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[date]

Dear Editorial Board at *Functional Ecology*,

Thank you for considering our manuscript (FE-2025-00191), now titled “The negative effects of an allelopathic invader on native plant photosynthesis are observed after tree canopy closure” for publication in *Functional Ecology*. We have slightly modified the title in accordance with suggestions to not overstate results. We appreciate the constructive feedback from the Associate Editor and reviewer and believe these changes have improved the manuscript. We are submitting a clean version of the revised manuscript and a marked-up version with changes tracked through the “Track Changes” feature in Microsoft Word.

In response to editor and reviewer feedback, we have streamlined the Introduction and Discussion sections to frame our study more clearly, minimize repetition, and avoid overstating conclusions. We have also modified figures and tables following suggestions from the Associate Editor and reviewer to improve readability. All other revisions are explained in our responses below. We provide a point-by-point response to each comment, with the editor/reviewer’s feedback in black-colored font and our response in red-colored font. Line numbers and text excerpts are included in our responses to facilitate review.

Importantly, we have updated our statistical approach to include only individuals with gas exchange measurements taken at both timepoints, in line with the study’s central focus on understanding the temporal component regulating native species responses to *A. petiolata*. We now also include individual identity as a random intercept term to account for repeated measures. This revised statistical approach strengthens support for the observed patterns reported in the previous manuscript submission and does not alter the main conclusions of the paper.

Please contact me if you have any questions regarding our revision.

Sincerely,

Evan A. Perkowski, Ph.D.

*On behalf of coauthors K. Carroll, Jessie Mutz, Snehanjana Chatterjee, Xianyu Yang, Lalasia Bialic-Murphy, Stephanie N. Kivlin, Susan Kalisz, andNicholas G. Smith*

ASSOCIATE EDITOR'S COMMENTS TO THE AUTHORS:  
Associate Editor

Comments to the Author:

This paper reports on an investigation into the plant photosynthetic and stomatal responses of two native species to an invasive plant with allelopathic effects, most notably at two different time points in a season. The authors measured gas exchange *Trillium* spp. and *Maianthemum racemosum* in the growing season while the tree canopy was open and again later in the growing season when the tree canopy was closed. The investigation made use of a long-term field experiment where *Alliaria petiolata*, an allelopathic invader that disrupts AM fungal communities, has been hand-weeded or left at ambient levels since 2006. Previous relevant results from this experiment were summarised. Both native species exhibited significantly reduced net photosynthesis rates under ambient *A. petiolata* levels compared to the weeded treatment. The results suggested that *A. petiolata* affects species differently, and reduced native plant net photosynthesis either by increasing nutrient stress, as indicated by the reduction in apparent photosynthetic capacity (Jmax and Vcmax) in Trillium spp., or by increasing water stress, as indicated by the reduction in stomatal conductance in *M. racemosum*. However, these results were found in one and not both of the time periods measured. The authors should take care not to overstate results, including in the abstract. That said, this manuscript has potential to contribute to our understanding the successful mechanisms of allelopathic invasive plants and illustrates the importance of understanding temporal patterns in the impacts of invasive species.

Thank you for the opportunity to revise our manuscript. We appreciate your suggestions and those of the reviewers. We have addressed all comments and provide our responses in blue colored font.

L44-46 – this repeats the definition. Perhaps better to say here, is that this has been demonstrated in the field/glasshouse many times.

We have modified this sentence to read: “Allelopathy is estimated to occur in 52% of invasive plant species (Kalisz et al., 2021) and has been shown to negatively affect native plant performance and soil microbial community composition in both field and greenhouse settings (Bialic-Murphy et al., 2020, 2021; Brouwer et al., 2015; Hale et al., 2011, 2016; Hale & Kalisz, 2012; Qu et al., 2021; Roche et al., 2021; Zhang et al., 2021).”

L65-69 – repetitive.

We removed these sentences.

L66 – it would be good to define ‘photosynthetic capacity’ as it is key to this research. There are lots of different terms used throughout the introduction, but pinning down what exactly is meant by this one seems key. Line 120 notes “Photosynthetic responses to *A. petiolata* invasion could be driven by changes in photosynthetic capacity, indicating nutrient limitation, or by changes in stomatal conductance, indicating water limitation.” Perhaps this could go earlier? Also are plant photosynthetic responses different to photosynthetic rates in this ms? If so, how are they different? I would expect to see Vcmax and Jmax referred to in the introduction – there is never any explanation about why these were calculated, meaning the reader has to bring significant knowledge to the paper. There is quite a bit of repetition in the introduction, including of methods, and I would instead use this word count to introduce the photosynthetic and stomatal measures more clearly and fully, and indicate why they are important for this study.

