

Invasion-induced root-fungal disruptions alter plant water and nitrogen economies

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2

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4 use efficiency, plant invasion, mutualism disruption, soil microbial community, AM soil fungi,
5 and plant traits.

6

7 **Abstract**

8 Despite widespread evidence that biological invasion influences the biotic and abiotic soil
9 environments, the extent to which each of these pathways underpins the effects of invasion on
10 native plant traits and performance is unknown. Leveraging a long-term (14-yr) manipulative
11 field experiment, we show that an allelochemical-producing invader, *Alliaria petiolata*, affects
12 native plants through biotic mechanisms, altering the soil fungal community composition, with
13 no apparent shifts in soil nutrient availability. These changes in belowground soil fungal
14 communities resulted in a high cost of resource uptake for native forest perennial herbs and a
15 shift in functional traits linked to their carbon and nutrient economies. Furthermore, we illustrate
16 that some species in the invaded community compensate for high nutrient costs by reducing
17 nutrient uptake and maintaining photosynthesis by expending more water. This demonstrates a
18 trade-off in trait investment that increases nutrient use efficiency as nutrient costs increase. Our
19 results show that invasion-induced disruptions in the soil fungal community belowground can
20 cascade to affect aboveground plant communities via shifts in physiological traits needed to
21 maintain plant water and nutrient economies. These complex above-belowground linkages
22 suggest that plant invasions should be evaluated at the system-level to fully understand and
23 predict their impact on native plants and communities.

24 **Main text**

25 Invasive plants have strong negative impacts on population dynamics of native species,
26 community assemblages, and ecosystem processes ¹. Two hypotheses may explain the impacts of
27 invasive species on native ecosystems: (1) shifts in soil resource availability ^{2,3} and (2) disruption
28 of belowground microbial-mediated resource provisioning ⁴. Both pathways result in the same
29 physiological signature of resource limitation for native plants and are therefore difficult to
30 disentangle with physiological trait measurements alone. Moreover, while evidence supports
31 both invasion hypotheses individually, invasive species commonly modify abiotic soil resources
32 and disrupt biotic interactions simultaneously and the cascading effects of invasion belowground
33 are rarely linked to aboveground impacts ^{5,6}. Gaining new insight into the mechanisms
34 underlying successful plant invasion requires tracing invasion-induced changes in belowground
35 soil dynamics to shifts in native plant traits and performance.

36
37 Many invasive species increase the quantity and quality of leaf litter and rhizodeposition, leaving
38 long-lasting effects on abiotic soil resource availability and carbon, nitrogen, and water cycles ^{2,7}.
39 If invasive species increase nutrient concentrations, belowground microbial mutualisms with
40 native species may be disrupted (Figure 1c) ⁸. Other invasive species may have specialized roots
41 or high root density, making the invasive species superior to natives at capturing limited
42 resources ⁹. If invaders are superior soil resource competitors, invasive species will deplete soil
43 resource availability to lower levels than can be tolerated by native species ¹⁰, resulting in
44 physiological stress and reduced native plant performance (Figure 1b).

45

46 While only recently investigated as a mechanism of invasion success, many of the world's most
47 successful invaders alter soil microbiome abundance and diversity and disrupt plant-microbial
48 interactions^{11,12}. Invasion-induced changes in soil microbiomes are driven by specialized
49 microbial associations with non-native plant hosts^{13,14}, altered plant diversity¹⁵, altered plant-soil
50 feedbacks¹⁶, and direct toxicity of soil microbes via the production of allelochemicals¹⁷. A shift
51 in soil microbiome abundance and diversity and the disruption of plant-microbial interactions
52 can have dramatic effects on soil resource provisioning to native plant partners¹⁸. Among the
53 most important plant-microbial symbionts for resource exchange is the association between
54 arbuscular mycorrhizal (AM) fungi (Glomeromycotina) that obligately live in plant roots of at
55 least 70% of land plant species¹⁹. The foundation of this symbiosis is a two-way exchange of
56 resources: AM fungi receive 20-40% of their host plant's carbon (C)²⁰, and in turn boost the
57 supply of soil resources (water, phosphorus (P), and nitrogen (N)) to their host plant²¹⁻²³. If the
58 mechanism driving reduced native plant performance following biological invasion is the
59 disruption of soil resource provisioning by microbial symbionts (mutualism disruption
60 hypothesis²⁴), then biological invasion would induce physiological stress of resident plants with
61 *no apparent change* in soil resource availability (Figure 1c).

62

63 Invasion-induced reductions in the availability and provisioning of soil nutrients and water can
64 leave unique physiological signatures on native plants (Figure 2). In response to invasion-
65 induced resource limitations, native plants can change their morphological, physiological, and
66 internal anatomical traits to increase resource use efficiency of leaf tissues. For instance, in
67 response to reduced water availability plants tend to decrease their specific leaf area (SLA)²⁵,
68 allowing for greater leaf-level photosynthetic water use efficiency (WUE) by reducing the

69 surface area from which water is lost (Figure 2). However, SLA shows only a limited response to
70 soil nutrients²⁶. Stomatal conductance also decreases with increasing water stress²⁷⁻²⁹, increasing
71 WUE, particularly if plants increase nutrient uptake to support photosynthesis under water
72 limitation^{30,31}. However, this results in a decrease in photosynthetic nutrient use efficiency
73 (PNUE) under water limitation³⁰. Conversely, reduced nutrient availability tends to increase
74 PNUE and decrease WUE³⁰. This trade-off leads to the prediction that plants under water stress
75 would have higher nitrogen per leaf area (N_{area}) for a given rate of stomatal conductance (g_s)
76 relative to plants under low water stress (Figure 2). Alternatively, one would expect a decrease in
77 N_{area} at a given g_s with soil nutrient stress. This trade-off has been confirmed in global
78 observations³², but has not been examined in an invasion context. We expect the optimal
79 combination of photosynthetic traits to shift if invasive species alter the availability or
80 provisioning of one or more soil resources. In this scenario, native plants in the invaded
81 community that are best able to shift their morphological, anatomical, and physiological traits are
82 likely to be the least impacted by invasion.

83

84 While physiological responses may help to maintain per-leaf-area rates of photosynthesis, they
85 may only have limited benefit to whole-plant performance (i.e., biomass accumulation and
86 reproduction) if the soil resources necessary to build new tissues are severely limited (Figure 1e).
87 Indeed, biomass responses to soil resource availability are very strong^{33,34}. This may cause a
88 negative feedback to invasion if invasion-induced reductions in resource availability limit
89 nutrient acquisition by suppressing root growth.

90

91 Here, we explore the effects of an allelochemical-producing invader on belowground processes
92 and explicitly link these changes to shifts in native plant traits, resource use efficiencies, and
93 growth. Our primary objective was to distinguish between altered soil resource availability
94 (depletion or enhancement) versus plant-fungal mutualism disruption as pathways underpinning
95 invasion-induced shifts in native plant traits and performance (Figure 1). The invader we
96 investigated, *Alliaria petiolata* (Brassicaceae), is a biennial herb from Eurasia that produces
97 secondary compounds toxic to AM fungi^{35,36}. This study leverages a 14-yr long manipulative
98 field experiment, in which *Alliaria* was weeded from one side of five paired plots and was left at
99 ambient densities on the other side of each plot. Our specific aims were to evaluate the extent to
100 which long-term removal of *Alliaria* leads to changes in (1) the availability of key limiting soil
101 resources (including nitrogen, phosphorous, soil moisture), (2) the soil AM fungal community
102 and abundance of AM fungi in native plant roots, (3) shifts in traits and resource use efficiencies
103 of native forest understory perennials, and (4) changes in whole-plant biomass accumulation.

104

105 To capture potential differences in abiotic soil properties and microbial communities, we
106 collected soil cores and root samples for a subset of native forest perennials within the *Alliaria*
107 ambient and weeded treatments. To trace the effects of altered belowground dynamics on native
108 plant traits and performance, we measured a suite of morphological and physiological traits and
109 recorded plant size annually for two consecutive years. To account for potential differences in
110 native plant traits due to variation in light availability, we also measured diffuse and direct beam
111 radiation in the forest understory. Our focal native plant species were *Arisaema triphyllum*,
112 *Trillium erectum*, and *Maianthemum racemosum*. These species were selected because they are

113 widespread perennial herbs throughout eastern North American forests that form root-AM fungal
114 associations³⁷.

115

116 If the focal native species follow the predictions of the mutualism disruption hypothesis, we
117 predict that the long-term removal of *Alliaria* would increase AM fungal root colonization and
118 alter the AM soil fungal composition. These changes in AM fungal associations would increase
119 soil resource provisioning to the native plant partners, detectable as a decrease in SLA and
120 increase in N_{area} . We expect similar shifts if *Alliaria* directly lowers soil resource availability for
121 native plant species. At the whole-plant level, we expect higher native plant growth in the
122 *Alliaria* weeded treatment than the ambient treatment due to increased soil resource availability
123 or provisioning.

