Dr. Elizabeth Bach
Topical Editor
SOIL

Dear Editor,

Re: Manuscript soil-2020-2 “Multi-cooperation of soil biota in the plough layer is the key for conservation tillage to improve N availability and crop yield” Shixiu Zhang et al.

Thank you very much for your careful review and constructive suggestions with regard to our manuscript. We sincerely appreciate the reviewer’s and Editor’s thorough reviews and helpful suggestions. I am sending here one copy of our revised manuscript, with the revised portion marked in blue, a file with highlights, and a revised appendix file.

The responses to the reviewer’s and Editor’s comments are listed below.

We believe that we have addressed all of the reviewers comments and that manuscript has been improved satisfactorily. We hope it will meet your approval.

Yours sincerely,

Dr. Shixiu Zhang

Topical Editor

Comments from the reviewers are in normal font and our responses are marked in blue. The line references in our responses refer to the line numbers in the revised manuscript.

1. However, both reviewers raise concerns with how models from the literature were applied to this specific study. Applying models can offer insights and predictions, but it is important to understand and report the uncertainties that arise from inputting field and laboratory data from one study into a model developed in another. A way to address this would be to conduct a sensitivity test, as suggested by reviewer 1. Additionally, caveats need to be incorporated throughout the results and discussion, especially to the conclusions, which both reviewers felt were overstating the underlying data.

Thank you for your suggestion. We are very appreciative of the reviewers' suggestions to obtain the reliable results and discussion. We extensively revised the manuscript to address the criticisms and shortcomings raised by the reviewers. We reconstructed the soil food web based on the trophic relationship among microbes, nematodes, collembolans and mites. And then these trophic groups were classified into six feeding guilds: bacteria, fungi, bacterivorous feeders, fungivorous feeders, herbivorous feeders and predators. The N mineralization of soil food web was also re-calculated according to de Ruiter et al. (1993). And according to the suggestion of reviewer1's suggestion, a sensitivity analysis was conducted to test the impact of uncertainty of assignment of feeding preferences of omnivorous collembolans in the model on the N mineralization results.

In addition, as we reanalyzed, the results and the discussion were also rewritten. According to the reviewer's suggestion, all inappropriate views were deleted.

2. Both reviewers mention ways the text could be improved for clarity. In some cases, there is confusion around methods, which may require some extensive rewriting. It is important to consider where grammatical changes can improve the text and where additional information is truly required. A third reviewer found the writing too confusing to do a full reviewer; however, the thorough review of the other two reviewers provides sufficient feedback to proceed with revision of the manuscript.

To make the text clear and concise to readers, we reorganized the structure of this manuscript and rewrote the abstract, introduction, results and discussion, and explained the material and methods in detail. We also invited the native English

speaking researcher to polish this manuscript. We believe that the revised manuscript will be satisfactory.

The content marked in blue in the revision is for the convenience of tracking the modification according to the reviewers' suggestion. But, other contents, such as abstract, introduction, results, discussion and conclusion, etc., were also revised intensively .

Reviewer 1

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 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 soil physicochemical parameters, but there was a slight difference in the abundance of soil collembolans and mites. Their abundance at the 20 cm depth 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.

Lines 142-143: We rewrote the description in the paper about how the soil bulk density was determined. The soil cores for the bulk density were 0-5 cm and 5-15 cm, and therefore, the bulk density data obtained represent the mean of these two depth bands, not 5 cm and 10 cm.

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 N mineralization by soil organisms among different tillage systems, not on the crop yield response to soil N input rate. Furthermore, the amount of soil input N as fertilizer was the same in all tillage systems; the amount of N fertilizer applied is 2/3 of the typical amount for soybean 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 applied; 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 applied N in soybean of the same variety.

We deemed it necessary to discuss whether soil organisms play a key role in N mineralization as nitrogen fertilizer application is reduced. As discussed in line 362-380 of the revision, N supply from soil mineral N is insufficient to achieve maximum soybean yield and must be supplemented by the N release from soil organisms.

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

These sentences described the determination of soil temperature were deleted from the revised manuscript because soil temperature was not used in the calculation of N mineralization of soil organisms according to the equation provided by de Ruiter et al. (1993).

4. 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).

Our purpose in the experiment 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 be lower averaged over 4 weeks incubation than that if they were allowed to stabilize for a week prior to initial measurement. So, in the revision, we used the inorganic

nitrogen content measured in fresh soil samples obtained from the field every month instead of the amount of mineralized N obtained through lab incubation (the method used in the original submission) to indicate the status of soil N during soybean growth; this avoids the problem raised by the reviewer. Field sampling was described in lines 138-140 and the method of determining soil mineral N was presented in the lines 146-148 of the revision.

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 in line 155-157. It was stated in lines 138-140 that the field soil samples were cores from the 0-15 depths, and the cores were separated into 0-5 and 5-15 cm depths, and subsamples from each depth were combined to form a single composite sample at each depth for each plot. This method of taking one deep core and separating the core into sections corresponding to two or more depths is widely used in field research to examine the effect of depth on a measured parameter; it avoids potential contamination by sloughing of the core sidewalls which can occur if a 0-5 cm core is first removed, and the coring probe reinserted into the same hole to get a separate 5-15 cm core. From this information, it is evident that the extracted organisms represent the mean of the entire range of the depths of the separated cores, 0-5 cm, and 5-15 cm.

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 than 0.2 g $individual^{-1}$ 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 which contains a high portion of cellulose and lignin. But cellulose and lignin are not the main food for herbivores. 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 root 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. This was clarified in Table S4 of the revision; the source of the C:N values is given in Table S4.

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.

Sorry, on checking, we realized that we had cited Berg et al. (1998) in the footnote for Table S4 (original submission), but we had neglected to include Berg et al. (1998) in the references. Thank you for pointing this out. The same information is in Berg et al. (2001) so we only cited this paper and did not cite Berg et al. (1998) in the revision.

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, Pt= 0.1 Wt/mt, where Pt, Wt and mt 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: Pt= 0.1 Wt/mt to calculate the production C. Please note that the production C is not equal to the biomass C. The production C is defined as the C used for anabolism (Zhang et al., 2019); the biomass C is the C contained within the living component of soil organisms.

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. The material in Table S5 in the original submission has been reformatted and moved to Table 3 in the revision.

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 soil organism taxa were added as the supplementary information in Tables S1 and S2. 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?

We agree that the N applied as fertilizer would have some effect on diluting the yield response to tillage, i.e. typical flattening of the yield N response curve at higher total N rates. However, the difference among tillage systems in total N mineralized by soil organisms (Table 3 in the revision) was over twice the applied amount, and we attributed the difference in yield to the difference in mineralized N. The yield followed the order NT > RT > CT, and although not significant, the trend was consistent with the differences in mineralized N among the tillage systems, NT > RT > CT (Table 3 in the revision). We attribute the lack of significance to normal within experiment variability.

