 400 μmol CO_2 mol^{-1} air. Each plant was measured in random order between 0900 and 1600 h at a saturating irradiance of 600 μmol photons$\cdot m^{-2}\cdot s^{-1}$ (Hale *et al.*, 2011). We recorded light saturated photosynthetic rate (A_{sat}), transpiration rate (E) and stomatal conductance to water vapor (g_{sw}) every 15 seconds for one minute.

To test for treatment effects on physiological traits, we used repeated measures linear mixed effects models (main effects: treatment, week of measurement (time), treatment×time interaction; covariates: air temperature, relative humidity, baseline physiological rates, initial plant wet mass). Data were transformed when necessary to meet the assumptions of normality. We selected the best covariance structure based on AIC.

Nutrient/carbohydrate concentration and allocation

Non-experimental plants (N = 4) were harvested prior to treatments to assess early season nutrient content and allocation patterns. Experimental plants were then harvested at three post-treatment time points (N = 6-8 plants/treatment/harvest): 9 July, 6 August, and at senescence (i.e. when >40% of their leaf tissue yellowed and light saturated photosynthetic rate A_{sat} was <1.0 μmol$\cdot m^{-2}\cdot s^{-1}$). We recorded the wet mass of roots at each harvest and a subset of roots from 10 plants (3 from the fungicide treatment, 3 from *Alliaria*, and 4 from *Hesperis*) were stained (Brundrett *et al.*, 1984) and examined under 200× magnification to confirm the presence of intact internal RFS structures. Leaf and the remaining root samples were dried at 70°C for 5-6 days. Rhizomes were flash-frozen in liquid nitrogen and lyophilized to a constant weight. Dried leaf, root, and rhizome samples were weighed and ground in a Wiley mill. Since bud formation in *Maianthemum* is not complete until the end of the growing season (LaFrankie, 1985), buds were counted only at the last harvest. For nitrogen and phosphorus analysis, leaf and rhizome samples

were sent to the Penn State Agricultural Analytical Services Laboratory (University Park, PA, USA). For carbohydrate analysis, rhizome samples were analyzed using HPLC to determine their fructan and soluble sugar content (Zuleta & Sambucetti, 2001). We report inulin and sucrose concentrations as they were the most abundant forms of carbohydrates in the rhizome throughout the season. All nutrient and carbon concentrations are expressed as a % of the sample dry mass.

We tested for significant treatment effects on leaf and rhizome nutrient concentration using two-way analysis of covariance (ANCOVA; model: trait = treatment + harvest date + treatment×harvest date + initial plant wet mass). For allocation to asexual reproduction, we used a one-way ANCOVA (model: bud number = treatment + initial plant wet mass).

Because inulin and sucrose concentrations ($r = -0.55$; $P < 0.0001$), rhizome and root mass ($r_s = 0.47$, $P = 0.0001$), and shoot mass and leaf area ($r = 0.81$, $P < 0.0001$) are highly correlated, we first used a multivariate analysis of covariance (MANCOVA) to test for an overall treatment effect (model: trait1, trait2 = treatment + harvest date + treatment×harvest date + initial plant wet mass). For each MANCOVA, we report F-statistics for Roy's greatest root (Scheiner, 2001). If a significant treatment effect was found by MANCOVA, we used two-way ANCOVA – and if significant, pairwise comparisons – to determine treatment effect on individual traits.

Soil colonization by fungal hyphae

Soil hyphal abundance was quantified following Baláz and Vosátka (2001). Mixed cellulose ester membranes (Millipore; pore size = 45μm) were inserted into each pot two weeks after the first treatment applications. We collected membranes at the first two post-treatment harvests only. [Note: Because plants senesced on different dates, membranes at final harvest are not comparable.] Membranes were stained and hyphal length determined following Cantor *et al.* (2011). We summed the hyphal length from all images/membrane to estimate hyphal length/membrane (mm), our proxy for soil hyphal abundance.

