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[date]

Dear Editorial Board at *Functional Ecology*,

Thank you for considering our manuscript (FE-2025-00191), titled “The negative effects of an allelopathic invader on native plant photosynthesis intensify as the growth season progresses” for publication at *Functional Ecology*. We appreciate the constructive reviews and have revised our manuscript in accordance with Associate Editor and reviewer feedback and believe these changes have improved the manuscript. The revised manuscript is attached along with a copy that contains changes that are noted through the “Track Changes” feature in Microsoft Word.

The revised manuscript now includes… XXX.

These and all other revisions are fully 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 of the revised manuscript and text excerpts are included in our responses to facilitate review.

Please contact me at the e-mail address listed above 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 kind words about our manuscript and its potential impact. We appreciate the suggestions to refine the language surrounding our results and have adjusted the manuscript per suggestions from the line comments below.

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

We agree and have modified the phrasing of this sentence accordingly. The new sentence reads: “Allelopathy is estimated to occur in approximately 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.

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 appreciate the suggestion to streamline the Introduction and replace repetitive content with clearer explanations of the photosynthetic traits measured in the study. The revised manuscript now includes a clearer explanation of the photosynthetic traits and clearly defines some of the phrases used in the study (e.g., that photosynthetic capacity refers to Vcmax and Jmax) in the Introduction. We have copied the paragraph in the Introduction that defines these traits below for ease of review:

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?

Thank you for the opportunity to clarify this. This line specifically indicates soil resource limitation, either water availability or soil nutrient availability. The revised sentence now reads: “However, plants cannot increase nutrient and water uptake to a similar extent in soil resource-limited environments, as soil resource availability is insufficient to acquire and satisfy the demand for photosynthetic enzymes.”

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

We have added “of coexisting native species” here. The sentence now reads: “However, the mechanisms that regulate these responses are not fully understood, in part because such studies have not quantified photosynthetic capacity responses of coexisting native species to *A. petiolata*”.

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

Yes, this is a good point. We have modified this sentence as follows: “”

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

We have removed these sentences from the Introduction.

L148-152: these lines repeat 120-122.

L170- expand PA and give country.

Done.

L175: ‘was’ to ‘has been’.

Done.

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.

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?

This is a great point and is the subject of some forthcoming work from a second field season. In the present manuscript, we appreciate the suggestion to be more careful with our wording and characterization of the results and have made adjustments accordingly.

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.

Thank you for the suggestion, we have removed duplicated axis labels in this figure and have extended this to remove duplicate axis titles in all other figures.

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

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.”

Thank you for the positive comments on our manuscript.

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.

Thank you for this suggestion and we agree. The revised version of the manuscript now streamlines content in 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.

Thank you for the positive comment about our Introduction. The reviewer raises some important points here and, indeed, Bialic-Murphy et al. (2021) find limited support for *A. petiolata* 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. Indeed, the link between AM fungal community structure and plant physiological responses are speculative and are intended to be presented as such. There are other works in the pipeline that more thoroughly address the mechanistic link between AM fungal communities and plant physiology from which this work was designed to provide the foundation for.

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?

This is a great question. We suspect that reduced water availability early in the growth season may have increased demand for acquiring water in *Maianthemum*

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?

There are many reasons as to why soil water content might be lower in the *A. petiolata*-ambient treatment.

I think a Conclusions is not necessary.

The conclusions section has been 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.

We agree with the reviewer’s suggestion and have revised the manuscript to be more cautious 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.

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

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.

This is a great idea. We have added temporal information to all plots. The x-axis labels now read “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.

Thank you for this suggestion. Table S1 and S2 have been moved into the main text as Tables 3 and 4. We have also 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.

We have standardized the Y-axis values between the two species for all figures.

*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.

The reviewer’s assessment of our results is correct; however, we call attention to revised results that now report marginal effects of *A. petiolata* independent of canopy openness on net photosynthesis. (*p*=0.062). Additionally, *M. racemosum* responses to *A. petiolate* are also strongest under closed canopy.

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.

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

Updated analyses suggest that net photosynthesis responses only occur later in the season

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*…”

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?

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

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

This is correct.

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.