As discussed in our response to comment 2 above there was no difference between the N input to soil (fertilizer + atmospheric deposition) among the three tillage systems. So, it is unlikely that the higher yield in conservation tillage, especially in NT, was related to the N fertilizer or atmospheric N deposition. The point is that, the amount of soil mineral N during the soybean growing season is distributed unevenly throughout the plow layer under conservation tillage systems. The amount of soil mineral N at 0-5 cm was higher in RT and NT than in CT; but, the opposite trend was observed in 5-15 cm (Table 1 in the revision). This poses a question, how did the deficit of

mineral N in 5-15 cm support the higher yield in RT and NT soils? We surmised 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.

Line 362-380: we rewrote the discussion to clarify why the mineralizable N mediated by soil organisms rather than the inherent soil mineral N plays a key role in meeting the requirements of plant growth in RT and NT soils. The soil organisms are producing mineralized N as it is being used by the plant, and thus, the yield in NT and RT was higher than CT even though the mineral N remained low in RT and NT soils.

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 revision. The material in Fig. S1 in the original submission is now included in Table 1 in the revision, and the letters indicating pairwise differences are included.

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. We reassigned the identified soil organisms (bacteria, fungi, nematodes, mites and collembolans) into six functional feeding guilds: bacteria, fungi, herbivorous feeders, bacterivorous feeders, fungivorous feeders, and predaceous feeders. And then their contributions to N mineralization were recalculated.

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 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 and the material in Fig. 1 was deleted in the revised manuscript. The focus of the revision was changed to differences among tillage systems in N mineralization by the different organism feeding guilds and the subsequent effect on soybean yield.

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?

It was 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 was on the comparison among different tillage systems, because these differences among tillage systems may be the primary reason for soil N mineralization and plant yield differences. So, we deleted these

unclear sentences in the revision.

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 we used stepwise regression analysis to relate the N mineralization of different channels with soybean yield, the results showed 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 different soil organisms dominate at different depths in driving N mineralization and plant growth. This was clarified in the revision. In lines 396-407 in the revision, we discussed about dominance of fungi in the 0-5 cm depth because fungi can transfer nutrients from surface residue via hyphae.

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. Furthermore, we re-calculated the N mineralization of soil organisms according to Ruiter et al. (1993). Sensitivity analysis was conducted to test the influence of the uncertainty in the feeding preference of omnivorous collembolans on the result of N

mineralization and the modelling performance was also discussed in the line 318-342. All ambiguous results were deleted, and the discussion was rewritten to obtain a concise and logical conclusion.

Reviewer 2

1. Title: needs re-working. “Multi-cooperation” isn’t correct. Perhaps simply “interaction”?

The title of this manuscript was re-worked.

2. Affiliations: I think there should be a better translation for “Key Laboratory of Mollisols Agroecology”. Even simply “Laboratory of Mollisol Agroecology”

The translation of our organization is the official translation and cannot be modified. This name was adopted several decades ago.

3. Ln 13: Please check English grammar. For example, “Conservation tillage systems may promote more complex and heterogeneous distributions of soil organisms relative to conventional tillage that may result in higher crop yield. However, the role of soil biota in N mineralization promoting plant growth remains limited.”

Thank you for suggestion. We have invited a native English researcher to help revise the paper.

4. Some introduction or definition of “trophic groups” and “energy pathways” is needed.

We reconstructed the soil food webs, and assigned the identified soil organisms (bacteria, fungi, nematodes, mites and collembolans) into six functional feeding guilds: bacteria, fungi, herbivorous feeders, bacterivorous feeders, fungivorous feeders, and predaceous feeders. Therefore, in the line 205-206, the definition of ‘trophic feeding guild’ was given in the revised manuscript.

5. Ln 27-31: Is the second to last sentence of the Abstract the main finding of the study? The last statement, on lines 30 and 31, is quite a broad generalization and is not overly useful. The second to last sentence here, lines 27 to 30 would seem to say that ploughed and non-ploughed systems are similar in terms of N supply

to plants, is that what you mean? Clarification may be needed.

These sentences were rewritten in the lines 48-51.

Reviewer 3

1. Specific comments One key concern is that N mineralization is measured under laboratory conditions and then corrected to field conditions, via a solely temperaturedependent Q10 equation (L112-114). It is well known that the simple Q10 relationship does not hold under realistic soil conditions, since temperature is not the only limiting factor. Soil moisture, substrate availability, etc also strongly co-determine the biogeochemical process rates in situ (see e.g. Davidson & Jansses 2006 Nature 440: 165-173 for SOM decomp). Therefore, I do not believe that the authors can capture realistic N mineralization rates in their field. I think this paper needs a thorough validation of this relationship.

The lab incubation method and the in situ method are the most common methods used in research to investigate the soil N mineralization rate. But, both methods have their own limitations (Hanselaman et al., 2004; Wienhold, 2007). So, obtaining the absolute real value is virtually impossible.

In the revision, we used the inorganic nitrogen content measured in fresh soil sampled from the field every month instead of the amount of mineralized N obtained through lab incubation to indicate the status of soil N under different tillage systems during soybean growth. Determination of inorganic N by direct monthly field measurements integrates the contribution of all of the factors mentioned by the reviewer affecting N mineralization rate. We want to emphasize that our core objective was to make a comparison among different tillage systems, not to obtain absolute values of N mineralization rates. In the revision, we reworked to objective to clarify that the focus was on comparing the tillage systems.

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.

2. Similarly, I am highly critical of the way the authors attribute N mineralization contributions from different soil biota groups. They use a series of equations from other authors to transform soil biota abundances into process rates (e.g. L170-L176, L177-188, L198-202). Mostly these steps seem to be based on Rashid et al 2014. These steps form the heart of their study. For instance, the conclusion that conservation tillage promotes N min (L21-23), hinges on these equations that all assume that more soil biota lead to more N min. The same goes

for the relative contributions of soil biotic groups to total N mineralization (L25-27). The parameter estimates (e.g. Q10 of 3, L116) used come from different systems in other countries, while it is know that N cycling processes are highly heterogeneous in space and time. I am therefore sceptical that the same relations and the same parameter estimates will hold in the system studied by the authors. In fact even in the source paper, Rashid et al 2014, the ecologicalproduction model is an improvement over the standard government rules, but still there is considerable error in the estimates (87-120% of observed N min rates) on the fields they studied. So I think the authors have to spend much more effort on convincing me and other readers that using these equations leads to valid inferences about this particular system. To be honest, as an empiricist, I think that to only realistic way to get to these questions is to use isotopic tracers in the field plots. However, what would help is if 1) we had realistic data on N min rates in the actual plots, and 2) the summed N contributions over the soil biota would have a strong predictive relationship with these independent field data. As it stands such a field validation is totally missing, which makes the study unconvincing.

