

Interactive comment on “Multi-cooperation of soil biota in the plough layer is the key for conservation tillage to improve N availability and crop yield” by Shixiu Zhang et al.

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Received and published: 26 May 2020

1. Material and Methods What was the motive behind choosing 0-5 and 5-15 cm soil layer for soil biota sampling and N mineralization when the plow layer for conventional tillage was 20 cm? For the latter case, tillage operation mixed the soil layer of 0-20 cm. Why bulk density was recorded at 5 cm and 10 cm and not 0-5 and 5-15 cm soil depth? The difference in bulk density might affect the soil N mineralization.

Soil stratification is a typical characteristic of conservation tillage, because there is a contrasting difference between top soil (usually means 0-5 cm) and the sub soil. Using either 5-15 cm or 5-20 cm to investigate the conservation tillage effect on the sub-soil

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depth is very common in the literature (for example, 5-15 cm in the study of Gómez-Rey, et al., 2012; 5-20 cm in Haplern et al., 2010). Our previous study found that there was no significant difference between these two soil depths (5-15 cm and 5-20 cm) in soil C, N, bulk density, soil water content, and the other parameters of soil physicochemistry, but there was a slight difference in the abundance of soil collembolans and mites. Their abundance at the 20 cm was very low. So, on this basis, we think it is more reasonable to use 5-15 cm to investigate the role of soil organisms. We rewrote the description in the paper about how the soil bulk density was determined.

2. Line 95, Zhang et al. (2019) used 40 kg N ha⁻¹ in the soybean field. Moreover, there might be atmospheric N deposition. Therefore, all or part of the N from the applied fertilizer and/or atmospheric N deposition can be taken up by soybean and help to increase the yield of soybean, how this effect of N fertilization on crop N uptake/yield was separated from N contribution by a different trophic group of soil organisms? Please explain why N fertilizer (40 kg N ha⁻¹) plot was considered as a suitable reference to estimate background crop yield/N response.

We focused on investigating the difference of soil organisms among different tillage systems, not on the soil input N. Furthermore, the amount of soil input N as fertilizer is the same in all tillage systems; the amount of N fertilizer applied is typical for soybean crop grown by local farmers. For the deposition of atmospheric N, its contribution can be neglected even if it is not uniformly distributed in the atmosphere, because it is very small relative to the amount of nitrogen fertilizer; further, all plots in the experimental site would receive the same deposition from the atmosphere. Therefore, in this context, there would be no significant difference in the utilization of N in soybean of the same variety.

3. Line 107, please add the soil depth at which temperature was recorded.

Soil depth has been added.

4. Line 119, Why the mineral N before incubation was measured and not after one

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week in the potential N mineralization method, ideally mineral N can be subtracted after 1 week of incubation. Since this time frame is used to enumerate the biota activity at optimal temperature and moisture content. Therefore, N mineralized during this time would be low and if this deleterious effects would not be adjusted then this effect may lead to underestimation of N mineral from the soil (Bloem et al. 1994).

In here, our purpose was to compare the difference between tillage systems rather than to obtain the absolute real value of soil N mineralization. Since the same test method was used for all tillage systems, errors or biases caused by the test method would be the same for samples collected from different tillage systems. But, we agree with the reviewer's suggestion that the activity of soil organisms may reduced after 4 weeks incubation. So, we used the inorganic nitrogen content measured in fresh soil every month instead of this amount of mineralized N obtained through lab incubation to indicate the status of soil N during soybean growth.

5.Line 148, please add the soil layer in cm where microarthropods were extracted? If the soil sample were collected from 15 cm soil depth, from the current unit it is not clear whether these organisms were extracted from 0-15 cm soil layer or 0-7.5 cm. How their contribution would be related to actual soil N mineralization from 0-5 and 5-15 cm? Although biota biomass from table 4 indicates the presence of these organisms in 0-5 and 5-15 cm, this should be explained in the methodology, in which depth actually the organisms were extracted.

The soil depths that soil organisms extracted from were added in the revised manuscript.

6.Earthworms were not present in this system or these organisms were not sampled from this experiment. Most of the published studies indicated their significant contribution after bacteria and fungi to N mineralization, it would be good to include their contribution in such systems.

The density of earthworms is less than 4 individual m⁻² and their fresh weight is less

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than 0.2 g individual⁻¹ m⁻² across all tillage systems. So, considering the low density and very small weight of earthworms in the studied region, we did not include them in this study.

7.Table S3, Why actual C:N ratio of the root of the soybean crop studied was not used. The currently used C:N ratio of soybean root is much less than the actual C:N ratio of the soybean, see for example (Kushwah et al. 2014; Redin et al. 2018). Such lower C:N ratio used in the calculation could lead to high N mineralization and hence overestimation of N mineralization in this category.

The C:N ratio of root in the literature (Kushwah et al. 2014; Redin et al. 2018) is based on the dry mass filled with the cellulose and lignin. But cellulose and lignin are not the main food for herbivoures. For example, plant-parasite nematodes primarily feed on the cytoplasm of root cells (Verschoor et al., 2002). So, using the actual C:N ratio of the soybean will underestimate the contribution of soil organisms to N mineralization. In our study, we used the C:N of the cytoplasm of root cells to indicate the C:N of root.

