**“Negative effects of an allelopathic invader on native plant species’ carbon assimilation are driven by changes in water use”**

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**Abstract**

Allelopathic plant invasion reorganizes belowground arbuscular mycorrhizal communities, which may have important consequences for coexisting native plant resource provisioning, uptake, and allocation to organs that support primary productivity and reproduction. Recent work has suggested that changes in arbuscular mycorrhizal community composition due to allelopathic plant invasion modify plant water and nutrient economies. However, mechanisms that drive these responses have not been well studied, limiting our ability to make inferences about the role of allelopathic plant invasion on plant primary productivity and growth. Here, we show that presence of *Alliaria petiolata*, an allelopathic invader that reorganizes arbuscular mycorrhizal community composition, decreased net photosynthesis rates in two native coexisting species that form associations with arbuscular mycorrhizal fungi. In both species, negative effects of *Alliaria petiolata* presence were driven by stronger reductions in stomatal conductance than indices of photosynthetic capacity, which increased stomatal limitation of net photosynthesis and intrinsic water-use efficiency. Interestingly, these patterns were only observed when the upper tree canopy had fully established. These results indicate that altered arbuscular mycorrhizal community composition due to allelopathic plant invasion may have resulted in greater costs of water uptake, leading to downstream negative impacts on native species carbon assimilation.

**Introduction**

Allelopathy is a common mechanism of invasive plant species (Kalisz et al. ), and describes a process where plants secrete

Allelopathy is a common mechanism of invasive

[what we’ve learned from TT so far]

[open questions about the study system]

Here, we collected gas exchange data from two native understory perennial species (*Trillium erectum* L. and *Maianthemum racemosum* L.) growing in a long-term *Alliaria petiolata* removal experiment to assess effects of *A. petiolata* invasion on native species physiology. Data were collected at two separate timepoints: (1) early in the growth season when the upper canopy was open and demand for soil resources was presumably high, and (2) later in the growth season when the upper canopy was closed and demand for soil resources was presumably low. We used these data to address the following hypotheses: (1) *Alliaria* presence will decrease native species net photosynthesis, which will be driven by a reduction in photosynthetic capacity, stomatal conductance, or both; (2) *Alliaria* presence will impact native species photosynthetic processes more strongly early in the growth season when the canopy is open, as demand for soil resources to support photosynthetic capacity will be greatest during this timepoint. We also expected that net photosynthesis and indices of photosynthetic capacity would increase with increasing soil nutrient availability independent of *Alliaria* presence.

**Methods**

*Study site and experimental design*

This study was conducted in a long-term *Alliaria petiolata* removal experiment at Trillium Trail Nature Reserve in Fox Chapel, PA (40.520 °N, -79.901 °W). Mean annual precipitation and mean annual temperature of the study area is 1006 mm yr-1 and 11°C, respectively (Palecki *et al.*, 2021). Wire fences (2.5 m tall) were set up in 2002 at five 14 x 14 m experimental plots to exclude deer. *Alliaria* *petiolata* individuals have been manually weeded from the left half of each experimental plot since 2006, with the right half of each plot left unweeded. Manual weeding of *A. petiolata* has been an effective invasion suppressant in this experiment, as weeded plots have been previously shown to have 0.08% mean abundance (Roche *et al.*, 2021). This long-term split-plot experiment occurs at a site with steep slope grades ranging from 25-75% and soils that have been broadly classified as Gilpin-Upshur-Atkins soils with dominant shale, sandstone, and red clay shale bedrock components. The *Alliaria* treatments were set up parallel to the slope in order to minimize chances of *Alliaria* allelochemical leaching into the weeded side of the plot.

Between April and July 2023, gas exchange data were collected from fully expanded leaves of two perennial understory native species: *Trillium erectum* and *Maianthemum racemosum*. Gas exchange data were collected at two timepoints: once during an initial measurement period where the upper canopy was establishing (April 19 through April 21 for *Trillium* and May 5 through May 6 for *Maianthemum*) and a second measurement period where the upper canopy had fully established (June 12 through June 15 for both species). The initial measurement period was conducted at separate timepoints for *Trillium* and *Maianthemum* because the timing of full leaf expansion differed between the two species.