Researchers have used theoretical methods to quantify the elemental energy flux of soil food webs for more than thirty years. The parameters, such as assimilation efficiency, the ratio of C:N of predator or prey, and feeding preference and so on, used in this method were almost constant over the past thirty years. The classic literature is de Ruiter et al. (1993), Didden et al. (1994) and Hunt et al. (1987), and the recent literature of Andrés et al. (2016, Soil Biology and Biochemistry), de Vries et al. (2013, PNANS) and Schwarz et al. (2017, Nature Climate Change) also used this method to explore the C or N flow through soil food webs in the grassland ecosystem of America, agroecosystem of Europe and the forest ecosystem of America. The method is well established and accepted by researchers. So far, as far as we know, there is no research using this theoretical method to quantify the energy flux of the soil food web in Asia or China.

In the revised manuscript, we re-calculated the N mineralization of soil organisms according to Ruiter et al. (1993), see line 217-233 for details. This method does not require the use of Q10. And we also discussed the influence of physiological parameters that are required for the calculation of N mineralization in the lines 329-342. As discussed in our response to comment 1 above, our objective was to compare tillage systems, not to obtain absolute values of N mineralization rates of the various soil biota. This objective was clarified in the revision.

We agree that the use of isotopic tracers would be a good way to obtain the actual N mineralization data independent of assumptions. However, that is a major study in itself and is well beyond the scope of this manuscript. We think that our findings will provide good background material for further studies in isotope tracing and we

mentioned this in the line 441-448 in the revision.

3. Data were missing in some months for nematode data and linear interpolation was used to fill these data gaps (L129). I find this a risky approach, especially since nematode population dynamics within season are non-linear, see e.g. the data in Rashid et al, but also other sources. I think the authors also need to show that their conclusions hold if the only work with the months where they have data on all soil groups.

The nematode populations for non-sampled months were estimated by linear interpolation between adjacent sampling dates. Ideally, more frequent sampling would be done, but as with most research projects, our resources were limited. This method (linear interpolation) is usually used in the literature (Didden et al., 1994; Berg et al., 2001; Zhang et al., 2019), which assumes that there is a linear course in biomass or abundance of soil organisms between sampling dates. This method can not track the short term trends of nematode population changes, but can yield a reasonably accurate mean value during the studied period.

Line 159-160: we rewrote these sentences to make it clear for readers.

4. The authors use the ratios of (calculated) mineral N delivery in the conservation tillage (ridge, and no tillage) to conventional tillage in their main figures. However, ratios are biased (e.g. Jasienski & Bazzaz 1999 Oikos 84: 321-326); a log(Treat/Control) has better statistical properties (Brinkman et al 2010 J Ecol 98: 1063–1073). Even better however would be if the main analyses and figures are directly based on the data from the three treatments directly, this approach would even give you a bit more statistical power. In that sense I find the supplementary figures to be much clearer.

Thank you for your suggestion. The tables and figures were reorganized in the revised manuscript. We used max-min normalization to compensate for the wide difference in ranges spanned by the various parameters, and ln(x+1) transformation to improve the normality of the data prior to statistical analysis. The detailed analysis information was presented in the line 246-266. In the revision, we deleted the calculations and figures on the various N delivery ratios for the tillage systems.

5. In general, I find that the writing is a bit to colloquial in tone and imprecise in many places. See some examples below. Also I find that the presentation of the energy channels to be a bit overstated, there have been many findings of cross-feeding across these ‘channels’, and really I think we need to adopt a

network view of the soil community and its links to biogeochemical processes.

The soil food webs were rebuilt in the revised manuscript. Sensitivity analysis was conducted to test the influence of the uncertainty of the assignment for omnivorous collembolans on the result of N mineralization. All ambiguous results were deleted, and the discussion was rewritten to obtain a concise and logical conclusion.

6. Minor comments - L44: what do you mean with ‘special species’? - L51: what are weak root infections - L55: what do you mean by capacity? Use of substrates? Process
rates? - L60: I would not use the word conquer here, maybe mediate? - L61: adverse effects on what? - L66: rich in what sense - L68: what is stratrified and in what way? -
L80: based on M&M I believe its 14 years, not 15. - L83: what do you mean coupling?
How will you quantify that coupling? - L85: it is a bit unclear what you mean by multiple spatial interactions in this hypothesis. How will you test this? - L94: how big were the plots? - L100: what was done with the maize residue?

These inappropriate points in the part of introduction were rewritten, please see Line 67-91 in the revised manuscript. And the hypothesis was also rewritten in the line 108-111 to avoid ambiguous and unclear words.

References

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Relationships between N mineralization of soil organisms and soybean yield in conservation tillage systems

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Abstract

It is increasingly being recognized that conservation tillage systems favoring rich and abundant soil organisms can achieve optimal crop production by increasing nitrogen (N) mineralization. However, our understanding of the role of soil organisms in N mineralization promoting plant growth remains limited. In this study, the relationship between N mineralization of soil organisms and soybean (*Glycine max* Merr.) yield was investigated under a long-term (initiated in 2001) tillage trial, comprising conventional tillage (CT), ridge tillage (RT) and no tillage (NT). The amount of N released from soil organisms at 0-5 cm and 5-15 cm during the growing season of soybean was calculated using the monthly biomass data of soil microbes, nematodes, mites and collembolans, and the food web energetic model. The results showed that the soil food webs of RT and NT released more N than that of CT throughout the plow layer. Similar results were also observed for soybean yield which decreased in the order of NT > RT > CT. Multiple regression models revealed that soybean yield was significantly related to the mineralized N in RT and NT through fungal and plant channels in 0-5 cm and bacterial channel in 5-15 cm, demonstrating the role of spatial variability of soil organisms in linking N mineralization to plant growth. Furthermore, RT and NT significantly enhanced the N mineralization of trophic feeding guilds in these energy channels, which is beneficial in providing sufficient N to plants. Our results suggest that different soil organisms dominate at different depths in driving N mineralization and plant growth, and that the enhanced N mineralization of soil organisms is a cornerstone for conservation tillage systems to

achieve the optimal crop productivity.

