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Effects of soil fungi on weed communities in a corn–soybean rotation – ADDENDUM

Authors' response to review comments

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At the Editor's suggestion, we are including a set of thoughtful review comments from one of our reviewers on the first revision of our manuscript, and our responses to the reviewer's comments.

Reviewer 1: Although the author's revision has improved the manuscript to some extent the primary criticism on the original manuscript has not been addressed adequately. The authors' decision to keep highly speculative (conclusive) statements in spite of minor and variable evidence supporting their hypothesis only undermines the quality of the discussion and the paper. For example, authors discounted the effects of fungicides on fungal diseases based on one glasshouse trial which showed variable result. The effects on AMF were also variable and moderate. Statements suggesting AMF as a potential biocontrol measure do little to support the main hypothesis of the study. Authors have not discussed the role of fungicide effects on nutrient availability when discussing the growth of weed species.

Overall there needs to be further modifications to the discussion section to focus on the main objective in order for this paper to be acceptable for publication.

Essentially, we see three issues raised in reviewer 1's comment:

I. Are we overstating our results with respect to the 'main objective' of our experiment, namely to assess whether soil fungal communities can have effects on field crop weeds that are significant in agronomic and agroecological terms?

The reviewer expressed concern that our conclusions are 'highly speculative', and that our results provide only 'minor and variable evidence supporting their hypothesis'. Of course, whether evidence is 'minor and variable' is a matter of opinion, but we believe that our findings do reveal that soil fungi can have significant effects on weeds.

If we discount results from the first year of fungicide treatments (a variety of other studies show that benomyl effects on soil fungi are cumulative and therefore we did not expect a substantial biological response after the first season of fungicide applications), then our results can be summarized as follows:

- Significant effects first appeared after the second season of fungicide applications, and a wider range of effects was evident in the third year.
- In the second year, there was essentially only one significant fungicide effect, but this was not 'minor' in agronomic terms: fungicide caused a 34% increase in total weed density in the corn crop, with associated effects on the composition and evenness of the weed community. Such an increase in weed density creates the potential for significant loss of yield, and for significantly increased weed pressure in the future.
- In the third year, fungicide caused effects in both crops that again are not 'minor' in agronomic terms. In corn, total biomass increased by 49%, an increase with significant implications for yield and future weed pressure, and there were associated effects on weed community composition and on the relative performance of host and non-host weeds. In soybean, there were large changes in the relative performance of these two weed classes, which was associated with large changes in the performance of two non-host weeds that can pose major management problems in corn–soybean production systems (lambsquarters and redroot pigweed).

Furthermore, a number of aspects of our design, especially the use of a fungicide known to be only partially effective on AM fungi (as demonstrated by the observed effects of the fungicide treatments) make for a relatively conservative test of our main hypothesis. Therefore, our observations of arguably 'non-minor' effects despite conservative aspects of our design further justifies the conclusions that we draw in the discussion section.

Finally, a recent report[1] emphasizes that caution is needed in interpreting fungicide effects on plant communities, but, consistent with our interpretations of our results, does not dispute that the major mechanism of these effects is through changes in plant–fungi interactions. They view the major complication as the question of separating direct and indirect effects of changes in these interactions, as we discuss.

We excerpt our summary statement of findings from the discussion:

Our findings indicate that the population and community ecology of these field-crop weeds can be strongly affected by the fungal component of soil microbiota. This result emerged despite the relatively low power of our experimental design, and suggests that the influence of

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soil fungi on population and community ecology of field-crop weeds can be large, given that our fungicide application rates apparently caused only moderate reductions in AMF symbiosis with AMF-host weed species (as indicated by colonization levels).

We see this statement as well justified, not excessively broad ('... field-crop weeds can be strongly affected ... can be large') and not 'highly speculative'.

II. The second issue raised by the reviewer's comments, we believe, is whether we have erred in attributing the fungicide effects we observed to responses mediated by soil fungi, as opposed to some other artifactual effect(s) of fungicide application. The reviewer identifies one artifactual effect that exemplifies this concern: 'Authors have not discussed the role of fungicide effects on nutrient availability when discussing the growth of weed species'.

We appreciate the potential importance of artifactual effects identified by the reviewer, but we do remain confident in attributing our results primarily to effects on soil fungi in general. First, as we note in the manuscript, there is a significant base of evidence supporting this conclusion from previous work: 'Benomyl has been repeatedly shown to alter soil fungal communities and thereby to affect plant population and community processes, and to primarily affect AMF'. In particular, a number of studies has examined the effects of benomyl applications on other soil attributes that might be relevant to the plant population and community attributes of interest. These have come to a unanimous conclusion that benomyl principally affects fungi, and apparently does not have large artifactual effects. To elaborate:

'The patterns of effects of the fungicides on soil nutrient cycling processes were not large and were specific to each fungicide. Captan appeared to have more pronounced overall effects on soil microbial activity and nitrogen dynamics than either benomyl or chlorothalonil'[2].

'The magnitude of benomyl effects on soil components and processes were small ($<33\%$ change with benomyl) relative to effects on mycorrhizal root colonization (80% decrease with benomyl). These results indicate that rather than having large non-target effects, benomyl applications principally affect mycorrhizal root colonization, thereby indirectly influencing soil biota and nutrient availability'[3].

'It can be concluded that although pesticides had transient effects on microorganisms and, possibly, some microbivorous animals, their influence on nutrient dynamics was negligible and they did not affect plant growth indirectly'[4].

On a related note, the reviewer provides this criticism: 'For example, authors discounted the effects of fungicides on fungal diseases based on one glasshouse trial which showed variable result (sic)'. This is a fair and useful comment, and we have revised the discussion to be more cautious in attribution of observed effects of AMF.

III. Finally, the reviewer remains concerned about our comments in the discussion section regarding the possibility of antagonistic effects of AMF to non-host species, stating that 'Statements suggesting AMF as a potential biocontrol measure do little to support the main hypothesis of the study'.

We wish to clarify that the comments that concern the reviewer do not seem to us to be directly relevant to the veracity of the main hypothesis, but rather represent our effort to interpret the significance and meaning of our findings relevant to that hypothesis. However, in response to the reviewer's concern, we have downplayed the discussion of AMF antagonism toward non-host species, since indeed our results did show that promotion of the relative performance of non-hosts by fungicide was species-specific, and did not indicate a more general response by the non-host species group. We would be reluctant to remove all discussion of this phenomenon, however, as we noted in the manuscript, a substantial number of independent studies (cited in manuscript) have observed quite powerful antagonisms of this sort in a variety of plant communities. Therefore, our suggestion that we may be observing such effects in a field-crop weed community is not 'highly speculative'.

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