8.Table S4, values of biotic biomass were expressed in mg C m⁻², but I could not find the reference of Berg et al. (1998) to confirm the average C content of 48% dry biomass used for microarthropods.

You can find the following sentences in the part of material of Berg et al. (1998): "The C content was set at 47.7% C for Acarida (Teuben 1991), —, and 47.5% C of the total dry weight for Collembola (Teuben 1991)."

9.For the nematodes biomass C, Ferris (2010) also adjusted this 0.1 C factor by using the formula, $P_t = 0.1 W_t / m_t$, where P_t , W_t and m_t are the C used in production, the body weight, and the cp class of taxon t, respectively. However, these factors may also influence the C biomass which may lead to over/underestimation of the biomass and therefore N mineralization by this group of soil biota.

Ferris (2010) used the formula: $P_t = 0.1 W_t / m_t$ to calculate the production C. Please

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be note that the production C in not equal to the biomass C. The production C corresponds to the respiration C, which were both used to calculate the metabolic footprints of nematodes. So, the definitions of production C, respiration C and biomass C are very different.

10.The table S5, the contribution of N mineralization by a different group of soil organisms, these result are the main result according to the objective of the study, therefore can be moved to the main manuscript.

Thanks for your suggestion, we have reorganized the tables and figures in the revision as per your suggestion.

11.Line 163, please add the data about the number of taxa or abundance of soil organisms (nematodes and microarthropods in supplementary information or main text).

Thank you for your suggestion. The identified taxon was added as the supplementary information. The biomass of the identified taxa was moved to the main text..

12.Line 250, No difference in soybean yield among different treatments might be linked with the applied N fertilizer dose and/or atmospheric N deposition. Moreover, can you please explain why the difference in soil N mineralization among different treatments would not result in yield increment of soybean among different treatments? It seems yield was tended to be higher but did not differ significantly among treatments. Would the presentation of crop N uptake rather than crop yield explain the difference?

Just as the above what we said, there is no difference between the N input to soil among there tillage systems. So, it is unlikely that the high yield in conservation tillage, especially in NT, is related with the N fertilizer or atmospheric N deposition. The point is that, the total N mineralization of soil during the soybean growing season is distributed unevenly throughout the plow layer under conservation tillage systems. The amount of N mineralization at 0-5 cm was higher in RT and NT than in CT; but, the opposite trend was observed in 5-15 cm (Fig. 1). This poses a question, how did the deficit of

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mineral N in 5-15 cm support the high yield in RT and NT soils? We thought that the contribution of soil organisms to N mineralization may offset this disadvantage. And this is main reason why we calculated the N mineralization of the soil organisms in this study.

13.Fig. S1a, the P-values presented in the figure indicate that tillage, depth, and their interaction were significant, please use multiple comparisons to differentiate the effects, if done already please add letters on the bar to differentiate the effect of the treatments within or between the two depths. These are the main result, therefore, I suggest presenting them in the main manuscript rather than supplementary information.

Thank you for your suggestion, this was done in the revisoin

14.Line 251, presenting the biomass or abundance data in the main manuscript would add more value, therefore I would suggest adding this data in the manuscript.

Thank you for your suggestion, this was done in the revision.

15.Line 263, indicate that bacterivorous nematodes and omnivorous-predaceous nematodes contributed highest to N mineralization that was not the case in Table S5. Can you please discuss this difference in detail in the discussion section? Or I could not understand from the current formulation what do you mean?

These sentences were rewritten. What we want to describe is that the relative changes were most pronounced in bacterivorous nematodes and omnivorous-predaceous nematodes, their contribution to N mineralization was greatly improved under RT and NT soils.

16.Fig. 1 The response ratio of soil N mineralized during the growing season and crop yield was calculated, is that a fair comparison. Is it not better to use the response ratio of soil N mineralization and crop N uptake? To check for the accuracy of modeling: did the temporal variation in calculated N-mineralization rates correspond with the temporal variation in measured N-mineralization rates (potential N mineralization)? I could not

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see this in the manuscript. The main aim of the manuscript is to examine the influence of soil biota on coupling N mineralization with soybean yield therefore the current fig. 1 did not meet the objective. Hence, I would suggest to also include the response ratio of soil N calculated based on the modeling and soybean yield.

The description of response ratio was deleted in the revised manuscript.

17. Fig. 2, what is the difference between mineralization N delivered by soil biota and of the contribution of soil biota to soil mineralization N? Please clarify it.

Their units are different. For the mineralization N delivered by soil biota, the unit is expressed as kg N ha⁻¹; for the contribution of mineralization N of soil biota to soil mineralization N, the unit is dimensionless based on standardization. The contribution of mineralization N of soil biota to soil mineralization N was deleted in the manuscript to make the text more clear to readers.