*Soil characteristics*

[need help here with methods]

*Gas exchange measurements and calculations*

Net photosynthesis (*A*net; μmol m-2 s-1), stomatal conductance (*g*sw; mol m-2 s-1), and intercellular CO2 (*C*i; μmol mol-1) concentrations were measured across a range of atmospheric CO2 concentrations (i.e., an *A*net/*C*i curve) using the Dynamic Assimilation™ Technique (Saathoff and Welles, 2021). *A*net/*C*i curves were generated along a reference CO2 ramp down from 420 µmol mol-1 CO2 to 20 µmol mol-1 CO2, followed by a ramp up from 420 µmol mol-1 CO2 to 1620 µmol mol-1 CO2 after a 90-second wait period at 420 µmol mol-1 CO2. The ramp rate for each curve was set to 200 μmol mol-1 min-1, logging every five seconds, which generated 96 data points per response curve. All *A*net/*C*i curves were generated after *A*net and *g*sw stabilized in a LI-6800 cuvette set to a 500 mol s-1 flow rate, 10000 rpm mixing fan speed, 1.5 kPa vapor pressure deficit, 25°C leaf temperature, 2000 μmol m-2 s-1 incoming light radiation, and initial reference CO2 set to 420 µmol mol-1. Snapshot *A*net, *g*sw, and leaf *C*i:*C*a measurements were extracted from the initial measurement of each *A*net/*C*i curve (*A*net; μmol m-2 s-1). Intrinsic water-use efficiency (*i*WUE; μmol CO2 mol-1 H2O) was calculated as the ratio of *A*net to *g*sw.

*A/Ci curve fitting and parameter estimation*

*A*net/*C*i curves were fit using the ‘fitaci’ function in the ‘plantecophys’ R package (Duursma, 2015). This function estimates the maximum rate of Rubisco carboxylation (*V*cmax; µmol m-2 s-1) and maximum rate of electron transport for RuBP regeneration (*J*max; µmol m-2 s-1) based on the Farquhar *et al.* (1980) biochemical model of C3 photosynthesis. Triose phosphate utilization (TPU) limitation was included as an additional rate-limiting step in all curve fits after visually observing clear TPU limitation for most curves. Dark respiration was estimated in curve fits as a fixed proportion of *V*cmax. We determined Michaelis-Menten coefficients for Rubisco affinity to CO2 (*K*c; μmol mol-1) and O2 (*K*o; mmol mol-1), and the CO2 compensation point *(Γ*\*; μmol mol-1) using leaf temperature and equations derived in Bernacchi et al. (2001). Specifically, *K*c *K*o, and *Γ*\* were each calculated as:

(1a)

(1b)

(1c)

In all three equations, *T*k is the leaf temperature (in Kelvin) during each *A*net/*C*i curve and R is the universal gas constant (8.314 J mol-1 K-1).

[temp standardization]

[Vcmax:gs]

*Data analysis*

We constructed a series of linear mixed-effects models to explore the role of *Alliaria* presence and canopy status on soil characteristics. Each model included *Alliaria* treatment and canopy status as individual categorical fixed effects, an interaction term between *Alliaria* treatment and canopy status, and plot designated as a random intercept term. Models were constructed with this independent variable structure for soil nitrate availability, soil ammonium availability, soil nitrogen (nitrate + ammonium) availability, and soil phosphorus availability.

Next, we then constructed a second series of linear mixed-effects models tot explore the role of *Alliaria* presence

**Results**

*Soil nutrient availability*

*Alliaria* presence had no effect on plant-available soil nitrogen availability (*p*>0.05; Table 1; Fig. 1a), a pattern true for both nitrate and ammonium concentrations (*p*>0.05 in both cases; Table 1) and observed regardless of canopy status (*Alliaria* presence-by-canopy status interaction: *p*>0.05; Table 1). Plant-available soil nitrogen availability was greater when the upper canopy was open compared to when the upper canopy became fully established (*p*<0.001; Table 1; Fig. 1a). *Alliaria* presence decreased plant-available phosphorus availability (*p*<0.05; Table 1), a pattern observed regardless of canopy status (*Alliaria* weeding-by-canopy status interaction: *p*>0.05; Table 1). Canopy status had no effect on plant-available phosphorus availability (*p*>0.05; Table 1).

**Table 1** Analysis of variance results exploring the role of *Alliaria* treatment and canopy status on plant-available soil nitrogen and phosphorus availability\*

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **Soil nitrogen availability** | | **Soil NO3-N availability** | | **Soil NH4-N availability** | | **Soil phosphorus availability** | |
|  | df | *χ*2 | *p* | *χ*2 | *p* | *χ*2 | *p* | *χ*2 | *p* |
| *Alliaria* presence (A) | 1 | 1.695 | 0.193 | 1.571 | 0.210 | 0.419 | 0.518 | 5.122 | **0.024** |
| Canopy status (C) | 1 | 70.151 | **<0.001** | 72.510 | **<0.001** | 38.321 | **<0.001** | 0.749 | 0.388 |
| A\*C | 1 | 1.974 | 0.160 | 1.422 | 0.233 | 0.781 | 0.377 | 0.026 | 0.872 |

\*Significance determined using Type II Wald χ2 tests (α=0.05). *P*-values less than 0.05 are in bold.