Robust regression provides estimates of means and standard errors for small sample sizes that are less affected by heterogeneous variance, non-normal residuals, and potential outliers (Wilcox, 1998). To test for a significant treatment effect on soil hyphal colonization, we used a robust regression (model: hyphal abundance = treatment + harvest date + treatment×harvest date) with bi-square MM-estimator using the R package *robustbase* (Rousseeuw *et al.*, 2015). Planned contrasts were performed between *Hesperis* and the two other treatments by pooling data across harvest dates (June and July) for each treatment.

Results

Evidence for mutualism disruption

We find that 100% of the *Maianthemum* plants examined are colonized by RFS (i.e. contain intact, diagnostic fungal structures inside of the roots; Fig. S2). However, the abundance of RFS hyphae outside the roots differs significantly across treatments ($\chi^2_{2,35} = 7.54$, $P = 0.023$). Linear contrasts reveal that membrane hyphal lengths in the *Hesperis* treatment are significantly greater than in the *Alliaria* and fungicide treatments ($z = 2.16$, $P = 0.031$). *Alliaria* and fungicide treatments are not different ($P = 0.56$, see Table 1 for raw means).

Physiology

Overall, physiological activities of *Maianthemum* from both the *Alliaria* and fungicide treatments are significantly and similarly reduced relative to the *Hesperis* treatment (Fig. 2). Compared to the *Hesperis* treated plants, plants in the *Alliaria* and fungicide treatments exhibit significantly lower photosynthetic rates (A_{sat} declines by 15% and 17%, respectively; $P = 0.031$, $P = 0.019$; Fig. 2a), stomatal conductance (g_{sw} declines by 23% and 28%, respectively; $P = 0.009$, $P = 0.001$; Fig. 2b) and maximum transpiration rates (E declines by 18% and 21%, respectively; $P = 0.020$, $P = 0.008$; Fig. 2c). A significant treatment×week effect is also evident for each of the physiological traits (Table S1). This can be attributed to declines in physiological function in weeks 4 and 5 for plants in the fungicide treatment. While the *Alliaria* treated plants do not exhibit the same decline in those weeks, over time their physiological functions was consistently lower than that of plants in the *H. matronalis* control. See Table S1 for a full summary of results from mixed model analyses of physiological traits.

Foliar and rhizome nutrient concentrations

Across all treatments, there is no significant difference in foliar or rhizome nitrogen (Fig. S3; $P = 0.11$ and $P = 0.37$, respectively) or foliar or rhizome phosphorus concentrations ($P = 0.23$ and $P = 0.96$, respectively). Because all of *Maianthemum*'s leaves are pre-formed eight to twelve months prior to emergence (LaFrankie, 1985), we did not expect treatment effects on foliar nutrient concentrations. Furthermore, our ability to detect treatment effects on rhizome nutrient concentration may be obscured by the ability of *Maianthemum* to resorb nitrogen and phosphorus from its leaves at the end of the growing season (DeMars & Boerner, 1997). Indeed, we find a significant effect of harvest date on all resource traits (foliar nitrogen $P < 0.0001$; foliar

phosphorous $P < 0.0001$; rhizome nitrogen $P < 0.0001$; rhizome phosphorous $P = 0.003$). Foliar nutrient concentrations slowly decline across the growing season and rhizome nutrient concentrations increase, indicating resorption.

Carbon allocation

Starch is not detectable in *Maianthemum* rhizomes. Instead, two major carbohydrates predominate: inulin, a storage carbohydrate, and sucrose, a mobile sugar. Rhizome inulin and sucrose concentrations are negatively correlated ($r = -0.55$; $P < 0.0001$). Treatment significantly affects total non-structural carbon concentrations in the rhizome (Roy's greatest root = 0.32, $P = 0.001$). Specifically, our treatments influence both inulin ($F_{2,55} = 5.42$, $P = 0.007$) and sucrose ($F_{2,55} = 7.52$, $P = 0.001$) concentrations. *Maianthemum* in the *Alliaria* treatment store 17% less inulin relative to the *Hesperis* control (Fig. 3a), but have significantly greater sucrose concentration (Fig. 3b). Fungicide treated plants do not show reduced rhizome inulin (Fig. 3a) and their sucrose content is intermediate between the *Hesperis* and *Alliaria* treatments (Fig. 3b).