124

125 **Results**

126 *Soil properties*

127 We found that hyphal length of AM fungi (i.e., aseptate hyphae) in the organic soil layer was
128 lower in *Alliaria* weeded plots compared to ambient plots ($P = 0.047$; Figure 3). However, this
129 effect disappeared in bulk soils ($P = 0.093$). Regardless of whether the organic and mineral
130 layers were analyzed separately or as a weighted mean (bulk soils), no other soil properties
131 differed between the *Alliaria* treatments ($P > 0.050$, Figure 3).

132

133 *Soil AM fungal communities*

134 In the organic soil layer, AM fungal Shannon-Wiener diversity was significantly lower in the
135 weeded treatment than the ambient treatment ($P = 0.039$; Figure 4b), but all other measures of

136 AM fungal diversity did not vary between treatments ($P > 0.050$). Soil AM fungal community
137 composition in the organic layer also did not vary between *Alliaria* treatments ($R^2 = 0.074$, $P =$
138 0.377 ; Figure 4a).

139

140 In the mineral soil, neither AM fungal Shannon-Wiener diversity ($P = 0.778$) nor Simpson index
141 ($P = 0.160$) varied between the *Alliaria* treatments (Figure 4e, f). However, the soil AM fungal
142 community composition in the mineral layer differed between treatments ($R^2 = 0.168$, $P = 0.023$;
143 Figure 4d).

144

145 *AM fungal root colonization*

146 Colonization of native plant roots by arbuscules ($P < 0.001$; Figure 5a) and aseptate hyphae ($P =$
147 0.009 , Figure 5d) in *Arisaema* was higher in the *Alliaria* weeded treatment than the ambient
148 treatment. For *Maianthemum*, there was no difference between treatments in the root
149 colonization by arbuscules ($P = 0.335$; Figure 5b) or aseptate hyphae ($P = 0.184$; Figure 5e).
150 Similarly, there was no difference between treatments in the root colonization by arbuscules ($P =$
151 0.608 ; Figure 5c) and lower colonization by aseptate hyphae ($P = 0.002$; Figure 5f) in the
152 weeded treatment for *Trillium*.

153

154 *Light availability and canopy openness*

155 Overall, total diffuse and direct beam radiation did not vary between the *Alliaria* treatments ($P =$
156 0.717). These results indicate morphological and physiological trait differences between
157 treatments were not driven by variation in canopy openness as measured by diffuse and direct
158 beam radiation.

159

160 *Leaf-level morphological and physiological traits*

161 For the non-reproductive life stage, N_{area} was consistently higher (7.8%) ($P = 0.023$, Figure 6a) in
162 the *Alliaria* weeded treatment as compared to the ambient treatment. There was also a lower g_s in
163 the *Alliaria* weeded treatment than the ambient treatment for one species (*Arisaema*; $P < 0.028$;
164 Figure 6b), but no change in g_s between treatment for *Trillium* ($P > 0.505$; Figure 6b) or
165 *Maianthemum* ($P > 0.373$; Figure 6b). Combined, these effects resulted in a higher N_{area} to g_s
166 ratio in the *Alliaria* weeded treatment compared to the ambient treatment for all three native
167 plant species ($P = 0.018$; Figure 6c). Light-saturated net photosynthesis (A_{sat}) was lower (20%) in
168 the *Alliaria* weeded treatment than the ambient treatment for all three native plants ($P = 0.044$;
169 Figure 6d), which, combined with the N_{area} response, resulted in a lower PNUE (16%) in the
170 *Alliaria* weeded treatment than the ambient treatment ($P = 0.013$; Figure 6e). The A_{sat} and g_s
171 responses reduced WUE for *Arisaema* ($P = 0.002$), but not for *Maianthemum* ($P = 0.282$) or
172 *Trillium* ($P = 0.298$) in the weeded treatment (Figure 5f). SLA was higher in the *Alliaria* weeded
173 treatment than the ambient treatment for all three native plants ($P = 0.004$; Supplementary Figure
174 1a). Leaf chlorophyll content (Chl) did not differ between treatments for any of the native
175 species ($P = 0.150$; Supplementary Figure 1a).

176

177 For the reproductive life stage, A_{sat} ($P = 0.004$) and PNUE ($P = 0.019$) were lower in the weeded
178 versus ambient treatment for *Maianthemum*. All other morphological and physiology traits did
179 not differ between treatments for any native plant species ($P > 0.050$).

180

181 *Whole-plant performance*

182 Plant growth differed across plant ontogeny, with smaller individuals growing faster than larger
183 individuals ($P < 0.001$). However, the change in plant size from 2018 to 2019 did not differ
184 between treatments for any of the focal native plant species ($P > 0.050$; Supplementary Figure
185 2).

186

187 **Discussion**

188 Invasive plants are commonly superior resource competitors, reducing soil nutrients to lower
189 levels than can be tolerated by native species. It is becoming increasingly clear that invasion can
190 also disrupt plant-fungal symbionts and reduce nutrient and water provisioning. What so far has
191 remained unclear is the extent to which these invasion-induced changes in belowground
192 dynamics impact aboveground plant traits and performance. Here we provide empirical support
193 for the mutualism disruption hypothesis as a mechanism of invasion success, demonstrating
194 aboveground changes in native plant traits and performance are driven by the disruption of
195 belowground root-fungal symbionts. Specifically, we found no apparent differences in soil
196 nutrient availability after 14-years of *Alliaria* removal. These results are similar to previous
197 studies, which found little evidence of recovery in abiotic soil properties
198 following *Alliaria* removal^{38,39}. Unlike abiotic soil resources, we find lower AM fungal
199 abundance in the roots of *Arisaema* and higher soil fungal diversity in the *Alliaria* ambient
200 treatment. These findings are congruent with previous works, which found plant invasion
201 increased microbial species richness^{15,38,40}. Together these results suggest that the main
202 mechanism of *Alliaria*'s impact on native plant performance is mediated through resource
203 provisioning, and not resource availability.

204

205 Invasion-induced reductions in soil resource provisioning via mutualism disruption should shift
206 native plant traits linked to carbon and nutrient economies, particularly if resources are limiting.
207 Under nutrient stress, plants may adjust their trait expression to maintain similar leaf-level
208 photosynthetic rates by trading efficient use of abundant resources for that of limiting resources
209 ³⁰. For the first time, our results from our 14-yr experiment support these predictions in an
210 invasion context. We detected divergent morphological and physiological traits for all three focal
211 native species in the weeded and ambient treatments. Specifically, plants in the ambient
212 treatment adopted a resource conservative strategy (low nitrogen concentration and specific leaf
213 area) ⁹. We also find evidence that plants in the *Alliaria* ambient treatment had a higher cost of
214 nitrogen uptake than plants in the *Alliaria* weeded treatment, as indicated by the greater PNUE
215 and lower ratio of N_{area} to g_s , consistent with previous results across nutrient availability
216 gradients ³². For one native species, *Arisaema*, there was evidence of differential trait investment
217 that increased PNUE and decreased WUE, as would be expected if nutrient availability was more
218 impacted than water availability by *Alliaria* invasion (Figure 2) ³⁰. Altogether, this evidence
219 demonstrates that plants in *Alliaria* invaded sites can maintain carbon fixation by expending
220 more water to compensate for the disruption of root-fungal symbionts and reduced nutrient
221 provisioning. More generally, these results provide some of the first evidence that invasion may
222 drive native plants toward novel regions of their trait space to maintain optimal carbon and
223 nutrient economies. Furthermore, our results suggest trait flexibility underlies a species' ability
224 to withstand invasion-induced resource limitation and may explain the observed variation in
225 mycorrhizal dependency of native plants for maintaining biomass accumulation ³⁵. However,
226 more research is needed to determine if these changes in plant traits are simply driven by a sheer

227 decrease in the abundance of beneficial root-fungal associations or a shift in the rate of resource
228 provisioning by effective fungal partners.