**Figure 1**

**A group of diagrams showing different types of trees

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**Figure 1** Effects of *Alliaria* treatment and canopy status on plot-level soil inorganic nitrogen availability (a), soil phosphorus availability (b), soil nitrate availability (c), and soil ammonium availability (d). Tree canopy status is represented on the x-axis. Blue points and boxplots indicate measurements collected in plots where *Alliaria* has been hand weeded since 2006, and orange points and boxplots indicate measurements collected in plots where *Alliaria* abundance has not been manipulated. Lettering above each treatment group indicates results from post-hoc Tukey’s tests, where different letters indicate statistically different groups (Tukey: *p*<0.05).

*Gas exchange*

Net photosynthesis decreased sharply with tree canopy closure in *Trillium* and *Maianthemum* (*p*<0.001 for both species; Table 2). An interaction between tree canopy status and *Alliaria* presence (*p*<0.05 for both species; Table 2)indicated that *Alliaria* presence had no effect on *A*net in either species when the tree canopy was open (Tukey: *p*>0.05 for both species), but *Alliaria* presence decreased *A*net in both species when the tree canopy was closed (Tukey: *p*<0.05 in both species; Fig. XX). Nitrogen availability increased *A*net in *Trillium* (*p*<0.05; Table 2), but not *Maianthemum* (*p*>0.05; Table 2), while phosphorus availability had no effect on *A*net in either species (*p*>0.05; Table 2).

Stomatal conductance decreased with tree canopy closure in *Trillium* and *Maianthemum* (*p*<0.001 for both species; Table 2). *Alliaria* presence decreased stomatal conductance in both species (*p*<0.05 for both species; Table 2) irrespective of tree canopy status (tree canopy-by-*Alliaria* presence interaction: *p*>0.05 in both species; Fig. XX). Nitrogen availability did not affect stomatal conductance in either species (*p*>0.05; Table 2). Increasing phosphorus availability increased stomatal conductance in *Trillium* (*p*<0.05; Table 2) but had no effect on stomatal conductance in *Maianthemum* (*p*>0.05; Table 2).

In *Trillium*, tree canopy closure, *Alliaria* presence, and phosphorus availability each decreased stomatal limitation of net photosynthesis (*p*<0.05 in both cases; Table 2). Nitrogen availability had no effect on *Trillium* stomatal limitation (*p*>0.05; Table 2). In *Maianthemum*, an interaction between *Alliaria* presence and tree canopy status indicated that the general positive effect of *Alliaria* presence on stomatal limitation (*p*<0.05; Table 2) was only apparent when the tree canopy was closed (Tukey: *p*<0.05), as there was no effect of *Alliaria* presence on stomatal limitation when the tree canopy was open (Tukey: *p*>0.05; Fig. XX). There was no effect of nitrogen or phosphorus availability on *Maianthemum* stomatal limitation (*p*>0.05 in both cases; Table 2).

**Table 2** Analysis of variance results exploring the role of *Alliaria* treatment, canopy status, plant-available soil nitrogen, and plant-available phosphorus availability on leaf gas exchange\*

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | **Net**  **photosynthesis** | | **Stomatal**  **conductance** | | **Stomatal**  **limitation** | |
|  |  | df | *χ*2 | *p* | *χ*2 | *p* | *χ*2 | *p* |
| *Trillium* | |  |  |  |  |  |  |  |
|  | *Alliaria* presence (A) | 1 | 0.489 | 0.484 | 3.921 | **0.048** | 4.239 | **0.039** |
|  | Canopy status (C) | 1 | 556.078 | **<0.001** | 16.493 | **<0.001** | 113.809 | **<0.001** |
|  | Soil N availability | 1 | 3.95 | **0.047** | 1.99 | 0.158 | 1.047 | 0.306 |
|  | Soil P availability | 1 | 0.142 | 0.706 | 5.763 | **0.016** | 5.892 | **0.015** |
|  | A\*C | 1 | 9.926 | **0.002** | 0.065 | 0.799 | 0.81 | 0.368 |
| *Maianthemum* | |  |  |  |  |  |  |  |
|  | *Alliaria* presence (A) | 1 | 6.989 | **0.008** | 20.808 | **<0.001** | 4.691 | **0.030** |
|  | Canopy status (C) | 1 | 73.455 | **<0.001** | 54.873 | **<0.001** | 0.656 | 0.418 |
|  | Soil N availability | 1 | 0.033 | 0.855 | 0.001 | 0.975 | 1.828 | 0.176 |
|  | Soil P availability | 1 | 0.997 | 0.318 | 1.700 | 0.192 | 0.215 | 0.643 |
|  | A\*C | 1 | 7.127 | **0.008** | 0.011 | 0.916 | 7.582 | **0.006** |

\*Significance determined using Type II Wald χ2 tests (α=0.05). *P*-values less than 0.05 are in bold.

