

Dear Editor,

Thank you for the evaluation of our revised manuscript. We conducted the minor revisions requested. Please find below a point-by-point response to the comments of the two referees. Please find the updated version without apparent track-change, followed by the revised version, with track-change. Please note that the line numbers refer to the revised version without track change. All co-authors had a final read of the manuscript and agreed with this final re-submitted version.

Best wishes,

Amandine Erktan, on behalf of the co-author team.

Report 1 - # Referee 3

The manuscript has been greatly improved. I am satisfied with the authors' responses to the comments. I suggest that it is ready for publication.

We are pleased that the corrections conducted satisfied Referee # 3.

Report 2 - # Referee 2

After reviewing this manuscript for a second time, I believe that the authors have done a nice job of addressing my comments. The introduction is far less repetitive and also emphasizes the broader scope of their study. I congratulate them on the hard work they have put into this revision.

We thank Referee # 2 for the positive evaluation of our revised manuscript.

My only main concern regarding their methodology is with regard to their CUE assessment. They attempt to discern whether C came from litter or from soil by examining the difference in ^{13}C in C4 versus C3 plants. However, their little mixture included both C4 and C3 plants, making it difficult to decipher the source of C. I request that they clarify their methodology, or include an important caveat into their methods/results about how C could have come from litter that was attributed to soil

We agree that a bias is possible in the estimation of the two C sources, however, this bias likely is small. As noted by # Referee 2, the presence of wheat in the chopped litter (otherwise mainly composed of maize) may introduce a bias in the estimation of the relative importance of the C sources. However, such bias can occur only if microbes preferentially feed on wheat or maize litter, resulting in under- or overestimating the proportion of the chopped litter as C source. Otherwise, the reference value for chopped litter already accounts for the mixture of wheat and maize. Notably, the $\delta^{13}\text{C}$ value of the chopped litter (13.71 ‰), is close to that of maize as C4 plant (about 11 ‰) reflecting the dominance of maize in the chopped litter. Not strictly proportional use of maize and wheat litter C by microorganisms therefore are unlikely to significantly affect our calculations. Importantly, for affecting our treatment effects on the use of litter C by microorganisms it would be necessary that microorganisms in the different treatments use wheat / maize litter C in different proportions, which is highly unlikely especially considering that the litter materials were thoroughly fragmented and the microbial communities in the different bacterial as well as fungal treatments differed little. Further, especially early during decomposition differential use of litter C

from maize and wheat is unlikely. In the revised version of the manuscript, we added the following sentence to point to the possible bias related to the heterogeneity in $\delta^{13}\text{C}$ signal of the chopped litter (l.198-201): "Using the mean $\delta^{13}\text{C}$ value of litter in this calculation assumes that carbon from maize and wheat litter in the litter mixture was used indiscriminately, which we assume to be justified considering the short period of time when predominantly labile litter compounds are used; further, considering that the litter mixture comprised predominantly maize litter differential incorporation of carbon from wheat is likely to affect the calculations only little." Finally, although the calculated relative importance of C sources may include some bias, we argue that the dominant use of soil C vs. litter C cannot be the result of an artefact.

One minor concern is that there are frequent locations where the grammar needs revising. I have tried to help point these areas out, but I may have missed a few. In general the manuscript is well written, but there are some consistent subject-verb tense issues as well as a few awkward phrasing locations.

We apologize for the errors in the grammar. We conducted the minor revision requested and double checked the English.

However, overall I find this to be a well designed study and of importance to the field.

We thank Referee # 2 for the positive comments on our manuscript.

MINOR COMMENTS:

Line 43: should be "has been" not "have been"

Corrections have been done accordingly (l.45)

Line 49: change "Since" to "For"

"Since a decade" was replaced by "In the last decade" (l.51)

Line 51: change linking to link

Corrections have been done accordingly (l.53)

Line 63: change to "higher contents of lipids and proteins" or "higher lipid and protein content"

We modified to "higher lipid and protein content" as suggested (l.68-69)

Line 80: change as well to also

"as well" was replaced by "also" as suggested (l.81)

Line 81: change to evidence, not evidences

"Evidences" was replaced by "evidence" (l.82).

Line 89: change to "has also been reported"

“as well have been reported” was replaced by “has also been reported” as suggested (l.90)

Line 125: were added

“was added” was replaced by “were added” as suggested (l.127).

Line 138-139: I feel like there is a word missing or something does not connect in these lines.

The sentence “A. castellanii is able to feed on our model strain of P. fluorescens (Jousset et al. 2009), but prefers less toxic strains” was rephrased as follow: “The amoeba species A. castellanii was shown to feed on our model strain P. fluorescens (Jousset et al. 2009), but prefers less toxic strains” (l.139-140)

Line 143: H. nitidus shows

The sentence was rephrased as follow: “The collembolan species H. nitidus was chosen because of its abundance in European temperate soils (Hopkin, 1997) and as it has been shown to preferentially feed on C. globosum (Pollierer et al., 2019); feeding on C. globosum it is able to survive and reproduce.” (l.142-144)

Line 144: “when fed this” drop “on”

“The collembolan species H. nitidus was chosen because of its abundance in European temperate soils (Hopkin, 1997) and for its appetite for C. globosum (Pollierer et al., 2019). H. nitidus is showing a strong preference for C. globosum and is able to survive and reproduce when fed on this saprotrophic fungus. ” was replaced by “The collembolan species H. nitidus was chosen because of its abundance in European temperate soils (Hopkin, 1997) and as it has been shown to preferentially feed on C. globosum (Pollierer et al., 2019); feeding on C. globosum it is able to survive and reproduce.” (l.142-144)

Line 144: change as well to also

“H. nitidus is as well” was replaced by “H. nitidus is also” as suggested (l.144).