229

230 At the whole-plant level, we found high variability in plant growth with small plants increasing
231 in size and large plants decreasing in size on average. Pronounced shrinkage of large individuals
232 is common for species with a high degree of modularity, such as our focal natives, and can be
233 adaptive for delaying senescence and preventing mortality during low resource years (e.g.,
234 drought years)^{41,42}. We also found no difference in biomass accumulation between the *Alliaria*
235 treatments for our focal native species. These results are congruent with our previous work, in
236 which we found no effect of the *Alliaria* treatment on *Trillium*'s biomass accumulation over a
237 six-year period⁴³. Despite no treatment differences in biomass accumulation, we did find a
238 decreased probability of *Trillium*'s reproduction (flowering and fruiting), seedling establishment,
239 and survival between the *Alliaria* treatments⁴³. Those changes in demographic performance
240 translated to a significant decrease (2.3-3.5%) in *Trillium*'s time-averaged population growth rate
241⁴³. Our results differ from studies on woody species, which found *Alliaria* invasion suppressed
242 biomass accumulation at the seedling stage⁴⁴. These mixed results suggest invasion may have
243 more pronounced effects on plant growth at the seedling stage and/or may differ among
244 herbaceous versus woody species. The disruption of root-fungal symbionts by *Alliaria* may also
245 induce trade-offs in resource allocation between life history traits (i.e., size at first reproduction
246 and life time reproductive success) via changes in demographic performance. Globally, our
247 findings empirically demonstrate that some plants can minimize the impacts of invasion in terms
248 of biomass accumulation by employing alternative trait strategies. However, the costs of
249 invasion-induced resource limitation may have stronger negative effects on other demographic

250 rates, as seen for *Trillium*⁴³. By allocating limiting resources to maintain biomass accumulation,
251 plants may decrease the availability of resources needed for future reproductive output (i.e., the
252 Principle of Allocation)^{45,46}. These findings emphasize the need to integrate perspectives from
253 life history theory and ecophysiology to better understand and predict species resistance to
254 invasion-induced shifts in belowground dynamics.

255

256 **Methods**

257 *Study site*

258 Our study site is in Fox Chapel, PA (50.520237, -79.900932) in the Trillium Trail Nature
259 Reserve. The experimental design includes five 14 x 14 m paired plots subdivided in 36 2 x 2 m
260 subplots. Beginning in 2006, we weeded *Alliaria* every spring from the left side of each plot and
261 carried the weeded material offsite. The right side of each plot was left at ambient *Alliaria* field
262 densities, resulting in a split plot design. To prevent seed dispersal and re-infestation of *Alliaria*
263 in subsequent years, we installed temporary mesh barriers between the weeded and ambient sides
264 of the plots prior to *Alliaria* seed maturation that were removed post seed dispersal each year. All
265 individuals of our three focal native perennial species within the study plots were permanently
266 tagged and monitored annually since the start of the experiment.

267

268 *Non-native invader*

269 *Alliaria petiolata* (Brassicaceae) is a biennial allelochemical-producing herb from Eurasia that
270 invades both disturbed environments and forest interiors throughout North America. *Alliaria*
271 produces glucosinolates that undergo hydrolysis when released into soils and are converted to
272 cyanide compounds that inhibit respiratory electron transport. These secondary compounds are

273 toxic to AM fungi ³⁶, soil pests and pathogens ⁴⁷, and insect herbivores ^{48,49}. A derivative of
274 glucosinolates, allyl isothiocyanate (AITC), inhibits AM fungal spore germination and reduces
275 soil respiration, root colonization, and arbuscule formation in plants associated with AM fungi
276 ^{24,36,44,50,51}. *Alliaria* invasion often increases soil N, P, Ca, and Mg availability and soil pH ^{40,52}.

277

278 Our previous work demonstrated that *Alliaria* can have cascading negative effects on the
279 performance and population growth rate of AM dependent understory plant species ⁴³. We also
280 previously found that *Alliaria* alters the diversity of native plant communities by reducing the
281 abundance of mycorrhizal plant species, with no subsequent changes in non-mycorrhizal plant
282 species ⁵³. In support of the mutualism disruption hypothesis, our previous findings suggest
283 *Alliaria* negatively affects the population- and community-level dynamics of native species
284 through the disruption of plant-microbial interactions.

285

286 *Focal native species*

287 The focal natives we used for this study are *Arisaema triphyllum* (L.), *Trillium erectum* (L.), and
288 *Maianthemum racemosum* (L.). These species are long-lived understory forest perennial herbs
289 common in eastern North American temperate forests. The focal species have belowground
290 rhizomes with coarse root systems that lack root hairs and form root-AM fungal symbioses and
291 produce aboveground stems and leaves that senesce annually ⁵⁴. The timing and duration of
292 aboveground phenology (period of active photosynthesis) differs among the focal species, with
293 aboveground emergence through senescence spanning April to July for *Trillium*, May to
294 September for *Maianthemum*, and April to August for *Arisaema* ^{55,56}. Leaves fully expand prior

295 to canopy closure for *Trillium* but continue to expand for up to a month following canopy closure
296 for *Arisaema* and *Maianthemum*.

297

298 *Soil samples*

299 To examine the effects of *Alliaria* on abiotic soil resources and AM fungal community
300 composition, two subplots within each paired plot were sampled in June 2018 following Burke,
301 et al. ³⁹. To obtain a sufficient amount of soil (50 g wet weight), five to ten soil cores in each
302 subplot were separated into organic and mineral horizons and pooled separately for a total of 20
303 soil samples (2 *Alliaria* treatments x 2 soil horizons x 5 plots). The depth of each soil layer per
304 soil core was recorded and the cores were separated into the organic and mineral layers
305 following Burke, et al. ³⁹.

306

307 *Soil properties*

308 To determine soil resource availability, phosphate (PO_4^{-3}), ammonium (NH_4), and nitrate (NO_3^-),
309 pH, and microbial biomass carbon (MBC) were extracted within 2 d and soil moisture within 3 d
310 of soil sampling. Soil moisture was determined gravimetrically as the difference between wet
311 soil and dry soil after drying samples at 105°C for two weeks. Soils were extracted in 0.5 M
312 K_2SO_4 at a ratio of 1:5 for extractable PO_4^{-3} , NH_4 and NO_3^- and the nutrients were quantified
313 colorimetrically ⁵⁷⁻⁵⁹. Soil pH was measured in a 1:2 ratio (w/v) of soil to H_2O slurry. Soil organic
314 matter was estimated at the plot level by loss-of-ignition method and combusting the soil in a
315 muffle furnace at 360°C ⁶⁰. Microbial biomass carbon (MBC) was estimated using chloroform
316 fumigation and direct extraction following Brookes, et al. ⁶¹ and expressed as $\mu\text{g C}$ per g of dry
317 soil and is corrected for extraction efficiency (0.45). To assess the length of AM fungal hyphae

318 in the organic and mineral layers, hyphae were extracted from 5 g of soil in 5% sodium
319 hexametaphosphate, filtered through nylon filter (47-mm diameter, 0.45- μ m pore size) and
320 stained with acid fuchsin¹⁵. The stained hyphae were visualized and scored using the grid-line
321 intersect method⁶² in 50 grids at 200X and the hyphal length was calculated as mm hyphae per g
322 of dry soil. To capture the average soil properties near the focal native plant roots, we used the
323 average proportion of each soil layer in each subplot to calculate the weighted mean soil
324 properties (i.e., total bulk soil). Overall, the depths of the soil cores before hitting claypan or
325 rock ranged from 2.93 to 15.00 cm, with a mean depth of 11.26 cm.

326

327 *Soil AM fungal communities*

328 To determine differences in AM fungal composition across *Alliaria* treatments, DNA was
329 extracted from 500 mg of soil using a Power Soil DNA Isolation Kit (MO BIO Laboratories, CA,
330 USA). DNA was quantified using a Qubit Fluorometer and stored at -80°C. To analyze the AM
331 fungal community composition, a nested PCR approach was used to first amplify a 1200 base
332 pair region of the 18S fungal DNA using NL1-NL4 eukaryotic primers⁶³. Each PCR contained
333 21.5 μ l of Platinum PCR Supermix (Invitrogen, Carlsbad, CA, USA), 1.25 μ l of each primer (10
334 μ M), 0.5 μ l of BSA (20 mg ml⁻¹), and 2 μ l (~20 ng) of DNA. The reactions for the first PCR
335 with NS1 and NS4 primers ran with a hot start at 94 °C for 3 min, followed by 30 cycles of 94
336 °C for 30 s, 58 °C for 60 s, and 72 °C for 60 s and a final extension step of 72 °C for 10 min. A 2
337 μ l aliquot of the PCR product from this PCR was used as template for the AM fungal-specific
338 PCR using the NS31 and AM1 primers^{64,65}. The reaction volumes were identical to the first
339 PCR. The reactions ran with a hot start at 94 °C for 5 min, followed by 40 cycles of 94 °C for 45
340 s, 63.1 °C for 60 s, and 72 °C for 90 s and a final extension step of 72 °C for 10 min. The PCR

341 with AM fungal-specific primers were run in triplicate, combined, cleaned with Agencourt
342 AMPure XP magnetic beads (Beckman Coulter, Brea, CA) and quantified with a Qubit
343 fluorometer. Each sample was sequenced in a paired end 2 x 275 b run on an Illumina MiSeq at
344 the University of Tennessee Genomics Core. All sequences are deposited in the NCBI SRA with
345 accession number (released upon publication). Contigs of forward and reverse sequences were
346 created using the DADA2 pipeline using default parameters ⁶⁶.