18. Discussion Line 285, In the case of Holtkamp et al. (2011) bacteria and fungi contributed about 77% of the total N mineralized which is in line with Rashid et al. (2014), who estimated that the aforementioned biota contributed to the 60% of the soil N mineralized. So, bacteria and fungi but not the higher trophic groups were responsible for most of the soil N mineralization in their systems. Even in your system Table S5, the contribution of fungi is the highest followed by bacteria and there is an insignificant contribution to N mineralization is coming from nematodes and microarthropods. What do you mean by the higher trophic group here?

We want to express that “For the mineral N delivered by soil organisms, the differences between CT and RT and NT were more pronounced for higher trophic groups than for lower trophic ones”. It is not our purpose to compare the amount of mineralization N of soil organisms or the contribution of mineralization N of soil organisms to soil N mineralization among different trophic groups. Our focus on the comparison between different tillage systems, because these differences among tillage systems may be the primary reason of soil N mineralization and plant yield differences.

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19. Lines 328-330, why fungal pathways were dominated in the soil layer 0-5 and bacterial pathways in the layer 5-15 cm in RT and NT tillage? Can you please mechanistically explain how these pathways contributed to soybean yield? In lines 335-341, I expected the discussion on why the fungal pathways were dominated contributors of soybean yield in 0-5 cm and bacterial pathways in 5-15 cm soil layer? Can you please discuss further how and why these pathways were dominated in these layer under RT and NT tillage operations.

We reconstructed the soil food web and calculated the mineral N delivered by soil organisms, and then found that RT and NT mainly drive the N mineralization through fungal and bacterial channels at the whole plow layer (0-15 cm). But, when use stepwise regression analysis to relate the N mineralization of different channels with soybean yield, the results have shown that at 0-5 cm, fungal channel was significantly related with soybean yield, while at 5-15 cm, bacterial channel was strongly related with soybean yield. These results suggest that the different pathways of soil organisms relating N mineralization and plant yield.

20. The manuscript uses modeling to estimate various fluxes of N in the soybean. In the model, a lot of parameters were taken from literature rather than from measurements in the actual sites. What the authors fail to discuss (and to mention), is that there is a degree of uncertainty associated with any model. Each estimate based on modelling equations comes with the error range. Depending on the model and the parameter in question, this error range can be small or large. Therefore, a sensitivity analysis should be carried out. Moreover, it needs to be mentioned, if any conclusions are to be drawn based on model-derived numbers. A model estimate for any parameter should never be presented as a single number without an error range. I encourage the authors to reflect this in the Discussion and Conclusion. Please provide the error range for the values you estimate based on models, and please adjust your Discussion of differences in soil N fluxes, and your Conclusions, to reflect the uncertainties associated with modeling.

Thanks for your suggestion. The soil food web was rebuilt in the revised manuscript.

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Furthermore, we re-calculated the N mineralization of soil organisms according to Rüter et al. (1993). Sensitivity analysis was conducted to test the influence of the uncertainty on the result of N mineralization. All ambiguous results were deleted, and the discussion was rewritten to obtain a concise and logical conclusion.

References 1.Berg, M.P, Kniese, J.P., Bedaux, J.J.M., Verhoef, H.A.: Dynamics and stratification of functional groups of micro- and mesoarthropods in the organic layer of a Scots pine forest, *Biology and Fertility of Soils*, 26, 268-284, 1998. 2.de Rüter, P.C., Van Veen, J.A., Moore, J.C. Brussaard, M.L. and Hunt, H.W.: Calculation of nitrogen mineralization in soil food webs, *Plant & Soil*, 157, 263-273, 1993. 3.Ferris, H.: Form and function: Metabolic footprints of nematodes in the soil food web, *European Journal of Soil Biology*, 46, 97–104, 2010. 4.Gómez-Rey, M.X., Couto-Vázquez, A., González-Prieto, S.J.: Nitrogen transformation rates and nutrient availability under conventional plough and conservation tillage, *Soil & Tillage Research*, 124, 144-152, 2012. 5.Verschoor, B.C.: Carbon and nitrogen budgets of plant-feeding nematodes in grasslands of different productivity, *Apply Soil Ecology*, 20, 15-25, 2002. 6.Wienhold, B.J.: Comparison of laboratory methods and an is situ method for estimating nitrogen mineralization in an irrigated silt-loam soil, *Communications in Soil Science and Plant Analysis*, 38, 1721-1732, 2007.

Interactive comment on SOIL Discuss., <https://doi.org/10.5194/soil-2020-2>, 2020.

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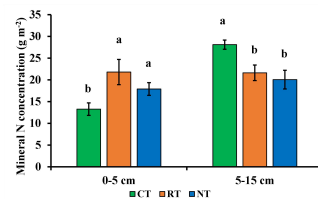


Fig. 1 Cumulative soil mineral N concentration during soybean growing season under different tillage practices (mean \pm standard error). Tillage practices capped by the same lowercase letter are not significantly different ($P > 0.05$). CT, conventional tillage; RT, ridge tillage; NT, no tillage.

Fig. 1.

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