Key words: conservation tillage, soil food web energetic approach, organism biomass, energy channels, soil N supply

1. Introduction

Nitrogen (N) is the most important growth-limiting nutrient for crops (Fageria et al., 2010). In order to achieve the maximum yield, N fertilizer is applied to crops all over the world; even legumes that fix N through symbiotic N-fixing microorganisms require additional chemical N application for maximum yield. However, globally, the N recovery rate by crops is only about 60% (Liu et al., 2010), which means that the rest of the fertilizer N is not available for the crop and is lost from the agroecosystems, resulting in undesirable environmental consequences. It is increasingly being recognized that exploiting the role of soil organisms in N mineralization is a promising approach to reduce the heavy dependence on N fertilizer without compromising the crop yield (Wall et al., 2015).

The process of N mineralization mediated by soil organisms is closely related to the predation in the food webs because soil organisms require carbon (C), N and other nutrients from the prey to support their metabolic activities (de Ruiter et al., 1993; Hunt et al., 1987). The N immobilized in the biomass of the lower trophic groups can be released by the predation of the higher trophic groups. Furthermore, the predators usually have a higher C:N ratio than their prey, which results in more N obtained than

73 their nutritional requirements, and the excess N is excreted into the soil (de Ruiter et
74 al., 1993; Hunt et al., 1987). It is estimated that the amount of N released by soil
75 organisms from predation accounts for 30%-80% of the annual N mineralization
76 under field conditions (de Ruiter et al., 1993; Holtkamp et al., 2011; Hunt et al., 1987;
77 Carrillo et al., 2016), and the value of this contribution varies with the biomass of soil
78 organism and the complexity of soil food webs (Carrillo et al., 2016; de Ruiter et al.,
79 1993; Holtkamp et al., 2011).

80 Conservation tillage, one of the most efficient practices to maintain optimal
81 productivity, has a prominent role in promoting the richness and abundance of soil
82 organisms (van Capelle et al., 2012). Several studies (Bender et al., 2015; Cole et al.,
83 2004; Thakur et al., 2014; Wagg et al., 2014) based on controlled (micro- or
84 meso-cosm) experiments found that the N mineralization of soil organisms increased
85 with the increase of soil biodiversity, which implies that a tillage system which forms
86 a complex soil food web is beneficial for releasing large amounts of N. However,
87 most of these cited studies have focused on the predation of microbial-feeding fauna
88 on microorganisms, and rarely consider the overall impact of all tropic levels of soil
89 organisms (bacteria, fungi, nematodes, mites and collembolans) on N mineralization.
90 As a result, our understanding of how the predation among soil organisms control the
91 N mineralization in the field is still limited.

92 Furthermore, relative to conventional tillage (CT), conservation tillage increases
93 the heterogeneity of soil organism distribution in the soil profile. For example,
94 bacteria and bacterivorous fauna dominate the whole plow layer of CT, while

conservation tillage is typically characterized by the fungi and fungivorous fauna near the surface and bacterial based communities at deeper soil depths (Hendrix et al., 1986; van Capelle et al., 2012). Moreover, conservation tillage also benefits by increasing the diversity of predaceous fauna since it reduces the tillage frequency. These changes in soil communities result in a more complex soil food web in conservation tillage, making it more difficult to understand the role of soil organisms in N mineralization promoting plant growth.

The objective of this study was to investigate the relationships between N mineralization of soil organisms and plant yield under contrasting tillage practices in a long-term (initiated in 2001) tillage trial. Soil food webs were composed of microbes, nematodes, mites and collembolans, and the amount of N released from soil organisms at each tropic feeding guild was quantified using the experimental data combined with the soil food web energetic model (de Ruiter et al., 1993). We hypothesized that (1) conservation tillage favors a greater release of N from soil organisms than CT, (2) soil organisms that play a key role in associating N mineralization and plant growth vary with soil depth in the conservation tillage system.

2. Material and methods

2.1 Experimental design and soil sampling

This study was conducted at the Experimental Station (44°12'N, 125°33'E) of the Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, in

Dehui County, Jilin Province, China. The station is located in a continental temperate monsoon zone. The soil is classified as Black soil (Typic Hapludoll, USDA Soil Taxonomy) with a clay loam texture. Tillage experiment was established in the fall of 2001 and included conventional tillage (CT), ridge tillage (RT) and no tillage (NT) with a two year maize (*Zea mays* L.) - soybean (*Glycine max* Merr.) rotation system. Each treatment had four replications. The soybean phase of the two-year maize-soybean rotation was sampled in 2015 in the present experiment.

Briefly, CT practice consisted of fall mouldboard plowing (20 cm) followed by the secondary seedbed preparation in the spring by disking (7.5-10 cm), harrowing and ridge-building. In RT, ridges were formed with a modified lister and scrubber and were maintained in June of each year with a cultivator. For the NT, no soil disturbance was practiced except for planting using a no-till planter. After harvest, the maize residue in the RT and NT plots was cut into about 30 cm pieces and left on the soil surface along with 30-35 cm standing stubble; soybean residue was directly returned to the soil surface. Residues in CT plots were removed prior to, and manually replaced on the soil surface after fall mouldboard plowing. Basal fertilizer was applied to the plots at rates of 40 kg N ha^{-1}, 60 kg P ha^{-1}, and 80 kg K ha^{-1}. The application rate of N is much lower than the local conventional application rate of 60 kg N ha^{-1}. Details of the experiment layout, tillage applications, crop rotations and fertilization were reported by Zhang et al. (2019).

Soil samples were taken at the end of each month from April to September during the soybean growing season when soil organisms are active. Seven soil cores

(2.5 cm in diameter) in each plot were randomly collected from a depth of 15 cm and each core was separated into 0-5 and 5-15 cm sections. Soil cores were combined to form a single composite sample for each plot and depth. Samples were immediately taken to the lab and stored at 4 °C. Soil bulk density for each plot was determined in the 0-5 and 5-15 cm depths using a slide-hammer probe with a 5 cm core diameter.

2.2 Soil mineral nitrogen and soybean yield

Soil mineral N was tested within 12 hours after soil samples were collected each month. Mineral N, including NO_3^- and NH_4^+, was extracted by 1 M KCl (soil : KCl = 1:2) and determined by a continuous flow analyzer (SAN++, Skalar, Netherlands).

Soybean yield was determined by hand-harvesting 3 m lengths of 6 interior rows from each plot after plants had reached the physiological maturity. Grain yield samples were dried to a constant weight at 75 °C in an oven, and then corrected to 13.5% grain moisture content.

2.3 Soil organism extraction

Soil organisms, including microbes, nematodes and microarthropods, were extracted from the soil taken from 0-5 cm and 5-15 cm depths within 2 weeks to obtain the reliable biomass data. All types of soil organisms were determined monthly except nematodes, which were only determined in April, June and August due to the limitation of labor. The nematode populations for non-sampled months were estimated by linear interpolation between adjacent sampling dates.