We have rewritten this paragraph to detail the main traits used in this study and what they tell us about whether plants may be limited by nutrients or water and to define *V*cmax and *J*max as indicators of photosynthetic capacity. The revised paragraph starts on line XX of the unmarked manuscript and is copied below:

“Photosynthesis links ecosystem carbon, nutrient, and water cycles in terrestrial ecosystems (Hungate et al., 2003). Through photosynthesis, plants convert carbon dioxide into simple sugars using enzymes such as Ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase (Rubisco). Building and maintaining these enzymes create a large nutrients and energy demand for the plant (Evans & Clarke, 2019; Evans & Seemann, 1989). Photosynthetic capacity, or the biochemically controlled rate at which a leaf can fix carbon, is typically estimated using the maximum rate of Rubisco carboxylation (*V*cmax) and the maximum rate of electron transport for RuBP regeneration (*J*max) (Ali et al., 2015; Farquhar et al., 1980). *V*cmax and *J*max are often positively correlated with leaf nitrogen and phosphorus content and are commonly used as a physiological indicator of nutrient stress (Ellsworth et al., 2022; Evans, 1989; Walker et al., 2014). Photosynthesis is also regulated by stomatal conductance, which controls CO2 uptake into the leaf from the atmosphere and supports transpiration (Farquhar & Sharkey, 1982). Transpiration allow for the uptake and transport of water and nutrients by the roots in the soil through the plant’s vasculat system to photosynthetic tissues. Stomates close and stomatal conductance generally declines with increasing water limitation, making it a useful physiological indicator of water stress (Medrano et al., 2002). Because leaf-level photosynthesis reflects both photosynthetic capacity and stomatal conductance, assessing how both respond to allelopathic invasion can help clarify the physiological mechanisms that drive native species responses.”

L70 – what is meant by resource limited environments? Most environments are limited by something – may have lots of light, but little water, or lots of water but little P. Are resource rich environments agricultural settings?

We clarify that soil resources (water and nutrients) are limiting.

L120: ‘have not quantified photosynthetic capacity responses…’ of what?

The sentence now reads: “However, the mechanisms that regulate the responses of coexisting native species to *A. petiolata* are poorly understood in part because they have not been previously quantified”.

L116-132: What about competition, irrespective of allelopathic effects. Something more is needed here to say ‘all else being equal’ the allelopathy should…

Line XX now reads: “Thus, all else being equal (e.g., competition for soil resources), disruptions in AM fungal mutualisms due to allelopathy could cause native plants to be unable to satisfy the demand to build and maintain photosynthetic enzymes and/or maintain optimal stomatal conductance, which may explain why native species exhibit reduced net photosynthesis rates in response to allelopathic invaders (Hale et al., 2011, 2016).”

L133-143 – this is methods and should not be in the introduction.

These sentences have been removed.

L148-152: these lines repeat 120-122.

Repeated content removed.

L170- expand PA and give country.

Done.

L175: ‘was’ to ‘has been’.

Changed as suggested.

L187: Could delete “These patterns have been observed despite evidence that…”. Also, are these soil measurements from the seasonal time periods relevant to this study? Or just one time point? This is key given the different temporal findings of this work.

The sentence now reads “Additionally, soil nutrient availability and soil water availability did not differ between *A. petiolata* treatments (Bialic-Murphy et al., 2021; Burke et al., 2019), at a single timepoint.”

L363: Was this change after canopy closure, or was this just when it was measured? Presumably there was a gradual change as the canopy closed. It is not a big deal, but it is currently saying something more than what is actually known. Take care with wording about this, also on line 400 – perhaps ‘measured after canopy closure’. Could you mark canopy closure on Figure 2? And photosynthetic measurement dates?

We have changed our phrasing in the Results and Discussion sections to clearly reflect the measurement timing.

Figure 3 could be simplified by removing duplicated axes titles in on the y-axes in the second column and the ‘Tree canopy status’ label on the x axis and potentially species headings in a, b, c, d.

We removed duplicated axis labels in this figure and all other figures that were presented in a similar way.

L487: What did Bialic-Murphy et al. (2021) find? Expand here or refer to Table 1, and specific rows.