**Figure 2**

**A graph of different trees canopy status

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**Figure 2** Effects of *Alliaria* treatment and canopy status on light-saturated net photosynthesis in *Trillium* (a) and *Maianthemum* (b), stomatal conductance in *Trillium* (c) and *Maianthemum* (d), and stomatal limitation in *Trillium* (e) and *Maianthemum* (f).Tree canopy status is represented on the x-axis. Blue points and boxplots indicate measurements collected in plots where *Alliaria* has been hand weeded since 2006, and orange points and boxplots indicate measurements collected in plots where *Alliaria* abundance has not been manipulated. Lettering above each treatment group indicates results from post-hoc Tukey’s tests, where different letters indicate statistically different groups (Tukey: *p*<0.05).

*Indices of photosynthetic capacity*

Canopy closure strongly decreased *V*cmax25 and *J*max25 in both species (*p*<0.001 for both traits in both species; Table 3). A marginal interaction between *Alliaria* presence and canopy status (*p*<0.1; Table 3) indicated that the marginal reduction in *V*cmax25 due to *Alliaria* presence (*p*<0.1; Table 3) was only observed under a closed upper canopy (Tukey: *p*<0.1), as there was no effect of *Alliaria* presence on *Trillium V*cmax25 under an open upper canopy (Tukey: *p*>0.05). A stronger interaction between *Alliaria* presence and canopy status (*p*<0.05; Table 3) indicated that reduced *Trillium* *J*max25 due to *Alliaria* presence (*p*<0.05; Table 3) was also only observed under a closed upper canopy (Tukey: *p*<0.05), as there was no effect of *Alliaria* presence on *Trillium* *J*max25 under an open upper canopy (Tukey: *p*>0.05). *Alliaria* presence had no effect on *Maianthemum* *V*cmax25 or *J*max25 (*p*>0.05 in both cases; Table 3), a pattern that was observed irrespective of upper canopy status (canopy status-by-*Alliaria* weeding interaction: *p*>0.05 in both cases). Soil nitrogen availability and soil phosphorus availability each had no effect on *V*cmax25 or *J*max25 in *Trillium* (*p*>0.05 in all cases; Table 3). Soil nitrogen availability had no effect on *V*cmax25 in *Maianthemum* (*p*>0.05; Table 3), but increased *J*max25 (*p*<0.01; Table 3). Soil phosphorus availability had no effect on *V*cmax25 or *J*max25 in *Maianthemum* (*p*>0.05 in all cases; Table 3).

Canopy status, *Alliaria* presence, soil nitrogen availability, and soil phosphorus availability each had no effect on *J*max25:*V*cmax25 in *Trillium* (*p*>0.05 in all cases; Table 3). In *Maianthemum*, *Alliaria* presence had a positive effect on *J*max25:*V*cmax25 (*p*<0.05; Table 3) while canopy closure had a marginal positive effect (*p*<0.1; Table 3). There was no effect of soil nitrogen availability or soil phosphorus availability on *J*max25:*V*cmax25 in either species (*p*>0.05 in all cases; Table 3).

**Table 3** Analysis of variance results exploring the role of *Alliaria* treatment, canopy status, plant-available soil nitrogen, and plant-available phosphorus availability on indices of photosynthetic capacity\*

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | *V*cmax25 | | *J*max25 | | *J*max25:*V*cmax25 | |
|  |  | df | *χ*2 | *p* | *χ*2 | *p* | *χ*2 | *p* |
| *Trillium* | |  |  |  |  |  |  |  |
|  | *Alliaria* presence (A) | 1 |  |  |  |  |  |  |
|  | Canopy status (C) | 1 |  |  |  |  |  |  |
|  | Soil N availability | 1 |  |  |  |  |  |  |
|  | Soil P availability | 1 |  |  |  |  |  |  |
|  | A\*C | 1 |  |  |  |  |  |  |
| *Maianthemum* | |  |  |  |  |  |  |  |
|  | *Alliaria* presence (A) | 1 |  |  |  |  |  |  |
|  | Canopy status (C) | 1 |  |  |  |  |  |  |
|  | Soil N availability | 1 |  |  |  |  |  |  |
|  | Soil P availability | 1 |  |  |  |  |  |  |
|  | A\*C | 1 |  |  |  |  |  |  |

\*Significance determined using Type II Wald χ2 tests (α=0.05). *P*-values less than 0.05 are in bold.