Microbial community was determined using the phospholipid fatty acid analysis (PLFA) as described by Bossio et al. (1998). Lipids were extracted from 8 g of freeze-dried soil with a Bligh and Dyer solution (chloroform: methanol: citrate buffer = 1: 2: 0.8 (v: v: v)). Polar lipids were separated from neutral lipids and glycolipids in a solid phase extraction column (Supelco Inc., Bellefonte, PA, USA) and transformed into fatty acid methyl esters with a mild alkaline methanolysis. Samples were then dissolved in hexane and analyzed in an Agilent 6850 series Gas Chromatograph with MIDI peak identification software (Version 4.5; MIDI Inc., Newark, DE, USA). Fatty acids were grouped as bacteria (14:0, i14:0, a14:0, 15:0, i15:0, a15:0, 15:1ω6c, 16:0, i16:0, a16:0, 16:1ω7c, 16:1ω9c, i17:0, a17:0, 17:1ω8c, 17:1ω9c,18:1ω7c, 18:0, 20:0), saprophytic fungi (18:1ω9c and 18:2ω6c) and arbuscular mycorrhizal fungi (AMF) (16:1ω5c) (Bach et al., 2010; Dempsey et al., 2013). Microbial biomass was estimated using the following conversion factors of fatty acid concentrations (nmol): bacterial biomass, 363.6 nmol = 1 mg C; saprophytic fungal biomass, 11.8 nmol = 1 mg C; and AMF biomass, 1.047 nmol = 1 μg C (Tsiafouli et al., 2015).

Nematodes were extracted from a 50 g soil sample (fresh weight) using a modified cotton-wool filter method (Liang et al., 2009). At least 100 nematode specimens from each sample were selected randomly and identified to genus level (see Table S1 for the list of identified taxa) using an Olympus BX51 microscope (OLYMPUS, Tokyo, Japan) according to Bongers (1994). Nematodes were assigned into four trophic groups: bacterivores, fungivores, plant-parasites and omnivores-predators (Ferris, 2010). Body length and maximum body diameter of

183 nematodes were measured using an ocular micrometer to calculate the nematode fresh
184 body mass (μg) (Andrássy, 1956). Nematode biomass was estimated by assuming that
185 the dry weight of a nematode is 20% of the fresh weight, and the C in the body is 52%
186 of the dry weight (Ferris, 2010).

187 Microarthropods were extracted from 200 mL fresh soil using modified
188 high-gradient Tullgren funnels (Crossley and Blair, 1991) for 120 h at room
189 temperature. Individuals were collected and stored in vials containing 95% ethanol for
190 identification. Mites and collembolans were identified to species or morphospecies
191 level (see Table S2 for the list of identified taxa) according to Christiansen and
192 Bellinger (1980-1981), Balogh and Balogh (1992), Bellinger et al. (2019), Pomorski
193 (1998) and Niedbala (2002). Soil microarthropods were allocated into four different
194 functional groups: fungivorous (oribatid) mites, predaceous mites, fungivorous
195 collembolans and omnivorous collembolans. Individual body length and width were
196 measured to estimate the dry weight based on regression equations from the literature
197 (Douce,1976; Hódar, 1996). Mite and collembolan biomass were estimated by
198 assuming the C in the body as 50% of the dry weight (Berg, 2001).

199 The unit of soil organism biomass was converted to mg C m^{-2} using soil bulk
200 density data. Taking into account the changes in abundance of soil organisms over
201 time, the biomass of soil organisms during the soybean growing season was estimated
202 by summing the monthly biomass.

203

204 **2.4 Modelling N mineralization of soil organisms**

Trophic feeding guild is defined as a group of species that exploit the same trophic resources (Burns, 1989). Before calculating the N mineralization of soil organisms, the identified soil organisms were first assigned into six functional feeding guilds: bacteria, fungi, herbivorous feeders, bacterivorous feeders, fungivorous feeders, and predaceous feeders to construct the structure of soil food webs (Fig. S1). Omnivorous-predaceous nematodes were assumed to feed on all other nematode groups (Yeates et al., 1993). Omnivorous collembolans, which mainly feed on bacteria, fungi, plant and microfauna (Barnes et al., 2014; de Vries et al., 2013), were proportionately assigned to bacterivorous, fungivorous, herbivorous and predaceous collembolans according to the assumption that their diet consists of 25% bacteria, 25% fungi, 25% plant and 25% other microfauna. The N mineralization of soil organisms was calculated with the food web energetic model (de Ruiter et al.,1993).

The calculation of N mineralization delivered by soil organisms is based on the assumption that the energy flowing into the biomass of a group is equal to the energy flowing out through natural death and predation. Following equations were used to calculate the N mineralization of soil organisms according to de Ruiter et al. (1993):

$$F_{ij} = \frac{w_{ij} B_i}{\sum_{k=1}^{n} w_{kj} B_k} \quad (1)$$

$$F = \frac{d_j B_j + P_j}{e_{ass} \times e_{prod}} \times F_{ij} \quad (2)$$

$$N_{min} = e_{ass} \times \left(\frac{1}{C:N_i} - \frac{e_{prod}}{C:N_j}\right) \times F \quad (3)$$

where, in equation 1, F_{ij} is the feeding preference of predator (j) on prey (i), which

was calculated based on the density independent feeding preference of j on i (w_{ij}, dimensionless; listed in Table S3), n is the total number of potential prey types (k = 1, 2, 3… n), and B is the biomass of prey (mg C m^{-2}). In equation 2, F is the feeding rate of predator on prey (mg C m^{-2} yr^{-1}); d_j is the natural death rate of j (yr^{-1}); B_j is the biomass of j (mg C m^{-2}); P_j is the energy loss of j due to the predation (mg C m^{-2} yr^{-1}); e_{ass} and e_{prod} is the assimilation efficiency and production efficiency of j, respectively. In equation 3, N_{min} is the N mineralization mediated by the predation of j on i (mg N m^{-2} yr^{-1}); $C:N_i$ and $C:N_j$ is the body C:N ratio of prey (i) and predator (j), respectively. The parameters of d, e_{ass}, e_{prod}, C:N of soil organisms are presented in Table S4.

The calculation of the N mineralization was started with the top predators, which are considered to have no energy loss from the predation, and then proceeded to the lower trophic groups. Based on the specific primary actors that drive energy flow from the basal resource to the soil food webs, the energy channels of the soil food webs can be divided into fungal channel (i.e. energy flux driven by fungi and then flow to fungivores and their predators), bacterial channel (i.e. energy flux driven by bacteria and then flow to bacterivores and their predators) and plant channel (i.e. energy flux driven by herbivores and then flow to their predators). The N mineralization of each channel was the sum of N mineralization of all functional feeding guilds within the channel.