347

348 *AM fungal root colonization*

349 To characterize differences in AM fungal colonization in native plant roots between the *Alliaria*
350 treatments, we collected root samples for each focal native species in June of 2018 (N= 53; 3-13
351 individuals x 3 native plant species x 2 *Alliaria* treatments). To control for variation in root
352 colonization across plant ontogeny, we restricted our samples to large non-flowering individuals.
353 Roots were cleared and stained following McGonigle, et al. ⁶⁷ and the presence or absence of
354 AM fungal structures (i.e., arbuscules or aseptate hyphae) were counted across 60-125
355 intersections at 200x.

356

357 *Leaf-level morphological and physiological traits*

358 To characterize the effect of the *Alliaria* treatments on morphological and physiological traits
359 associated with water and nutrient use efficiency for the focal native understory species, we
360 quantified the instantaneous physiology of a subset of reproductive and non-reproductive plants
361 in 2018 (N= 468; 29-57 individuals x 2 *Alliaria* treatments x 3 species x 2 life stages). We
362 measured the net photosynthetic rate (A_{sat}), and stomatal conductance (g_s) using a Li-COR LI-
363 6400XTR portable photosynthesis system, equipped with a CO₂ control module, 2 x 3 cm leaf

364 cuvette and a red-blue light-emitting diode (LED) light source (Li-Cor, Lincoln, NE, USA).
365 Measurements were taken at a saturating light level of $800 \mu\text{mol m}^{-2}\text{s}^{-1}$, ambient temperature and
366 humidity, and a reference chamber CO_2 concentration of $400 \mu\text{mol mol}^{-1}$, following Heberling, et
367 al.⁶⁸. To minimize within-plant variability for *Maianthemum*, we restricted our sampling to
368 either the 2nd or 3rd leaf from the terminal end of the stem following Cornelissen, et al.⁶⁹ and only
369 used leaves without signs of senescence or damage (e.g. insect herbivory). Specific leaf area
370 (SLA) and leaf carbon and nitrogen concentration were measured on a subset of individuals (11-
371 18 individuals x 2 *Alliaria* treatments x 3 species x 2 life stages). To measure SLA, we collected
372 two to three 5.32 cm^2 leaf tissue samples from each individual and calculated the mean leaf
373 surface area per g dry mass ($\text{cm}^2 \text{ g}^{-1}$). Leaf carbon and nitrogen concentration were measured on
374 dry leaf samples using an elemental analyzer (Costech Inc., Valencia, CA). We used these
375 physiological measurements to calculate water use efficiency (WUE) (A_{sat} / g_s), photosynthetic
376 nitrogen use efficiency (PNUE) ($A_{\text{sat}} / \text{percent nitrogen} * \text{SLA}$), and nitrogen per leaf area (N_{area})
377 for a given rate of stomatal conductance (g_s). The mean chlorophyll content (Chl) of three
378 leaflets per plant was measured using a SPAD 502 Chlorophyll Meter (spectrum Technologies,
379 Inc, Aurora, IL, USA); (N=284; 34-57 individuals x 3 species x 2 *Alliaria* treatments x 2 life
380 stages). To standardize our sampling, we chose to measure Chl from the darkest green section of
381 each leaflet. The Chl-sampled individuals are a subset of plants for which the other functional
382 traits were measured and the same plants for which whole-plant performance were measured
383 (below).

384

385 *Light availability and canopy openness*

386 To test if focal species' trait characteristics were driven by differences in ambient light levels in
387 the *Alliaria* ambient and weeded treatments, we took hemispherical 180° image digital
388 photographs with a Pentax K-50 camera fitted with a Lensbaby™ circular fisheye lens. To take
389 the photographs, the camera was held ~ 80 cm above the forest floor on a tripod in the center of
390 each subplot in which functional trait data were collected. The camera was set to the full wide-
391 angle mode, with the flash and macro setting turned off. To maintain consistent image alignment,
392 we standardized the directionality of each photograph (e.g., top of photograph facing North and
393 left side facing West). Photographs were post-processed and analyzed using Gap Light Analyzer
394 2.0 software to calculate the gap light index ⁷⁰, a metric of diffuse and direct beam radiation.

395

396 *Whole-plant performance*

397 To capture the effect of the *Alliaria* treatment on plant growth, we selected N = 501 individuals
398 across the three species and measured aboveground size in June-July in 2018 and 2019. Plant
399 biomass was estimated using species-specific allometric equations previously developed ^{43,71,72}.

400

401 **Statistical analysis**

402 *Soil properties*

403 To evaluate the effects of the *Alliaria* ambient and weeded treatment on abiotic soil
404 concentrations of PO_4^{-3} , NH_4 , NO_3 , MBC, and the biomass of fungal hyphae, we conducted
405 univariate analyses for each variable separately using a generalized linear mixed effect model.
406 To improve normality of the residuals, PO_4^{-3} and soil moisture were square root transformed and
407 pH, NH_4NO_3^- , and MBC were natural log transformed.

408

409 *Soil AM fungal communities*

410 To characterize differences in alpha diversity between treatments, we calculated the Shannon-
411 Wiener index and Simpson diversity index. To test for significant differences in Shannon-
412 Wiener, we used a linear mixed effect model and for the Simpson index we used a mixed effect
413 model with a beta distribution. To capture differences in the soil fungal community composition
414 between the *Alliaria* treatments based on species abundance, we used a permutation multivariate
415 analysis of variance (PERMANOVA) using the `vegan` package and the `adonis` function
416

417 *AM fungal root colonization*

418 To quantify differences in the 2018 root colonization rates of AM fungi between the *Alliaria*
419 ambient and weeded treatments, we used a beta regression model⁷³. For each model, we
420 included the *Alliaria* treatment, the focal native species, and an interaction term as a fixed effect.
421 The response variable for each model was the proportion of arbuscules or aseptate hyphae
422 present across 60-125 fields of view.

423

424 *Leaf-level morphological and physiological traits*

425 To evaluate the effect of the *Alliaria* treatments on the morphological and physiological traits,
426 we conducted univariate analyses for each trait separately using a linear mixed effects model.
427 The fully parameterized models included the *Alliaria* (ambient or weeded) treatment, the focal
428 native species (*Arisaema*, *Maianthemum*, and *Trillium*), initial plant size (2018), and a treatment
429 by species interaction term as predictor variables. The response variables were A_{sat} , Chl, WUE,
430 g_s , SLA, PNUE, N_{area} , and N_{area}/g_s . Examining differences in morphological and physiological
431 traits for the reproductive life stage was not possible for *Arisaema* because few individuals

432 flowered in 2018, so the morphological and physiological traits for reproductive and non-
433 reproductive life stages were analyzed separately. To improve nonnormality of the residuals, we
434 log transformed PNUE WUE, and g_s .

435

436 *Light availability and canopy openness*

437 To test for differences in light availability between *Alliaria* ambient or weeded treatment, we
438 calculated the gap light index and applied a generalized linear model. The fully parameterized
439 model included the *Alliaria* treatment as the predictor variable and the total diffuse and direct
440 beam radiation as the response variable.

441

442 *Whole-plant performance*

443 To examine if the *Alliaria* treatments influenced plant growth of our focal native species, we
444 used a linear mixed model. To improve nonlinearity, size at time t_{2018} and t_{2019} were log
445 transformed. The fully parameterized models included the *Alliaria* (ambient and weeded)
446 treatment and initial plant size as fixed effects.

447

448 All statistical analyses were conducted in R v. 3.4.3⁷⁴. Generalized linear models were fit using
449 the `glm` function and generalized linear mixed models were fit using the `lmer` and `glmer`
450 functions in package `lme4`⁷⁵. To account for the nested experimental design and non-random
451 spatial structure, we included treatment nested within plot as a random factor in our regression
452 models. To test for significant main effects and interaction terms, we used backward stepwise
453 regression. Non-significant interactions and main effects were dropped sequentially, removing
454 non-significant interaction terms first. Comparisons between the fully parameterized models and

455 reduced models were made using a likelihood ratio test. Significant differences were determined
456 using type III analysis of deviance for interactions and type II for main effects, using the `Anova`
457 function in the `car` package ⁷⁶. To identify significant difference between factor levels, we
458 calculated differences of least squares means and confidence intervals using the `lmerTest`
459 package and the `diffLsmeans` function ⁷⁷.