**Figure 3**

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**Figure 3** Effects of *Alliaria* treatment and canopy status on the maximum rate of Rubisco carboxylation in *Trillium* (a) and *Maianthemum* (b), the maximum rate of electron transport for RuBP regeneration in *Trillium* (c) and *Maianthemum* (d), and the ratio of the maximum rate of Rubisco carboxylation to the maximum rate of electron transport for RuBP regeneration in *Trillium* (e) and *Maianthemum* (f). Tree canopy status is represented on the x-axis. Blue points and boxplots indicate measurements collected in plots where *Alliaria* has been hand weeded since 2006, and orange points and boxplots indicate measurements collected in plots where *Alliaria* abundance has not been manipulated. Lettering above each treatment group indicates results from post-hoc Tukey’s tests, where different letters indicate statistically different groups (Tukey: *p*<0.05).

*Nitrogen-water use tradeoffs*

Canopy closure decreased intrinsic water use efficiency in *Trillium* (*p*<0.001; Table 4) and marginally decreased intrinsic water use efficiency in *Maianthemum* (*p*<0.1; Table 4). *Alliaria* presence decreased intrinsic water use efficiency in both species (*p*<0.05 in both species; Table 4) regardless of canopy status (canopy status-by-*Alliaria* weeding interaction: *p*>0.05 in both cases; Table 4). There was no effect of soil nitrogen availability on intrinsic water use efficiency in either species (*p*>0.05 in both cases; Table 4). Increasing soil phosphorus availability had a negative effect on intrinsic water use efficiency in *Trillium* (*p*<0.05; Table 4), but not *Maianthemum* (*p*>0.05; Table 4).

In *Trillium*, *V*cmax25:*g*sw decreased with canopy closure (*p*<0.001; Table 4) and marginally decreased with increasing soil phosphorus availability (*p*<0.1; Table 4). There was no effect of *Alliaria* presence or soil nitrogen availability on *V*cmax25:*g*sw in *Trillium* (*p*>0.05 in both cases; Table 4). An interaction between canopy status and *Alliaria* presence (*p*<0.05; Table 4) indicated that the general positive effect of *Alliaria* presence on *V*cmax25:*g*sw was only observed when the upper canopy was closed (Tukey: *p*<0.05), as there was no effect of *Alliaria* presence on *V*cmax25:*g*sw when the upper canopy was open (Tukey: *p*>0.05).

**Table 3** Analysis of variance results exploring the role of *Alliaria* treatment, canopy status, plant-available soil nitrogen, and plant-available phosphorus availability on nitrogen-water use tradeoffs\*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  |  | *iWUE* | | *V*cmax25:*g*sw | |
|  |  | df | *χ*2 | *p* | *χ*2 | *p* |
| *Trillium* | |  |  |  |  |  |
|  | *Alliaria* presence (A) | 1 |  |  |  |  |
|  | Canopy status (C) | 1 |  |  |  |  |
|  | Soil N availability | 1 |  |  |  |  |
|  | Soil P availability | 1 |  |  |  |  |
|  | A\*C | 1 |  |  |  |  |
| *Maianthemum* | |  |  |  |  |  |
|  | *Alliaria* presence (A) | 1 |  |  |  |  |
|  | Canopy status (C) | 1 |  |  |  |  |
|  | Soil N availability | 1 |  |  |  |  |
|  | Soil P availability | 1 |  |  |  |  |
|  | A\*C | 1 |  |  |  |  |

\*Significance determined using Type II Wald χ2 tests (α=0.05). *P*-values less than 0.05 are in bold.

**Figure 4**

**A graph of different trees

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**Figure 4** Effects of *Alliaria* treatment and canopy status on intrinsic water-use efficiency in *Trillium* (a) and *Maianthemum* (b) and the ratio of the maximum rate of Rubisco carboxylation to stomatal conductance in *Trillium* (c) and *Maianthemum* (d). Tree canopy status is represented on the x-axis. Blue points and boxplots indicate measurements collected in plots where *Alliaria* has been hand weeded since 2006, and orange points and boxplots indicate measurements collected in plots where *Alliaria* abundance has not been manipulated. Lettering above each treatment group indicates results from post-hoc Tukey’s tests, where different letters indicate statistically different groups (Tukey: *p*<0.05).

**Discussion**

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