2.5 Statistical analyses

Data were ln(x + 1) transformed to increase normality prior to statistical analysis.

Two-way analysis of variance (ANOVA) was performed to test the effect of tillage, soil depth and their interaction on the biomass of each feeding guild, and the N mineralization of soil food webs. When their interaction was significant, multiple comparisons were performed based on post hoc test to determine if tillage effects were significant in each soil depth. Tukey's honestly significant difference test was used for means comparisons and a difference at the $P < 0.05$ level was considered statistically significant.

Forward stepwise multiple linear regression (MLR) was used to identify the main channel that most accurately affects the crop yield at each soil depth. In stepwise regression, only one independent variable is considered at a time and another variable is added to the model at each step until no significant (*P*-value was set at 0.05) improvement in the percentage of explained variance is obtained. Prior to MLR, all parameters were min-max normalized to accurately preserve all relationships of data value and prevent potential bias from the domination of large numeric ranges over those with small numeric ranges. Min-max normalization subtracted the minimum value of an attribute from each value of the attribute and then divided the difference by the range of the attribute. The normalized value lay in the range [0, 1] (Jayalakshmi and Santhakumaran, 2011). All statistical analyses were performed using the R software (R 3.4.0, R Development Core Team 2017) using the car package for ANOVAs and the stats package for MLR analyses.

3. Results

3.1 Soil mineral N and soybean yield

Tillage effect on the soil mineral N varied with soil depths. At 0-5 cm, the amount of soil mineral N was higher ($P < 0.05$) in RT and NT than in CT, while the entire plow layer (0-15 cm) and the deep layer (5-15 cm) showed an opposite trend decreasing in the order of CT > RT > NT. There was no statistical significance for soybean yield among tillage treatments (Table 1); however, the yield of RT and NT increased by 6.6% and 26.5%, respectively, in comparison with CT.

3.2 Soil organism biomass

For soil microbes, a higher ($P < 0.05$) biomass of bacteria and fungi was observed under RT and NT than that under CT at both soil depths (Table 2). The similar trend was also found for the bacterivores and predators with a significant ($P < 0.05$) increase in biomass under RT and NT at both soil depths. For herbivores, a higher ($P < 0.05$) biomass was found under NT than that under CT, while for fungivores, RT significantly ($P < 0.05$) increased the biomass at both soil depths (Table 2).

3.3 Mineralization N of soil food webs

A greater ($P < 0.05$) amount of mineralized N of the whole soil food web was found under RT and NT than CT throughout the plow layer (Table 3); however, these positive effects varied with the energy channels. Compared to CT, RT and NT significantly ($P < 0.05$) increased the amount of mineralized N delivered by bacterial

and fungal channels at both soil depths. The components within these channels exhibited similar trends. For the components in the bacterial channel, the amount of mineralized N from the basal resource to the bacteria, and then from the bacteria to the bacterivores was greater ($P < 0.05$) under RT and NT than that under CT at both soil depths. However, RT and NT significantly ($P < 0.05$) increased the mineralized N from the bacterivores to the predators only at 5-15 cm. For the components in the fungal channel, the amount of mineralized N from the basal resource to fungi was significantly ($P < 0.05$) increased under RT and NT at both soil depths, while the amount of mineralized N from the fungi to the fungivores was only significantly ($P < 0.05$) increased under NT at 0-5 cm. For the plant channel, a greater ($P < 0.05$) quantity of mineralized N was released from RT and NT than from CT at 0-5 cm (Table 4). A similar result was also observed in the amount of N mineralized from basal resource to herbivores in RT and NT at the same soil depth.

3.4 Relationship between soil organisms and soybean yield

At 0-5 cm, 83.6% of the variation of the soybean yield was explained by the combined influence of fungal and plant channels (Table 4). Their relative contributions to the soybean yield decreased in the order of fungal channel (0.557) > plant channel (0.550), which means that when the min-max normalized fungal channel and plant channel increases by one unit, the min-max normalized soybean production would correspondingly increase by 0.557 and 0.550 times respectively. At 5-15 cm, only the bacterial channel significantly affected soybean yield and accounted

for 37.3% of the yield variance. The yield of soybean would increase by 0.656 times when the bacterial channel is increased by one unit.

4. Discussion

4.1 Performance of modelling N mineralization of soil organisms

The calculation of N mineralization of soil organisms was based on the predation relationship of soil food web structure (de Ruiter et al., 1993; Hunt et al., 1987), which highly depends on the assignment of species into functional feeding guilds. In this study, one of the weaknesses is that omnivorous collembolans were assumed to be divided in equal proportions among bacterivores, fungivores, herbivores and predators. To test how this assumption might affect the calculation of N mineralization, a sensitivity analyses was performed by re-assigning omnivorous collembolans into fungivores and herbivores (50% each) according to Barnes et al. (2014). This resulted in a very small deviation between these two models and an overall decrease of up to 0.24% among the tillage systems (Table S5), suggesting that the presented approach in this study is robust to estimate the mineralized N in the food webs.

The physiological parameters, such as assimilation efficiency, production efficiency and death rate, of trophic groups required for the calculation of N mineralization, are very difficult and impractical to determine under the field conditions because soil organisms have high spatiotemporal heterogeneity. Therefore, these physiological parameters are often cited from the literature (de Ruiter et al., 1993; de Vries et al., 2013; Hunt et al., 1987), and kept the same in all treatments to

facilitate the calculation of C and N mineralization of soil organisms (Holtkamp et al., 2011). Although this may lead to a certain deviation (maximum 30%) between the simulated and observed values (Carrillo et al., 2016; de Ruiter et al., 1993), a series of studies across natural and agricultural systems (Barnes et al., 2014; Carrillo et al., 2016; de Ruiter et al., 1993; Holtkamp et al., 2011; Schwarz et al., 2017) demonstrated that this approach is very useful in simulating C and N mineralization in soil organisms and can effectively reflect the changing trend of mineralization among treatments.

The biomass of organisms can be used to predict the potential of mineralized N because the biomass is the predominant factor in the calculation of N mineralization (Carrillo et al., 2016; de Ruiter et al., 1993; Holtkamp et al., 2011). In this study, the biomass of trophic feeding guilds under RT and NT increased significantly relative to CT, leading to the corresponding increase in N mineralization of the food webs. For example, higher biomass of bacterivorous feeders in RT and NT resulted in higher N released from bacteria at both soil depths. But, this predictable relationship between biomass and N mineralization of soil organisms is not consistent for the higher trophic level groups, i.e. predaceous feeders. The biomass of predaceous feeders was significantly increased under RT and NT soils throughout the plow layer, while the corresponding N mineralization increase occurred only from bacterivores to predators at the lower soil depth (5-15 cm). This may be mainly due to the existence of more than one prey resource for predators, and consequently, it is difficult to predict which prey has the greatest contribution to changes in N mineralization. Overall, modelling

N mineralization of soil organisms can effectively integrate soil organism communities and their functions related to N process, which may provide mechanistic predictions of the response of soil organisms to different tillage systems.