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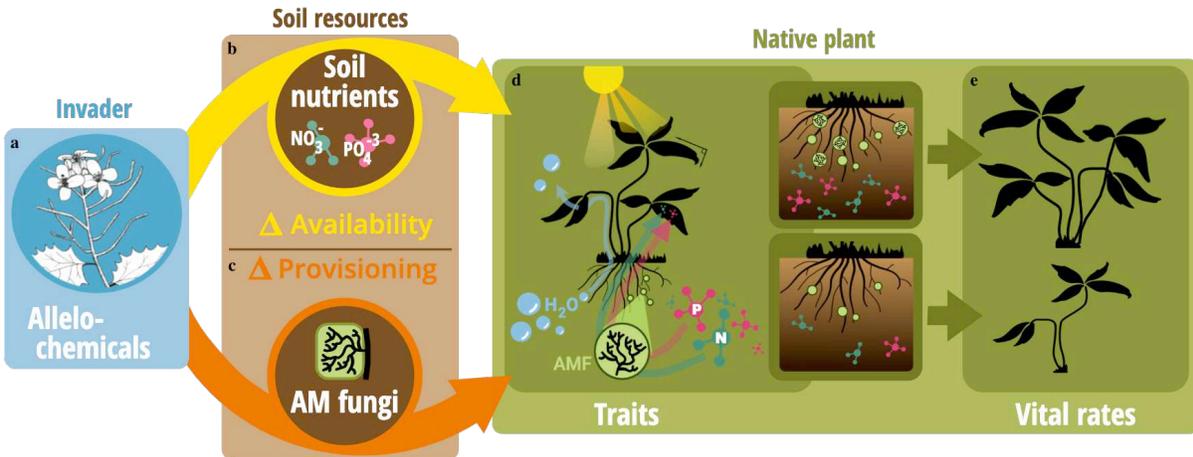
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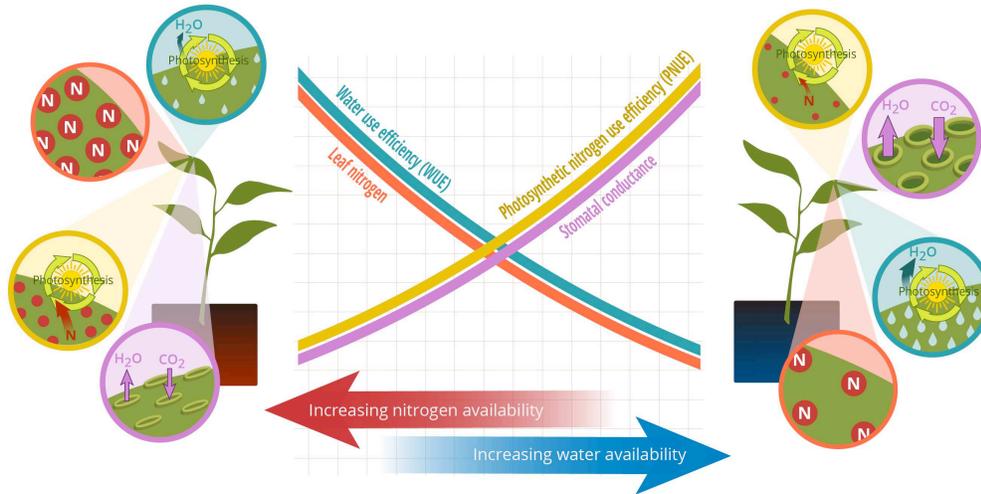
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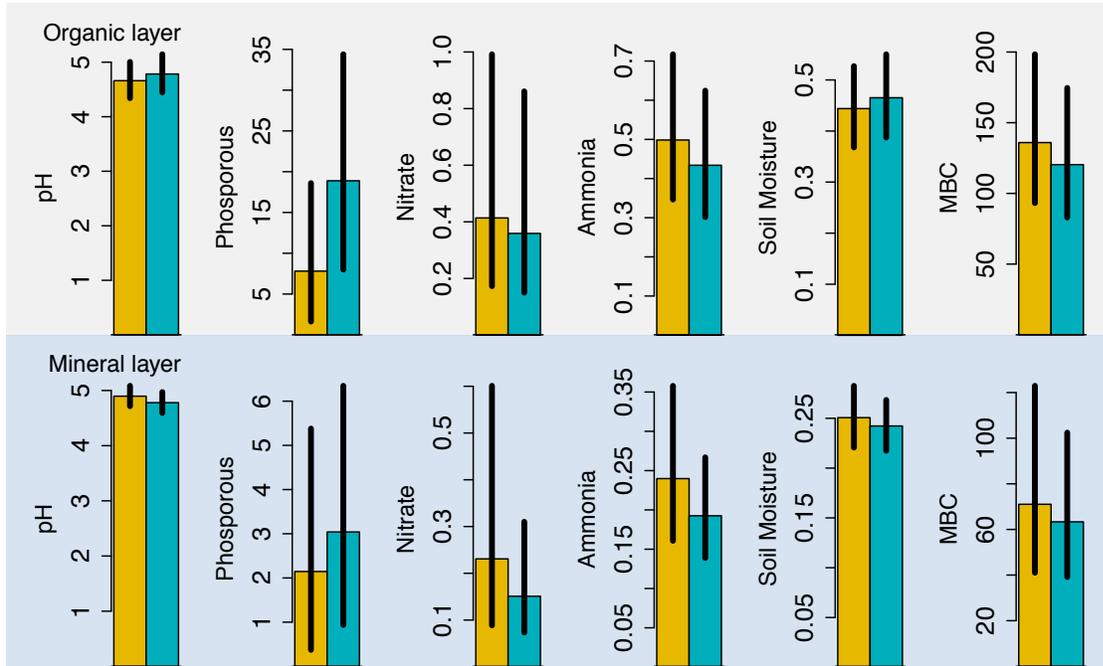
472
 473 Figure 1: Invasive plants can lead to resource limitation through the depletion of soil resources (panel b)
 474 or the disruption of mutualistic interactions respectively (panel c). These alternative or concurrent
 475 pathways of invasion-induced resource limitation can be detected as a shift in native plant traits linked to
 476 their carbon and nutrient economies (panel d). Plants that can adjust their trait space to maintain leaf-level
 477 photosynthetic output are likely to be the least impacted by invasion. However, metrics of whole-plant
 478 performance (i.e., survival, growth, and reproduction) can be constrained if resources necessary to build
 479 new tissues are significantly limited (panel e).

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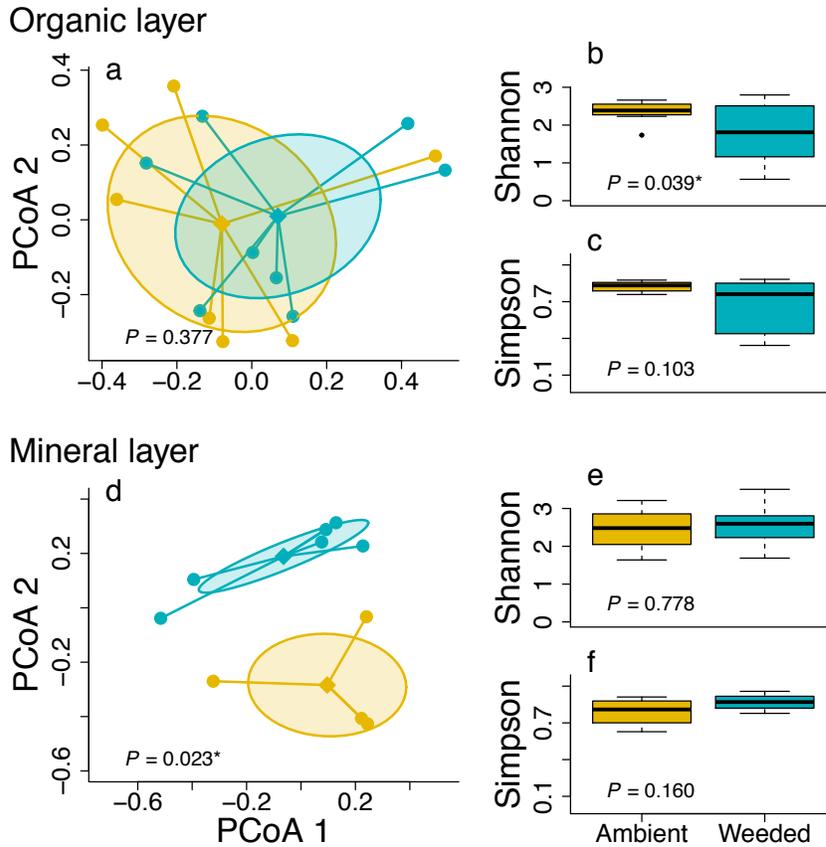


491
 492 Figure 2: Plant responses to altered nutrient and water availability and/or provisioning. Plants
 493 tend to increase stomatal conductance and decrease leaf nitrogen in low nutrient environments
 494 and vice versa in low water environments. In order to maintain similar leaf-level photosynthetic
 495 rates in low resource environments, plants can trade the efficient use of abundant resources for
 496 that of limiting resources. For example, plants in nutrient limited environments can increase
 497 PNUE and decrease WUE. These tradeoffs lead to the prediction that plants under high nutrient
 498 stress will decrease leaf nitrogen per area at a given stomatal conductance.