L487 now reads: “Building on results reported in Bialic-Murphy et al. (2021), who showed that *A. petiolata* treatments modify AMF community structure and native plant nutrient and water economies (Table 1), our new results suggest that *A. petiolata* invasion modifies net photosynthesis rates by altering nutrient uptake and allocation to photosynthetic enzymes in *Trillium* spp. and, by altering water uptake and use for photosynthesis in *M. racemosum*.”

Figures – overall great colour selection, really clear figure legends. Consistency throughout makes them easy to follow.

Thank you.

REVIEWER'S COMMENTS TO THE AUTHORS:

Reviewer: 1

Comments to the Corresponding author  
The paper is well written and certainly falls within the aims of Functional Ecology, as it “provides important insight into understanding the mechanisms that drive photosynthetic responses to allelopathic plant invasion and are a critical piece of empirical data needed to link the effects of allelopathic plant invasion on belowground soil microbial communities with its effects on plant population and community dynamics.”

The main drawback is the Discussion that must be shortened; the same concept of the underlying mechanisms is overly reiterated throughout this section. The Introduction needs to be shortened as well.

The revised manuscript streamlines both the Introduction and Discussion sections.

The Introduction provides a quite robust background upon which the authors build up their mechanistic explanation. However, a lack of direct measurement of the colonisation rate and of its presumable link with native plant physiology responses to *A. petiolata* makes the suggested mechanisms quite speculative. From the paper of Bialic-Murphy 2021 (*Ecology Letters*) on the same species, I do not see such a significant difference. No differences emerged between ambient and weeded treatments in terms of root colonisation by arbuscules for both species and aseptate hyphae only for *Mainanthemum*, despite a significantly different fungal community composition in the mineral soil layer. The only difference was a significant reduction in aseptate hyphae roots of *Trillium* under weeded treatment, not the ambient.

Bialic-Murphy et al. (2021) found limited support for *A. petiolata’s* effects on root colonization and aseptate hyphae. However, differences in fungal community composition in the mineral soil layer could have lasting impacts on plant communities, especially if shifts in AM fungal communities are associated with either more generalist fungi or an increase in the relative proportion of pathogenic fungi. We make it clear that the link between AM fungal community structure and plant physiological responses that we present is speculative. The results of this paper provide the foundation for ongoing research that directly addresses the mechanistic link between AM fungal communities and plant physiology.

The main findings indicated that *Trillium* spp. responded to the presence of *A. petiolata* only under closed canopy conditions, while *M. racemosum* always responded to *A. petiolata* independently of canopy openness (timing). If the lack of AM fungal partners later in June may explain the reduced Anet and Jmax for *Trillium* spp., the lack of water may explain the reduced Anet, g and increased stomatal limitation for *Maianthemum* only later in June, not in early May when water is available, even if slightly lower than weeded treatment? Why this kind of response? Why does *Maianthemum* respond to the presence of *A. petiolata* in the first half of May?

In the previous analysis, we used models that included individuals that had measurements at a single timepoint as well as both timepoints. In the statistical approach presented in the revision, we only include individuals that were measured at both timepoints and included individual identity as an additional random intercept term to account for repeated measures. This allows us to more robustly assess the temporal effects of treatments on gas exchange. While the main results are unchanged, net photosynthesis and stomatal conductance responses to *A. petiolata* treatments in *M. racemosum* are now only observed after tree canopy closure. *Trillium* spp. and *M. racemosum* responses to *A. petiolata* treatments follow the same pattern, with no treatment differences observed early in the season and significant negative effects of *A. petiolata* observed after canopy closure.

Moreover, SWC is lower in the *A. petiolata*-ambient treatment, particularly in June. If AM partners are absent, why is the soil water content lower? If mycorrhizas facilitate water supply to the plant and its consequent reduction into the soil, shouldn't their absence have the opposite effect, i.e., a less pronounced reduction?

Sure, this is a possible explanation; however, the presence of the allelopathic invader in the *A. petiolata*-ambient treatment, especially at high densities, should promote reduced plot-level water availability due to the increased water uptake of the invasive species.

I think a Conclusions is not necessary.

Removed.

In conclusion, I suggest resubmitting a revised version with a Discussion mainly focused on the investigated traits and a more cautious approach to the underlying mechanisms for the observed outcomes.

The revised the manuscript is more cautious in assigning causal mechanisms that link the observed responses.

L198. To underline the perennial status of this type of species, i.e., geophytes, I suggest rewriting it as, "... are each understory perennial herbs that form rhizomes (geophytes), with widespread…”. for example.

Done.

L235. Maybe is it “Restrictions on triose phosphate utilisation were included as an additional rate-limiting step.” or not?

Done.