4.2 Relationships between N mineralization of soil organisms and soybean yield

Soybean is a legume and can obtain some N through the colonization of rhizobia in the root system, but the N provided by rhizobia cannot meet its requirement (Thilakarathna and Raizada, 2017). Therefore, soil N supply is an important determinant of achieving the maximum yield of soybean. Soil N supply is highly dependent on the level of mineral N and mineralizable N regulated by soil organisms (Whalen et al., 2013). In this study, the content of mineral N in the plow layer (0-15 cm) decreased in the order of CT > RT > NT over the whole growing season of soybean. This is counter intuitive as the soybean yield followed the reverse order, NT > RT > CT. At the critical growth stage, due to the strong demand for N by the crops, the soil mineral N content may decline (Fageria et al., 2010). However, this decline is short-lived and does not last the entire growing season.

Mineralization N delivered by soil organisms, which is another important source of soil N supply, was prominently improved in RT and NT soils. The multiple linear regression analysis further showed that there was a positive correlation between the N mineralization of soil organisms and soybean yield. These results suggest that the mineralized N from soil organisms produced over the growing season plays a key role in meeting the requirements of plant growth in RT and NT soils; it could also explain

379 the apparent inconsistency of higher soybean yield but lower decline in soil mineral N
380 over the growing season in RT and NT soils than in CT. Our result is consistent with
381 the reports of Carrillo et al. (2016) and Evans et al. (2011) that were also conducted in
382 field conditions and suggests that farming practices favoring a rich and abundant soil
383 organisms can improve crop yield by increasing N availability to plants. Although the
384 amount of mineralized N in RT and NT soil was increased, it does not mean that all
385 mineralized N may be taken up by the plant. For example, at the upper soil layer (0-5
386 cm), only the trophic feeding guilds within fungal and plant channels strongly linked
387 N mineralization with plant yield. This implies that the N released from other soil
388 organisms in the corresponding soil layer might be re-utilized by organisms or
389 leached from the soil, reducing the N availability to plants (Bender et al., 2015;
390 Thakur et al., 2014).

391 Numerous studies (Hunt et al., 1987; Thakur et al., 2014; Wagg et al., 2014;
392 Whalen et al., 2013) have demonstrated that the presence of predators that feed on
393 microbes can promote the N mineralization and the absorption of N by crops. This is
394 consistent with our results, which found that the association between N mineralization
395 in fungal and bacterial channels and soybean yield was enhanced in RT and NT soils.
396 However, there was a spatial difference in the distribution of fungal channel and
397 bacterial channel in the plow layer, in which the fungal channel at 0-5 cm and the
398 bacterial channel at 5-15 cm were the driving factors in mediating N mineralization.
399 This difference may largely result from the location of residues in RT and NT soils,
400 which were placed on the surface of the soil instead of being mixed with the soil.

Unlike bacteria, fungi are less dependent on nutrient spatial distribution in soils because they can transfer nutrients from surface residues to mineral soil via the hyphal growth (Frey et al., 2003). Additionally, the residue layer can serve as a habitat for many microarthropod groups, such as collembolans, which prefer to feed on fungi (Schwarz et al., 2017). These soil communities favored by the surface residues may account for why fungal channel plays a dominant role in mediating the N supply in the upper layer (0-5 cm) of RT and NT soils.

Fungal channel and bacterial channel are the main regulatory channels for N mineralization but they differ in turnover rate for processing N (de Vries et al., 2013; Wardle et al., 2004). In contrast to the "slower" fungal channel, which favors N retention in the soil (de Vries et al., 2011), the bacterial channel supports a faster N turnover rate and provides more mineralized N for crop production (de Vries et al., 2013; Whalen et al., 2013). This suggests that the dominant bacterial channel at 5-15 cm in RT and NT soils promotes the supply of N to plants. Furthermore, along this bacterial channel, the N mineralization from the bottom bacteria to the intermediate bacterial feeders, and then to the top predaceous feeders was greatly enhanced in RT and NT soils. There is general agreement with other researches (Carrillo et al., 2016; Wagg et al., 2014) that the tight interlinkage within trophic levels in the food web stimulates the release of N from soil organisms. The enhanced N mineralization of bacterial-channel may partially explain why the severe shortage of soil mineral N at 5-15 cm in RT and NT soils during the growing season did not result in a compromise of soybean yield.

Plant channel has been considered to have a very minor effect on N mineralization (Holtkamp et al., 2011). In this study, the amount of N mineralization in the plant channel was indeed the least among the different channels across tillage systems. However, to our surprise, a positive association between plant channel and soybean yield at 0-5 cm was evident in RT and NT soils. This may primarily due to the significant increase of mineralized N delivered by herbivores in plant channel under RT and NT soils, indicating that herbivores play a non-negligible role in the process of associating N mineralization with plant growth. Verschoor (2002) reported that the N mineralization of herbivores accounted for 10% of total N mineralization in a grassland system, and attributed these beneficial effects of herbivores to the activity of soil microbes that was stimulated by the increase in root exudates after infection by herbivores. In our study, most groups classified into herbivores are the facultative feeders. For example, herbivorous collembolans can switch their diet from plant roots to decaying litter (Endlweber et al., 2009). Therefore, we propose that the positive role of herbivores at 0-5 cm in RT and NT soil may partly be due to their manipulation on surface residues by fragmenting and mixing. Therefore, the surface area of litter in contact with soil microbes would be increased, which is beneficial for N mineralization (Soong et al., 2016).

In this study, the N mineralization of soil organisms was quantified using the experimental data and the food web energetic model based on the steady-state assumption. This method yields relatively static data that cannot reflect the dynamics nutrient flow of the soil food webs. However, it can filter some useful information

from the complex food web to help us better understand which soil organisms play a key role in N mineralization promoting crop growth. This forms background information for further study on the dynamics of the soil food web in N mineralization using ^{15}N tracer technology.

5. Conclusion

Our results showed that, during the whole growing season, almost all soil organisms in the food webs of RT and NT released more N than CT throughout the plow layer. However, the ability of soil organisms to supply N for soybean growth varied with energy channels and soil depths. Soil organisms in the fungal and plant channels at 0-5 cm and in the bacterial channel at 5-15 cm were the main drivers in associating N mineralization with crop yield. In conclusion, the long-term application of conservation tillage systems has promoted the N mineralization of soil organisms, which is favorable for achieving the optimal crop yield.