Treatment also significantly alters allocation to belowground structures (Roy's greatest root = 0.43, $P = 0.0001$), which is driven by changes in root mass ($F_{2,54} = 9.40$, $P = 0.001$), not rhizome mass ($F_{2,54} = 0.22$, $P = 0.80$). Plants from both the *Alliaria* and fungicide treatments produce ~25% less root mass than the *Hesperis* controls (Fig. 3c). Our analyses show no significant treatment effect on shoot mass ($F_{2,54} = 0.66$, $P = 0.52$) or leaf area ($F_{2,52} = 0.04$, $P = 0.96$). Due to leaf preformation in *Maianthemum* (LaFrankie, 1985), we did not expect responses in aboveground tissues to our experimental treatments.

Treatment has a marginally significant effect on asexual reproduction ($F_{2,15} = 3.01$, $P = 0.079$). On average, *Maianthemum* in the *Hesperis* control produce two more asexual buds compared to the *Alliaria* treatment; fungicide treated plants produce intermediate bud numbers (Fig. 3d).

Discussion

Our results mechanistically link allelopathic mutualism disruption of root fungal symbionts (RFS) to plant fitness declines and provide clear support for the predictions of our model (Fig. 1).

Prediction 1. Soil hyphal length was reduced in the *Alliaria* and fungicide treatments relative to the *Hesperis* control (Table 1). These results corroborate the findings of Hale *et al.*

(2011) and Cantor *et al*. (2011), which showed that a similar *Alliaria* treatment reduced soil respiration rates around *Maianthemum* and that soil hyphal abundance was lower in *Alliaria* invaded field sites, respectively.

Prediction 2. *Alliaria* treatment significantly diminishes plant physiological activities (Fig. 2). In fact, *Alliaria* treated plants displayed nearly identical patterns of physiological suppression to those observed in the non-systemic fungicide treatment. The observed declines in *Maianthemum* physiological activity following loss of the soil RFS network could be the result of declines in RFS-delivered benefits, including plant nutrition, water availability, and/or changes in sink strength (Hale *et al.*, 2011). However, foliar and rhizome nitrogen and phosphorus concentrations did not differ among treatments (Fig. S3). Thus, nutrient limitation is not responsible for the reductions in *Maianthemum* physiological activities, but could become important if the experiment continued for multiple growing seasons. The significant photosynthetic and stomatal conductance declines in *Maianthemum* seen here point to reductions in water availability (Augé, 2001) and are consistent with our previous field study (Hale *et al.*, 2011).

Prediction 3. RFS mutualism disruption manifested as changes in carbon allocation in *Maianthemum* plants. Significantly lower inulin concentrations, root mass and asexual reproduction in the *Alliaria* treatment are consistent signatures of carbon limitation (Fig. 3a,c,d). Reduced root mass with disrupted RFS is a somewhat surprising result, as other forest herbs have shown the opposite outcome (i.e. increased root growth) when RFS are disrupted (e.g. Lapointe & Molard, 1997). However, fungicides have been shown to reduce root length in some mycorrhizal plants (e.g. Sukarno *et al.*, 1993). The reduced root mass seen in our study further supports the idea that *Alliaria* and fungicide treatments cause carbon limitation in *Maianthemum*.