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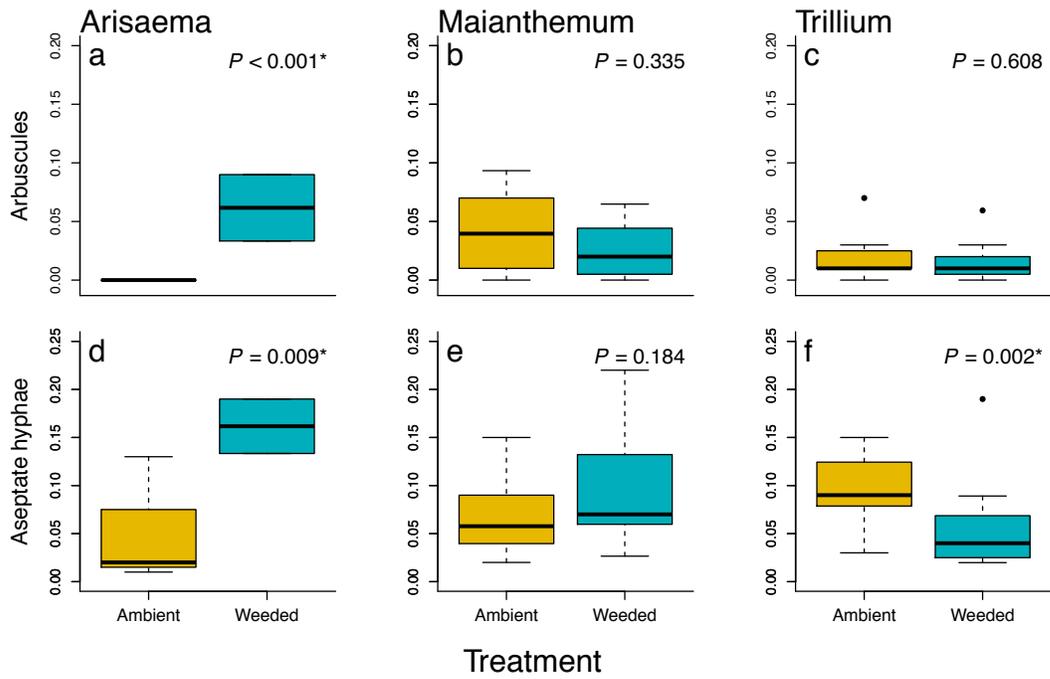


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 508 Figure 3: Soil properties in the organic (top) and mineral (bottom) layers. All soil properties were
 509 non-significant between the ambient (gold) and weeded (teal) treatments at $P = 0.05$.
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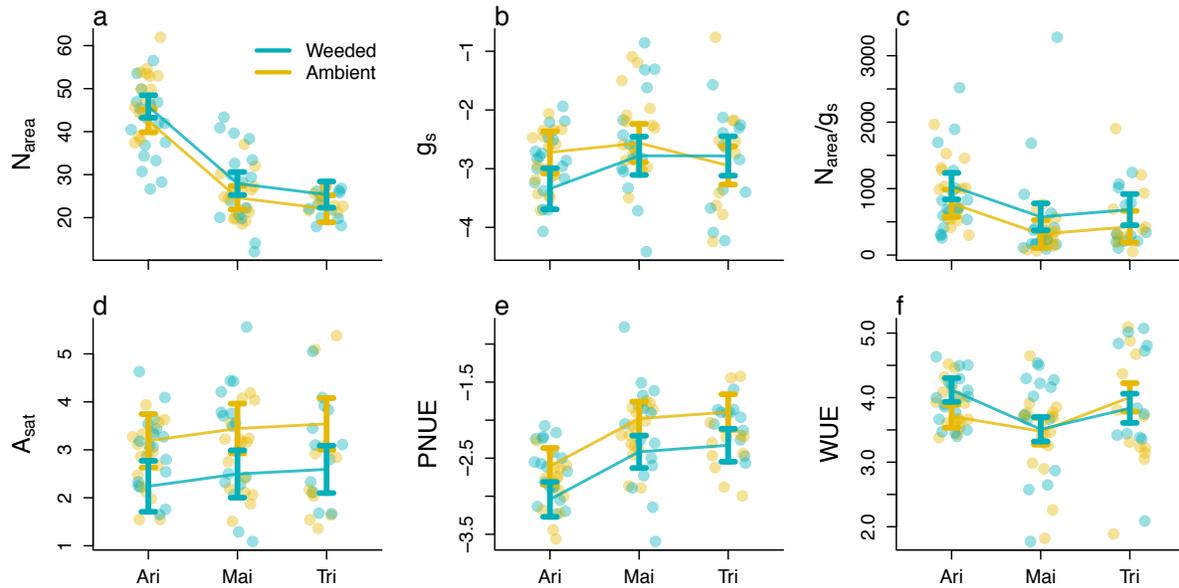
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512 Figure 4: Comparison of *Alliaria* treatment effects (ambient or weeded) on soil fungal
 513 community beta (a, d) and alpha diversity (b, c, e, f) for two soil layers: organic (top) and
 514 mineral (bottom). The Principal Coordinates Analysis graphs (left) illustrate the similarity or
 515 dissimilarity (beta diversity) of the soil fungal community between treatments based on species
 516 abundance. The graphs to the right show differences in soil fungal Shannon-Wiener diversity and
 517 Simpson Index between the ambient (gold) and weeded (teal) treatments.
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Figure 5: Root colonization of the focal native perennials (*Arisaema*, *Maianthemum*, and *Trillium*) in the *Alliaria* ambient and weeded treatments in 2018. The response variable was the proportion of arbuscules or aseptate hyphae present across 60-125 fields of view.



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Figure 6: Physiological traits and resource use efficiencies for the non-reproductive life stage in the *Alliaria* weeded (teal) and ambient (gold) treatments. The interaction term between the *Alliaria* treatment and species is significant at $P = 0.05$ for WUE and g_s . The main effect of treatment is significant for A_{sat} , PNUE, N_{area} , and N_{area}/g_s .

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Figures

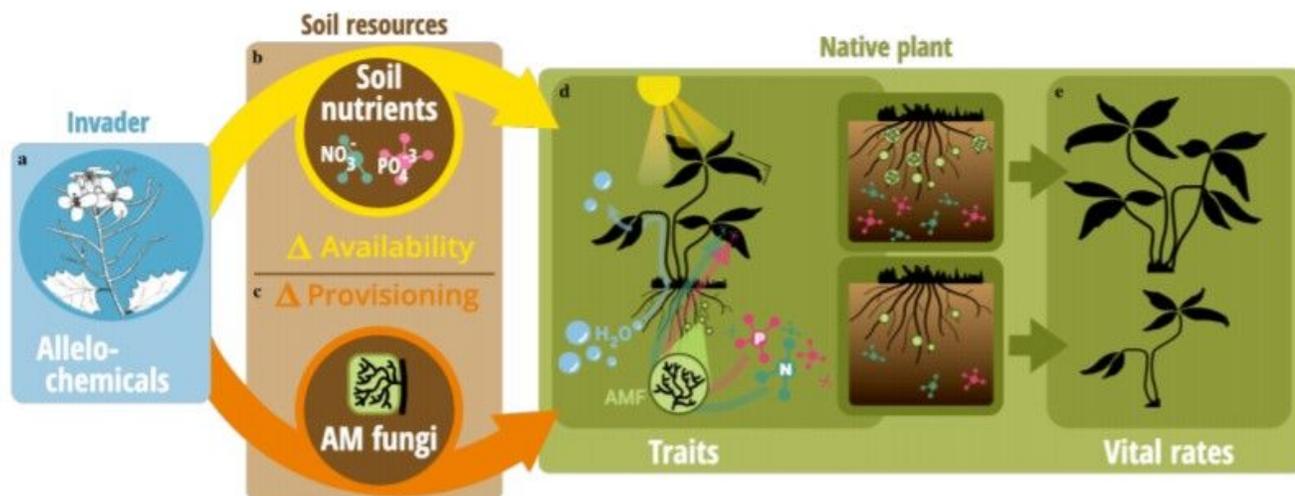


Figure 1

Invasive plants can lead to resource limitation through the depletion of soil resources (panel b) or the disruption of mutualistic interactions respectively (panel c). These alternative or concurrent pathways of invasion-induced resource limitation can be detected as a shift in native plant traits linked to their carbon and nutrient economies (panel d). Plants that can adjust their trait space to maintain leaf-level photosynthetic output are likely to be the least impacted by invasion. However, metrics of whole-plant performance (i.e., survival, growth, and reproduction) can be constrained if resources necessary to build new tissues are significantly limited (panel e).