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Date accessibility: all data are included in the manuscript and its supporting information.

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625 **Table 1** Effects of tillage systems on the crop yield and the soil total N and
626 cumulative mineral N concentrations (means (SE)).

627

	CT	RT	NT
Yield (kg ha^{-1})	1242 (96) a	1324 (189) a	1570 (221) a
Mineral N (g m^{-2})			
0-5 cm	15.27 (1.44) b	20.09 (2.90) a	17.90 (1.46) ab
5-15 cm	28.10 (1.05) a	21.33 (1.79) b	20.06 (2.14) b
0-15 cm	21.68 (0.65) a	20.71 (1.86) ab	18.98 (0.67) b

628 CT, conventional tillage; RT, ridge tillage; NT, no tillage. Same lowercase letter in the same row
629 indicates no significant difference among tillage systems ($P > 0.05$).

Table 2 Cumulative soil biotic biomass (expressed as mg C m^{-2}) under different tillage practices (means (SE)).

	0-5 cm			5-15 cm			ANOVA		
	CT	RT	NT	CT	RT	NT	Tillage (T)	Depth (D)	T × D
Bacteria	6077 (499)	7367 (363)	8452 (1408)	9000 (1362)	11393 (1324)	12780 (733)	< 0.001	< 0.001	ns
Fungi	16386 (1309)	22375 (1639)	26646 (7661)	18558 (2409)	23938 (3622)	26168 (1769)	< 0.001	ns	ns
Herbivorous feeders	67 (5)	90 (23)	95 (13)	73 (4)	87 (25)	110 (18)	0.017	ns	ns
Bacterivorous feeders	78 b (15)	168 a (17)	128 a (30)	56 b (14)	93 a (13)	112 a (11)	< 0.001	< 0.001	0.045
Fungivorous feeders	58 (15)	98 (12)	99 (19)	34 (12)	57 (16)	55 (24)	0.023	0.002	ns
Predaceous feeders	60 (14)	88 (14)	78 (8)	96 (15)	123 (22)	176 (49)	0.002	< 0.001	ns

CT, conventional tillage; RT, ridge tillage; NT, no tillage; ns indicate no significant difference ($P > 0.05$). Means for the different tillage systems at the same depth and followed by the same lowercase letter are not significantly different ($P > 0.05$).

635 **Table 3** The amount of mineral N delivered by soil food webs (expressed as mg N m^{-2} $year^{-1}$) under different tillage practices (means (SE)).

636

Channel	Feeding guild	0-5 cm			5-15 cm			ANOVA		
		CT	RT	NT	CT	RT	NT	Tillage (T)	Depth (D)	T × D
Plant channel	Total Nmin	52.55 c (2.80)	63.30 b (3.28)	75.95 a (4.76)	140.50 a (27.74)	118.40 a (7.21)	159.77 a (23.46)	< 0.001	< 0.001	0.020
	Nmin (resource→herbivores)	41.69 c (3.25)	53.16 b (2.69)	61.65 a (3.19)	72.29 c (7.72)	77.35 b (4.38)	86.09 a (12.00)	< 0.001	< 0.001	ns
	Nmin (herbivores→predators)	10.85 ab (2.40)	10.15 b (0.78)	14.30 a (2.22)	68.21 ab (20.22)	41.04 b (8.95)	73.68 a (13.67)	0.003	< 0.001	ns
Bacterial channel	Total Nmin	4517.74 b (353.44)	5855.59 a (307.55)	6425.15 a (916.86)	6550.21 b (970.00)	8830.57 a (145.38)	9565.72 a (438.29)	< 0.001	< 0.001	ns
	Nmin (resource→bacteria)	4271.71 b (349.71)	5205.55 a (257.29)	5951.09 a (822.55)	6314.26 b (954.98)	8457.56 a (103.94)	8979.14 a (512.34)	< 0.001	< 0.001	ns
	Nmin (bacteria→bacterivores)	225.41 b (30.23)	622.04 a (46.52)	449.51 a (133.83)	186.04 c (40.46)	296.38 b (36.76)	428.84 a (47.20)	< 0.001	< 0.001	0.002
	Nmin (bacterivores→predators)	20.62 a (3.70)	28.00 a (10.27)	24.54 a (2.52)	49.91 b (11.11)	76.64 ab (20.63)	157.71 a (74.42)	0.002	< 0.001	0.013
Fungal channel	Total Nmin	5447.57 b (436.59)	7434.05 a (551.69)	7646.12 a (794.07)	6537.00 b (302.66)	7949.78 a (990.23)	8468.86 a (313.37)	< 0.001	0.007	ns
	Nmin (resource→fungi)	5421.75 b (433.21)	7402.99 a (542.76)	7613.55 a (798.64)	6509.06 b (299.44)	7919.58 a (521.04)	8414.91 a (325.48)	< 0.001	0.007	ns

Nmin (fungi→fungivores)	20.09 b (4.24)	25.49 ab (6.92)	26.74 a (4.11)	17.32 b (1.00)	21.53 ab (4.57)	29.44 a (4.25)	0.003	ns	ns
Nmin (fungivores→predators)	5.72 ab (2.13)	5.57 b (2.58)	5.83 a (2.13)	10.61 ab (3.99)	8.68 b (3.83)	24.52 a (10.89)	0.034	< 0.001	ns
Mineral N of the whole soil food web	10017.85 b (789.55)	13352.94 a (687.93)	14147.22 a (1549.39)	13227.71 b (1065.70)	16898.76 a (1177.10)	18194.35 a (568.77)	< 0.001	< 0.001	ns

Resource is a collective resource of residues and plant roots; residues and plant roots supply energy to microbial channel and plant channel, respectively.

CT, conventional tillage; RT, ridge tillage; NT, no tillage; Nmin(i→j) indicates the mineral N delivered by the predation of j on i; ns indicates no significant difference ($P > 0.05$); Same lowercase letter in the same row and same depth indicates no significant difference among tillage systems ($P > 0.05$).

640 **Table 4** Relationships between N mineralization of different energy channels and
641 soybean yield based on multiple linear regression. Data were min-max normalized
642 and are dimensionless.

Soil depth (cm)	Variable	Beta standardized coefficient	T value	Adjusted R^2	F value of the regression
0–5	Fungal channel	0.557	2.886*	0.836	19.737**
	Plant channel	0.550	2.437*		
5–15	Bacterial channel	0.656	2.745*	0.373	7.555*

643 * and ** indicate significant at 0.05 and 0.01, respectively.