

Manuscript Title: Multi-cooperation of soil biota in the plough layer is the key for conservation tillage to improve N availability and crop yield

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The manuscript examines the influence of soil biota on coupling N mineralization with soybean crop yield under conventional and conservation tillage regimes. Many studies already existed in the literature, which investigated the influence of soil biota to soil N mineralization using foodweb modeling (De Ruiter et al, 1993a, b; De Ruiter et al, 1994; Hunt et al. 1987; Schon et al. 2013) approach in different ecosystems (grassland or arable). In addition, other studies are also available in the literature who used production ecological calculation to study contribution of soil biota to N mineralization (Persson et al. 1980; Persson 1983; Didden et al. 1994, de Goede et al. 2003, Van Vliet et al. 2007; Rashid et al. 2014; Deru et al. 2019). Most of these studies are carried out in the Netherlands and/other European countries however the current study investigated N mineralization and coupling that with crop yield under different tillage regimes in China. Therefore, it would be a good addition to the scientific knowledge in this field. However, there are many flaws in the parameters used to calculate N mineralization or methodology that need to be considered carefully to make this manuscript acceptable for the publication.

#### 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.

Line 95, Zhang et al. (2019) used 40 kg N ha<sup>-1</sup> 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<sup>-1</sup>) plot WAS considered as a suitable reference to estimate background crop yield/N response.

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

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

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.

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.

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.

Table S4, values of biotic biomass were expressed in mg C m<sup>-2</sup>, 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. 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.

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.

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).

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?

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.

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.

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?

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 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.

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.

## 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?

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.

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.

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