Line 160: You mention that the litter was a mixture of maize and wheat, though more dominated by maize. Because the agricultural soil C is also mainly from wheat, how are you able to split your understanding of whether the soil C consumption was from the litter or soil organic matter?

Indeed, the difference in the signal from maize (C4) and wheat (C3) plant was used to calculate the relative importance of the two C sources, namely the soil C and the added chopped litter. The added litter was indeed not composed by pure maize litter. We argue, however, that this little affected our calculations. We agree with referee # 2 that there is a risk of a potential bias in the calculation of the relative importance of the two C sources because of the heterogeneity of the $\delta^{13}\text{C}$ signal of the added chopped litter, but this risk remains low (see response to the 2nd comment of Referee # 2). We added the following sentence to point to this potential bias: “Using the mean $\delta^{13}\text{C}$ value of litter in this calculation assumes that carbon from maize and wheat litter in the litter mixture was used indiscriminately, which we assume to be justified considering the short period of time when predominantly labile litter compounds are used; further, considering that the litter mixture comprised predominantly maize litter differential incorporation of carbon from wheat is likely to affect the calculations only little.” (l.197-200)

Line 232: “were” captured

“was” was replaced by “were” as suggested (l.235)

Line 243: remove the word “only”

“only” was removed as suggested (l.246)

Line 250: change as well to also had

“as well” was replaced by “also” (l.254)

Line 253: remove as well

“as well” was removed as suggested (l.256)

While not an essential change (and admittedly a preference of this reviewer), it would be much better to see the actual p-values instead of > or < 0.05 values.

We agree with Referee # 2 and modified the text to show the real P-values instead of the significance levels.

Line 271: insert the at “in THE presence of”

“in presence of” was replaced by “in the presence of” (l.275)

A more general opening paragraph to the discussion would be helpful. The authors jump into results again and then focus on specific results quite quickly. I would highly recommend a summarizing paragraph that emphasizes the key results and their relevance before jumping into such detail.

*We added a general summary of the main results at the beginning of the discussion (l.331 – 344): “Our results showed that simplified trophic interactions modified microbial community composition and soil aggregation, but did not or only little affect C dynamics. Overall, the effects were stronger in the fungal-based system than in the bacterial-based system. In the latter, the inoculation of *P. fluorescens* as dominant bacterial strain in large drove the changes in microbial community composition, whereas the addition of the amoeba predator *A. castellanii* did not induce further changes, presumably because *P. fluorescens* is a less preferred and toxic strain for *A. castellanii*. However, *A. castellanii* enhanced the formation of soil aggregates, presumably related to changes in the production of bacterial EPS in response to the attack by *A. castellanii*. In the fungal-based system, conform to our expectations, the inoculation of *C. globosum* increased fungal biomass and the addition of the grazer *H. nitidus* reduced it. These variations in fungal biomass were positively related to changes in soil aggregation, suggesting a detrimental effect of collembolans on soil aggregation. Surprisingly, the inoculation of *C. globosum* and *H. nitidus* resulted in significantly modified bacterial biomass and composition, and this was related to changes in soil aggregation. Finally, in the bacterial- and fungal-based systems, soil organic matter was the dominant C source and inoculation steps only weakly modified the relative importance of soil vs. added chopped litter as microbial C source. Notably, the inoculation treatments did not significantly affect SOC concentrations*

and CO2 emissions, suggesting that despite trophic interactions significantly modify microbial communities and soil aggregation this may not change soil C dynamics.”

Additionally, the first paragraph of the discussion is currently quite long and addresses multiple ideas. Could the authors break it up into more than one paragraph?

We split the first paragraph in two paragraphs. The first one (l.346-351) deals with the effect of the inoculation of P. fluorescens on the microbial community. The second one (l.353-373) deals with the lack of effect of A. castellanii on the microbial community.

Line 388: awkward phrasing in “Despite of that”

“Despite of that” was replaced by “However”. Sorry for the awkward phrasing (l.408)

Line 406: change to “also feeding on bacteria”

“feeding as well on bacteria” was replaced by “also feeding on bacteria” as suggested (l.426).

Line 414: as well to also

“as well may have modified” was replaced by “also may have modified” as suggested (l.433)

Line 414: it has “long been assumed”

“it has been assumed for long” was replaced by “it has long been assumed” as suggested (l.434)

Line 416: “a” microbial wash

“addition of microbial wash” was replaced by “addition of a microbial wash” as suggested (l.436)

Line 432: change to “were observed”

“Effects, however, were minor as no overall changes in bacterial C source was observed between treatments and soil C remained the main source of carbon for bacterial communities.” Effects, however, were minor and did not affect bacterial C source which remained to be based mainly on soil C.” (l.451)

Table 2: Again it would be preferable to have real p-values

We replaced the significance code by the real P-value in the table 2 and in all other tables and figures (as well as in the text).

Figure 2b: What does NRD stand for?

Thank you for pointing this mistake. RND meant “natural reduced diversity” in a previous version of the manuscript. We forgot to change it here. In the revised version, we now modified RDN to RMB, standing for “remaining microbial background”.