**Introduction**

[what we’ve learned from TT so far]

[open questions about the study system]

Here, we collected gas exchange and chlorophyll fluorescence 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) when the upper canopy was open and demand for soil resources was presumably high, and (2) when the upper canopy was closed and demand for soil resources was presumably low. We used these data to address the following hypotheses:

1. *A. petiolata* invasion will negatively impact native species’ photosynthetic capacity. This response will be associated with reorganized belowground microbial communities that will decrease provisioning of soil resources to native species
2. Negative effects of *A. petiolata* invasion will be most prominent when the upper canopy is open, as greater demand to build and maintain photosynthetic enzymes will enhance negative effects of inhibited plant-AM fungal symbioses
3. Of the two native species, *A. petiolata* invasion will more negatively affect photosynthetic capacity in *M. racemosum*, as *M. racemosum* emerges later than *T. erectum* and will therefore rely more strongly on plant-AM fungal symbioses for nutrient and water acquisition.

**Methods**

*Study site and experimental design*

This study was conducted in a long-term garlic mustard (*Alliaria petiolata*) removal experiment at Trillium Trail Nature Reserve in Fox Chapel, PA (40.520 °N, -79.901 °W). The study, which has been in operation since 2006, boasts steep slope grades ranging from 25-75%, with soils at the site broadly classified as Gilpin-Upshur-Atkins soils with dominant shale, sandstone, and red clay shale bedrock components. Mean annual precipitation and mean annual temperature (2006-2020) of the study site is XX mm yr-1 and XX °C, respectively (CITE).

Fences were set up in 2002 at five 14 x 14 m experimental plots to exclude deer. Since 2006, *Alliaria petiolata* has been manually weeded from the left half of each experimental plot, with the right half of each plot left unweeded.

In 2002, fences were set up at 5 14m x 14m experimental plots to exclude deer and other

*Leaf gas exchange measurements*

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 each *A*net/*C*i curve at 420 µmol mol-1 CO2 (*A*net,420; 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 also 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).

*Chlorophyll fluorescence*

A MultispeQ device (PhotosynQ, Lansing, MI, USA) was attached to leaves after *A*net/*C*i curves were produced.

*Data analysis*

To investigate the role of

**Results**

*Soil nutrient availability*

*Alliaria* weeding had no effect on plant-available soil nitrogen availability (*p*>0.05; Table 1; Fig. 1a), a pattern true for both NO3-N and NH4-N concentrations (*p*>0.05 in both cases; Table 1) and observed regardless of canopy status (*Alliaria* weeding-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* weeding increased 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* weeding 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* weeding (W) | 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 |
| W\*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 graph of different sizes of plants

Description automatically generated with medium confidence**

**Figure 1** Effects of *Alliaria* treatment and canopy status on plot-level soil nitrogen availability (a) and soil phosphorus availability (b). *Alliaria* treatment is represented on the x-axis. Red points indicate measurements collected while the upper canopy was open while blue points indicate measurements collected while the upper canopy was closed. Larger circular points indicate the treatment mean, while error bars indicate upper and lower 95% confidence intervals. 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 upper canopy closure in *Trillium* and *Maianthemum* (*p*<0.001 in both species; Table 2). An interaction between canopy status and *Alliaria* weeding treatment (*p*<0.05 in both species; Table 2)indicated that the negative effect of *Alliaria* presence on net photosynthesis was only apparent when the upper canopy was closed (Tukey: *p*<0.001 in both species), as there was no effect of *Alliaria* weeding on net photosynthesis in either species when the canopy was open (Tukey: *p*>0.05 in both species). Net photosynthesis also generally increased with increasing soil nitrogen availability, but this pattern was only observed in *Trillium* (*p*<0.05; Table 2). Soil phosphorus availability did not influence net photosynthesis in either species (*p*>0.05 in both species; Table 2).

Stomatal conductance decreased with canopy closure in *Trillium* and *Maianthemum* (*p*<0.001 in both species; Table 2), a pattern that was not modified by *Alliaria* weeding (canopy status-by-*Alliaria* weeding interaction: *p*>0.05 in both species; Table 2). *Alliaria* presence decreased stomatal conductance in *Maianthemum* (*p*<0.001; Table 2), and marginally decreased stomatal conductance in *Trillium* (*p*<0.1; Table 2). Increasing soil phosphorus increased stomatal conductance in *Trillium* (*p*<0.05; Table 2) but did not affect stomatal conductance in *Maianthemum*(*p*>0.05; Table 2).

Stomatal limitation of net photosynthesis decreased with canopy closure in *Trillium* (*p*<0.001; Table 2), but not *Maianthemum* (*p*>0.05; Table 2). There was no effect of *Alliaria* weeding on stomatal limitation in *Trillium* (*p*>0.05; Table 2). However, an interaction between canopy status and *Alliaria* weeding (*p*<0.05; Table 2) indicated that the positive effect of *Alliaria* presence on stomatal limitation (*p*<0.05; Table 2) was only apparent when the upper canopy was closed (Tukey: *p*<0.05), as there was no effect of *Alliaria* presence on stomatal limitation when the upper canopy was open (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).

*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).