Comparison of leaf longevity and carbon storage across treatments provides insight into the carbon stress observed here. Plants in the *Alliaria* treatment senesced, on average, seven days later than those in the *Hesperis* treatment (data not shown). This observation is consistent with other studies of forest herbs that show increased leaf longevity in response to photosynthetic rate suppression and carbon stress (Muraoka *et al.*, 1997; Tomimatsu & Yoshimichi, 2008). Further, in just a single growing season, inulin concentrations in rhizomes of *Alliaria* treated plants were 17% lower (Fig. 3a) relative to the *Hesperis* controls. This large magnitude decline mirrors the

observed decline in physiological traits (Fig. 2) and should affect plant fitness in the long run if RFS disruption is chronic as in heavily invaded forests.

Interestingly, in addition to reducing storage carbohydrates, the *Alliaria* treatment significantly increased mobile sucrose concentrations (Fig. 3b). We offer two potential, non-exclusive mechanisms for this contrasting result. First, sucrose may be in transit to the internal root symbionts for re-establishment of the external soil hyphal network. Sucrose is the precursor to the hexose sugars that are exchanged with AMF arbuscules in roots (Parniske, 2008). Repeated inhibition of the external hyphal network as it is regrown and disrupted by *Alliaria* could create an open-ended carbon sink further reducing carbon storage and increasing carbon stress. While such an open-ended sink is expected to up-regulate photosynthesis and carbohydrate production, we observe a decrease in photosynthetic rates following disruption of the soil hyphal network in the *Alliaria* treatment. Our results point to limited water availability constraining *Maianthemum's* photosynthetic capacity and suggest an inability of *Alliaria* treated plants to respond to the increased sink strength. Second, the observed sucrose concentration could result from changes in gene expression. Sugar produced via photosynthesis acts as both a substrate for construction and as a signal that modulates gene expression in plants (Smeekens, 2000; Rolland *et al.*, 2006). In general, when sugars from photosynthesis are scarce, genes controlling storage and growth are repressed, while genes controlling photosynthesis, nutrient mobilization, and export are enhanced (Rolland *et al.*, 2006). It is possible that the low carbon fixation rates we observed in *Alliaria* and fungicide-treated *Maianthemum* could repress genes directing carbon storage and root growth. Similarly, increased sucrose availability in the rhizome (Fig. 3b) could be explained by enhanced expression of export genes. The possibility for invaders to alter gene expression in native plants by mutualism disruption is an intriguing possibility that, to our knowledge, is completely unexplored. This avenue of research warrants future study, as it could lead to a deeper mechanistic understanding of the processes underlying the impacts of mutualism disruption.

Finally, we note that the results of our study could be due to direct, phytotoxic effects of *Alliaria* on *Maianthemum*. However, *Alliaria*'s effects were significantly greater than the fungicide treatment for only a single trait (inulin, Fig. 3a) and slightly, but not significantly, greater than the fungicide treatment for only two traits measured (sucrose, Fig. 3b, and asexual reproduction, Fig. 3d). In all other comparisons, *Alliaria* and the non-systemic fungicide