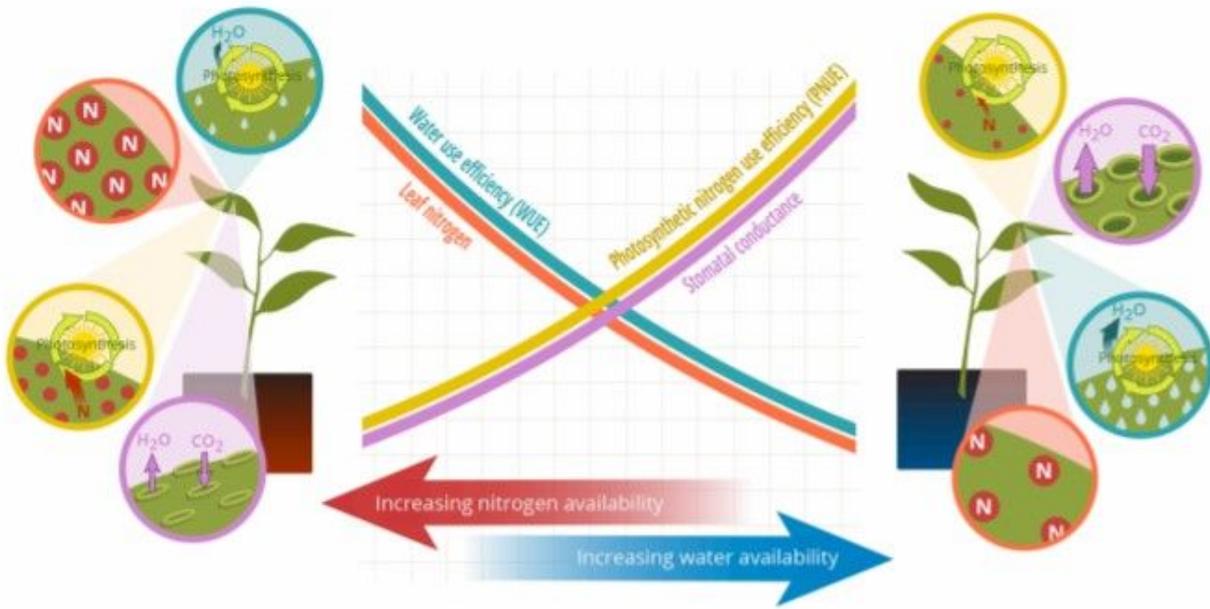


Figure 2

Plant responses to altered nutrient and water availability and/or provisioning. Plants tend to increase stomatal conductance and decrease leaf nitrogen in low nutrient environments and vice versa in low water environments. In order to maintain similar leaf-level photosynthetic rates in low resource environments, plants can trade the efficient use of abundant resources for that of limiting resources. For example, plants in nutrient limited environments can increase PNUE and decrease WUE. These tradeoffs lead to the prediction that plants under high nutrient stress will decrease leaf nitrogen per area at a given stomatal conductance.

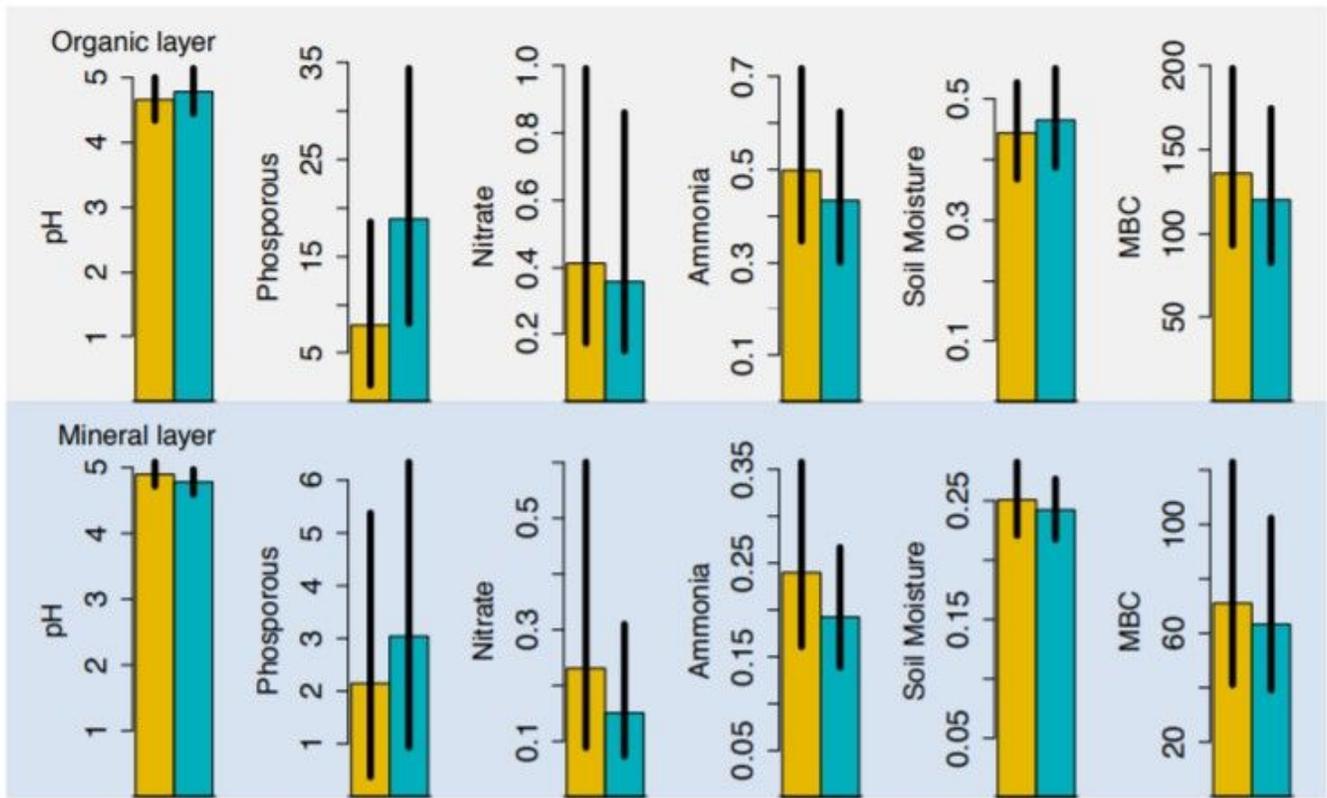


Figure 3

Soil properties in the organic (top) and mineral (bottom) layers. All soil properties were non-significant between the ambient (gold) and weeded (teal) treatments at $P = 0.05$.