In the figures, in my opinion, the temporal information is missing. Open and closed canopies may also indicate two different forest management stands that are adjacent to each other in space. I think the reading could improve by adding the months “open apr-may” and “closed June” with the months in the line below. It is just an idea.

We have added temporal information to all plots. The x-axis labels now read as “Open (April-May)” and “Closed (June)”.

The table in the supplementary material concerning the ANOVA on chemical traits needs the first place. I suggest making Table S1 a main-text-table, (it should become Table 3 if I am correct). To make it not large, I suggest transposing treatments in columns and all six variables in rows: Soil nitrogen availability, Soil NO3-N availability, Soil NH4-N availability, Soil phosphate availability, Soil N:P, Soil water content.

We moved Table S1 and S2 into the main text as Table 3 and transposed treatments into columns and variables in rows as suggested.

Figure 4. As for Figure 3, standardising the Y axis should improve readability, particularly when comparing species, despite the contraction of the box for *M. racemosum*. So, 200 for Vcmax25 and 300 for Jmax25 for both species. As stated in L556-559, Vcmax and Jmax of *Maianthemum* are nearly half of those of *Trillium*, but this is not easily observable from the current graphs.

Done.

*M. racemosum* responded to the presence of *A. petiolata* independently of canopy openness (timing); *Trillium* spp. responded to the presence of *A. petiolata* only under closed canopy. That’s all.

Our new analyses, discussed above, show that both species respond similarly (after canopy closure only). This comment is no longer applicable.

L480-515. This section emphasises the effect of the presence of *A. petiolata* on both species; however, on *Trillium* spp., it was only late in June, as explained more thoroughly in the following sections. In fact, in L480-490, I disagree with this point. The response was very different between the two species. From graphs and Table 3 and 4, in *Trillium* this reduction was significant for Anet and Jmax only under closed canopy. This species reacts mainly to a closed canopy. Based on the data distribution in the box plot, the significance of *A. petiolata* treatment for Anet (P=0.016) in *Trillium* and for stomatal limitation (P=<0.001) in *Maianthemum* is quite ambiguous. For these two variables, the response pattern inferable from the box plot graphs justifies the interaction AxC effect (in fact, it is significant) rather than the main effect. Anyhow, for Anet, the great difference between the P values, i.e., 0.016 for *Trillium* spp. compared with <0.001 for *Maianthemum* is not of secondary importance.

Please see previous comment/response.

Why does *Maianthemum* respond to the presence of *A. petiolata* in early spring, when water is available even if slightly lower than weeded treatment?

Please see previous comment/response.

L497. What does it mean “strongest”? It is best to stick to an intensity indication, i.e. higher-lower, increase-decrease, and similar. For example: “Indeed, the stronger late-season photosynthetic rate reduction to ambient levels of *A. petiolata*…”

We have changed our wording throughout the rest of the manuscript to indicate direction and magnitude.

L505. Maybe, but SWC is lower in the *A. petiolata*-ambient treatment. If AM partners are absent, why is the soil water content lower? If mycorrhizas facilitate water supply, shouldn't their absence have the opposite effect?

This is plausible. However, it is also likely that presence of additional plants in the *A. petiolata*-ambient treatment (i.e. *A. petiolata* allowed to persist) results in greater plot-level water uptake, rendering these plots with less water availability compared to the *A. petiolata*-weeded treatment. We now discuss these options.

L521-533. Again, I agree with these explanations, but only under closed canopy conditions, i.e., with lower nutrient and water availability.

We added the sentence: “It is important to note that this study cannot separate the effects of canopy closure from those of soil resource availability. Further research is needed to understand the causal mechanism driving these temporal effects.”

L584. Again, the soil moisture content between the weeded and ambient treatments differs only slightly in the first half of May.

This is not a correct characterization of our results. Model results and Fig. 2c suggest that reductions in soil moisture persist across the measurement period when *A. petiolata* is present. Model results indicate that there is no interaction between day of year and *A. petiolata* treatment. This is supported by Fig. 2c, where the slopes (i.e. day of year effect on soil moisture) is similar between *A. petiolata* treatments, but the treatments exhibit different y-intercepts.

L602. Except for soil NH4-N availability, canopy status significantly affected all the traits investigated. That’s why canopy status has been the main environmental factor contributing to the observed responses, there might be some surprises coming out.

As noted above, we now discuss how we cannot disentangle canopy effects from soil resource availability effects: “It is important to note that this study cannot separate the effects of canopy closure from those of soil resource availability. Further research is needed to understand the causal mechanism driving these temporal effects.”