355 treatments were statistically indistinguishable and responded in a similar fashion. While direct
356 allelopathic effects may explain slight differences between these treatments, the tandem declines
357 in soil hyphal abundance in both *Alliaria* and fungicide treatments point to loss of the mutualism
358 function as the key effect of the allelochemicals.
359 Many herbaceous species in northeastern North American temperate hardwood forests
360 possess similar life histories (Bierzychudek, 1982; Whigham, 2004; Jolls and Whigham, 2014),
361 growth patterns, and root traits (i.e. lack fine roots, exhibit 2–4 orders of root branching, and
362 sustain long-lived fungal structures inside their roots (Fig. S2; Brundrett & Kendrick, 1988)). We
363 suggest that many species will be highly susceptible to RFS disruption following *Alliaria*
364 invasion (e.g. species in the genera *Maianthemum*, *Polygonatum*, *Trillium*, *Sanguinaria*,
365 *Arisaema*, *Erythronium*, *Asarum*, *Allium*, and others). If the allocation changes observed by
366 *Maianthemum* in the *Alliaria* treatment are multiplied over many growing seasons in the field,
367 the ability of native plants to compete and tolerate additional environmental stresses could be
368 compromised. Declines in hyphal length, as observed here and under field conditions (Cantor *et*
369 *al.*, 2011), and our observed declines in root mass (Fig. 3c) indicate that on *Alliaria* invaded
370 sites, plants that rely on belowground mutualisms will be less able to forage and compete for soil
371 resources and water. Additionally, the observed declines in inulin storage (Fig. 3a) suggest that
372 plants on invaded sites may be unable to store sufficient quantities of carbohydrates and tolerate
373 additional stresses, such as high herbivore pressure. For example, white-tailed deer (*Odocoileus*
374 *virginianus*) can consume up to 100% of flowering plants in *Maianthemum* populations annually
375 (Ruhren & Handel, 2003; Kraft *et al.*, 2004). High concentrations of storage carbohydrates are
376 critical for herbaceous perennials to survive such repeated herbivory episodes (Lapointe *et al.*,
377 2010). Lastly, clonal reproduction, which is linked to bud number, is likely important in
378 maintaining population growth in many forest herb species (Honnay *et al.* 2005), which exhibit
379 slow growth and low germination rates (Bierzychudek, 1982; Whigham, 2004; Jolls and
380 Whigham, 2014). Overall, mutualism disruption by *Alliaria* could lead to population decline and
381 may generally explain the reduced abundance of native plants on invaded sites (Rodgers, 2008;
382 Stinson *et al.*, 2007).
383 In summary, we have shown that allelochemicals from a widespread invader reduce
384 fungal hyphal growth in the soil and may alter the carbon fixation capacity and allocation
385 patterns in native plants. Reduced water absorption leads to persistent declines in plant

photosynthetic rates, and this source limitation coupled with an increased RFS sink demand culminates in reduced allocation to functionally important traits like storage, growth, and reproduction. Ultimately, these impacts could facilitate further invasion of the ecosystem by reducing the competitive ability of mutualism dependent native plants and compromise their ability to respond to other environmental stressors. Many exotic and invasive species produce novel chemicals that are toxic to root fungal symbionts (e.g. Bainard *et al.*, 2009; Meinhardt & Gehring, 2012), yet the majority remain untested for allelopathic potential. Allelopathic mutualism disruption may be an important, but under-recognized, mechanism contributing to ecosystem invasion. As the number of invasive species and their corresponding impacts continue to increase, studies of invasion employing the powerful tools of eco-physiology may be critical in revealing invasion mechanisms and informing management decisions.

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Table 1. Raw means and log-transformed means from robust regression for soil fungal hyphal length/membrane (mm) in each treatment at June and July harvests. Each treatment has 6-7 replicates comprised of pots containing one *Maianthemum racemosum* individual and one membrane. Because plants were harvested on different dates for the last harvest in August, hyphal data were not comparable.

			Raw data		**Log-transformed data from robust regression**	
Harvest	**Treatment**	**N**	**Mean**	**SE**	**Mean**	**SE**
June	*Hesperis*	7	2.43	0.60	0.70	0.25
	Fungicide	7	3.59	1.23	1.06	0.51
	Alliaria	7	1.71	0.83	0.01	0.26
July	*Hesperis*	6	19.51	4.46	2.82	0.31
	Fungicide	7	6.38	1.37	1.70	0.26
	Alliaria	7	12.11	2.72	2.34	0.30

Figure 1. Hypothetical model depicting the costs and benefits to the plant of supporting root fungal symbionts in A) the absence of *Alliaria* and B) in the presence of *Alliaria*. Solid arrows represent carbon costs; dashed arrows represent resource benefits. Thickness of arrows represents the magnitude of the costs and benefits.