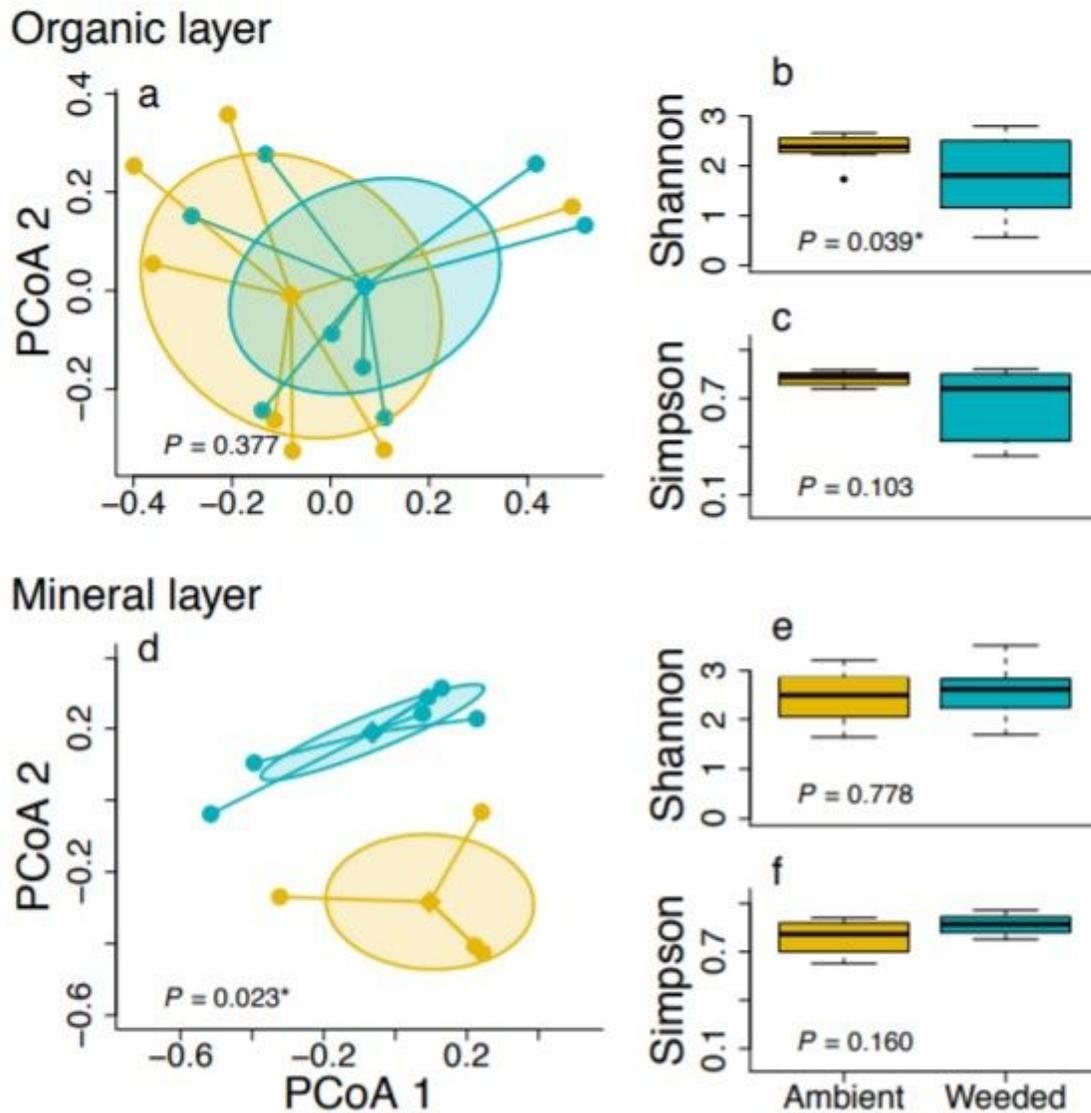


Figure 4

Comparison of *Alliaria* treatment effects (ambient or weeded) on soil fungal community beta (a, d) and alpha diversity (b, c, e, f) for two soil layers: organic (top) and mineral (bottom). The Principal Coordinates Analysis graphs (left) illustrate the similarity or dissimilarity (beta diversity) of the soil fungal community between treatments based on species abundance. The graphs to the right show differences in soil fungal Shannon-Wiener diversity and Simpson Index between the ambient (gold) and weeded (teal) treatments.

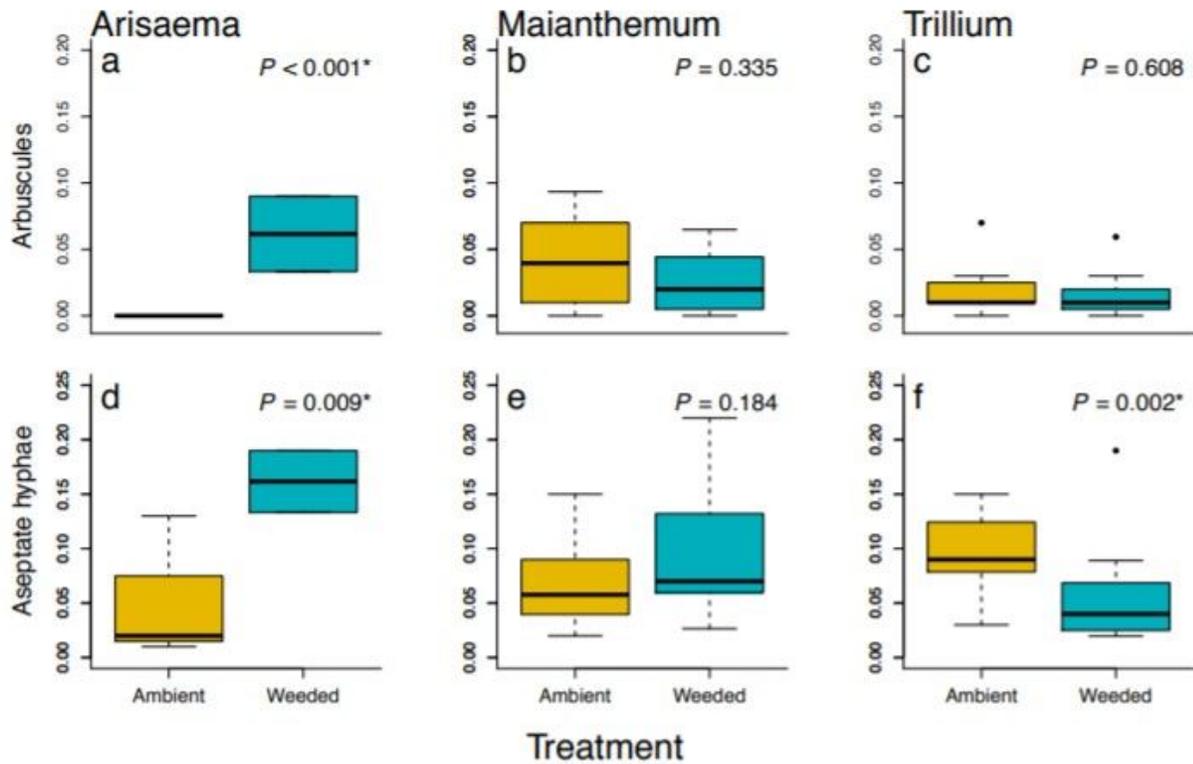


Figure 5

Root colonization of the focal native perennials (Arisaema, Maianthemum, and Trillium) in the Alliaria ambient and weeded treatments in 2018. The response variable was the proportion of arbuscules or aseptate hyphae present across 60-125 fields of view.

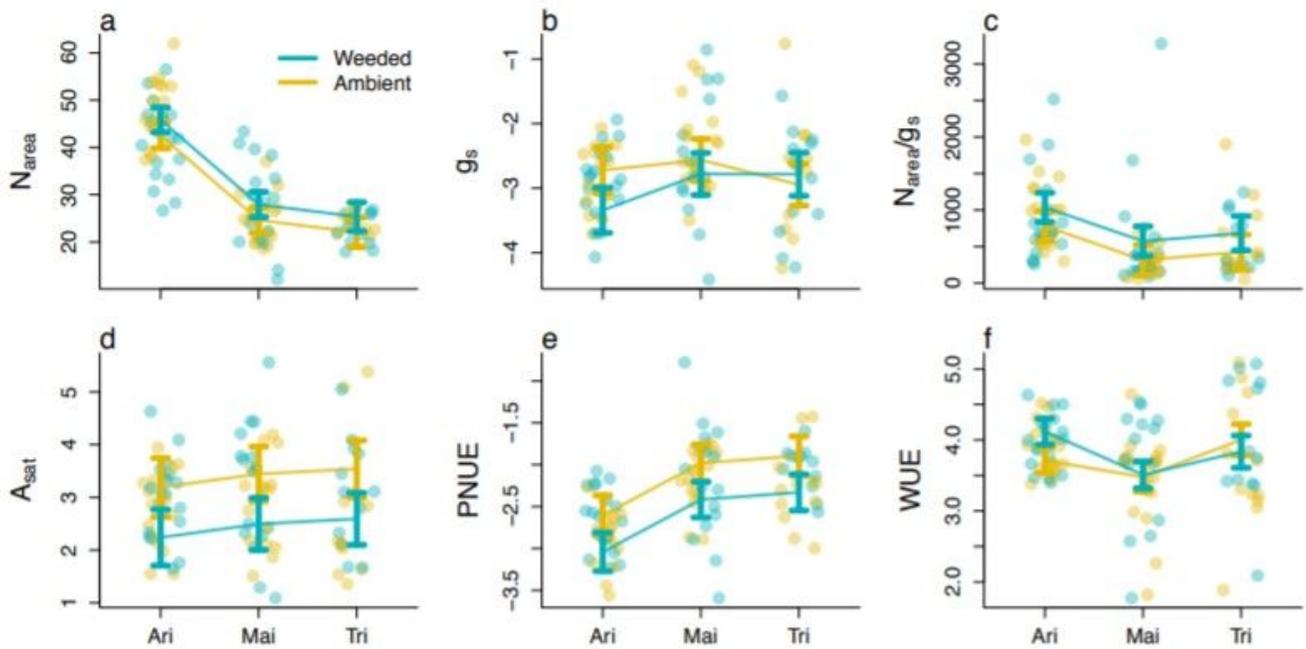


Figure 6

Physiological traits and resource use efficiencies for the non-reproductive life stage in the *Alliaria* weeded (teal) and ambient (gold) treatments. The interaction term between the *Alliaria* treatment and species are significant at $P = 0.05$ for WUE and g_s . The main effect of treatment is significant for A_{sat} , PNUE, N_{area} , and N_{area}/g_s .

Supplementary Files

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- [BialicMurphyetalSupplementaryanonymous.pdf](#)