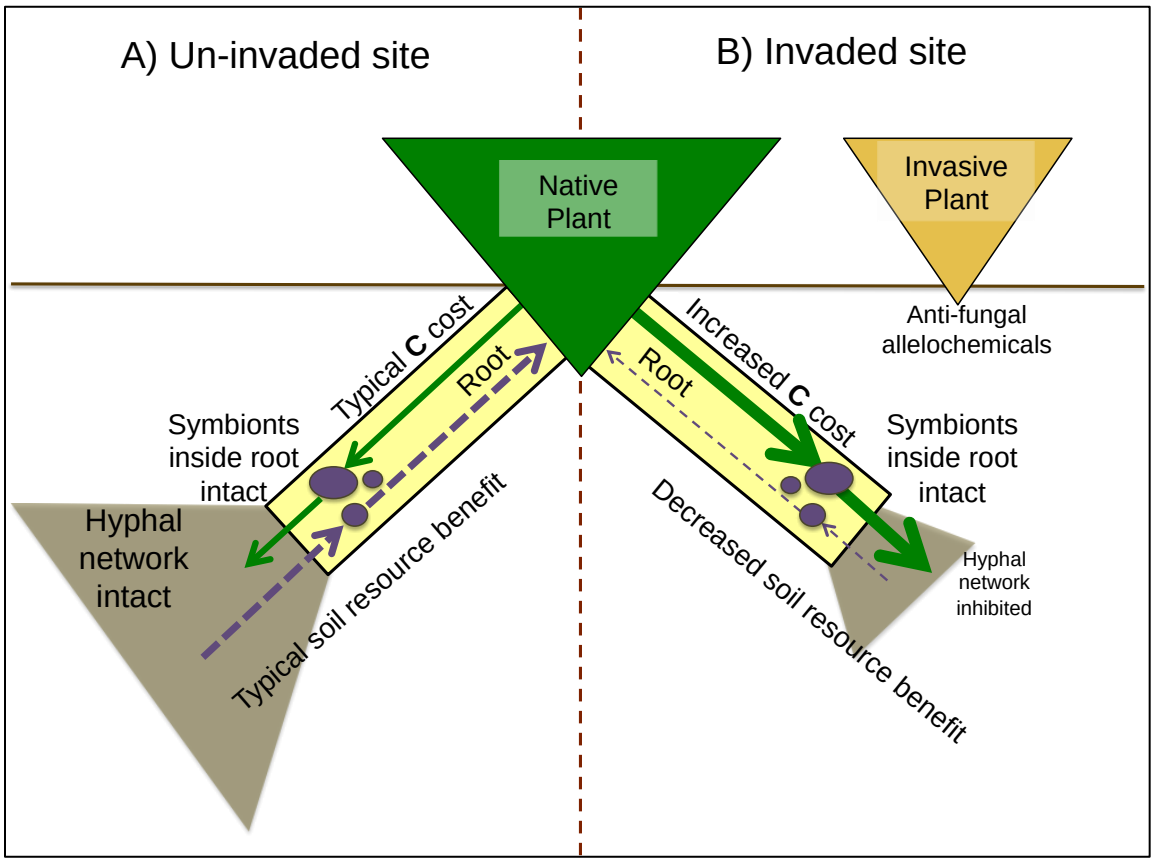

Figure 2. Five-week average physiological performance of *Maianthemum* plants exposed to *Hesperis*, fungicide or *Alliaria* treatments. A) Light saturated photosynthetic rate (A_{sat}), B) stomatal conductance (g_{sw}), and C) transpiration rate (E). Different letters are significantly different ($P < 0.05$; values are least squares means ± 1 standard error).

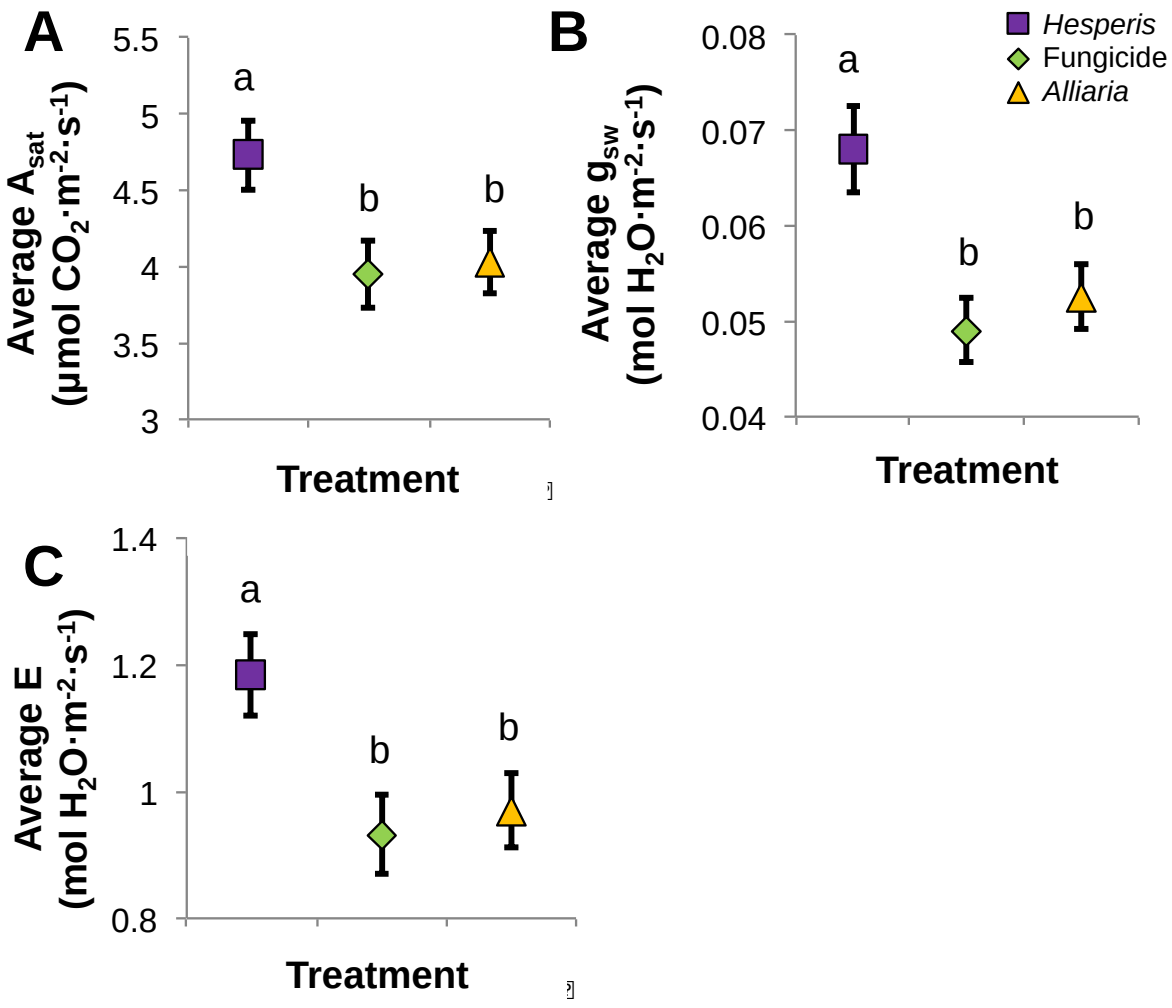

Figure 3. Average carbon allocation in *Maianthemum* plants exposed to *Hesperis*, fungicide or *Alliaria* treatments. Carbon can be stored as inulin (A), maintained in a mobile form as sucrose (B), allocated to root mass (C), or allocated to asexual reproduction (D). Asexual reproduction was measured as new bud production along the rhizome at the last harvest. As determined by pairwise comparisons, treatments with different lowercase letters are significantly different from each other ($P < 0.05$; values are least squares means ± 1 standard error).