

Dr. Elizabeth Bach  
Topical Editor  
SOIL

Dear Editor,

Re: Manuscript soil-2020-2 “Relationships between N mineralization of soil organisms and soybean yield in conservation tillage systems” 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 red, and a file with a revised appendix.

The responses to the reviewer’s comments are listed below. The content marked in red in the revised manuscript 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.

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

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.

### **Reviewer 1**

1. I think the manuscript can benefit from a discussion (either in the Introduction or Methods) on the utility and limitations of energetic food web modeling. Indeed, these methods have been used for several decades now, but they are not mainstream, and many readers interested in this study will likely be less familiar. From my understanding, energetic food webs are highly parameterized theoretical models that are meant to give crude estimates of C and N cycling rates through food webs; the results are not meant to be interpreted as absolute. Addressing these expectations/limitations upfront should help eliminate the confusion of why so many parameter values are derived from different systems while also elucidating the usefulness of this method.

Thank you for your suggestion. We added the description regarding the utility and limitations of food web energetic modeling approach in the part of Introduction (Line 130-135) and Methods (Line 283-287) to help readers better understand this approach.

2. Furthermore, there should be a figure that illustrates the energetic food web models (similar to Figure S1), ideally showing all three tillage treatments so that they can be compared visually (see Pressler et al. 2018 for example). The line size of each pathway should indicate the strength of the interaction between food web compartments (the 'igraph' package in R is one way to do this). I would also suggest eliminating (or reducing) Table 3 that shows the same information or placing that information in the appendix.

Referring to the example presented in the study of Pressler et al. (2018), a vividly visible diagram Figure 2 showing the N flux across multi-trophic feeding guilds among different tillage system was added in the main text. Table 3 was moved to the supplementary file and renumbered as the Table S7.

3. I propose adding a separate section for the Sensitivity Analysis under the 'Statistical Analysis' heading in the Methods. Explicitly state what was done and if the results are consistent when using different feeding preferences and/or different food web configurations (see Koltz et al. 2018 for example) -- simply stating that "a sensitivity analysis was performed by re-assigning omnivorous collembolans into fungivores and herbivores (50% each)" is not sufficient. A formal sensitivity analysis would help evaluate the robustness of the results in this ecological context. There should be text explaining how it was performed in the Methods, reported in the Results, and discussed in the Discussion.

Thank you for your suggestion. After carefully reading the reports of Koltz et al. (2018) and other studies (Barnes et al., 2014; Carrillo et al., 2016; Schwarz et al., 2017; Zhang et al., 2018) that also conducted Sensitivity Analysis to test the robustness of the data, we adhered to our approach but made some modifications to thoroughly test the changes in all functional feeding guilds. The presented Sensitivity Analysis was based on Barnes et al. (2014) and more detailed information was added in the part of 'Statistical Analyses'

(Line 302-308), 'Results' (Line 358-366) and 'Discussion' (Line 413-438).

The primary reason for we did not refer to Koltz et al. (2018) to perform the Sensitivity Analysis is that we think their assumption is impractical for our study. Koltz et al. (2018) conducted the Sensitivity Analysis based on the assumption of incomplete food webs (feeding groups removed from the network at one time) and creating an additional food web (feeding groups have no specified feeding preferences).

Although this assumption can ensure a formal sensitivity analysis, it is not suitable for our study because almost all feeding guilds, except omnivorous collembolans, have explicit feeding preference and position in the detritus food web network. Therefore, we did the Sensitivity Analysis according to Barnes et al. (2014) which is more reasonable for field conditions. Furthermore, we made a modification to not only show the changes in the whole food web, but also to show the changes in each feeding guild to fully understand the consequences of variation in feeding preferences of omnivorous collembolans. This can provide more useful, practical and realistic information (Line 424-438) than that method of Koltz et al. (2018).

4. The various types of tillage treatments need to be clearly defined in both the Introduction and Methods section. What is the difference between conservation tillage vs. conventional tillage? Which one of the experiment treatments is considered to be conservation tillage? Is the NT the control treatment? There appears to be a disconnect between the terminology used in the Introduction and Methods; the terms should be consistent and clear throughout the manuscript. Not all readers will be familiar with agricultural practices.

More detailed information about the difference between conventional tillage and conservation tillage (including reduced tillage and no tillage) was added in the part of Introduction (Line 90-93). The terms and abbreviations of different tillage practices have been checked and used consistently throughout the whole text in the revised manuscript.

5. The hypotheses in the last paragraph of the Introduction need to be developed further. Why do you expect there to be more N release from conservation tillage from conventional tillage? Why would soil depth be pertinent for determining the influence of soil organisms regarding N mineralization?

The hypotheses were rewritten in the line 121-125 to make them clear to readers.

6. Overall, the Discussion is long and disorganized. I suggest starting with a short summary of the results, making sure to highlight the key new insights, followed by a discussion of if the hypotheses were supported or not. The middle paragraphs should be used to explain what the findings mean within a broader context, drawing on support (or contrasting support) from the literature. I will reserve the section on study limitations and commentary on the sensitivity analyses for the section right before the Conclusions paragraph.

Thank you for your suggestion. We reorganized discussion in the Line 399-521, and used the writing technique you mentioned to ensure that readers can easily find the

summary results in the opening paragraph of each subtitle and whether these results support our hypothesis.

To make it more logical and smoother for readers to follow, the discussion was partitioned into the following subsections: 4.1 performance of modeling N mineralization within the food web (line 413-438), 4.2 tillage effects on the N mineralization within the food web (Line 441-458), and 4.3 relations between N mineralization within the food web and soybean yield (Line 461-521).

7. The Abstract is confusing. I'm not sure what results are from the energetic modeling, N mineralization measurements, or other associated analyses. For example, in Lines 46-48, is this the energetic modeling result, or is this from the biomass estimates of the trophic guilds? In general, the Abstract needs to be summarized more effectively, as the current take-home message is unclear.

The Abstract was rewritten in lines 30-57.

8. The way the experiment is described is somewhat misleading, as at first read it appears that the experiment and its sampling have been going on since 2001, which was not the case. To make the experimental design clearer, I suggest stating the time interval of the tillage treatments (e.g., 2001 – 2015) and mention that this study only sampled for N mineralization and soil organisms in 2015.

The description of the time over which this study was conducted was revised and it was highlighted in the parts of 'Title' (Line 8), 'Abstract' (Line 36), 'Introduction' (Line 128) and 'Methods' (Line 176) to make it clear for readers.

9. The extraction of 120 hours at room temperature is not standard for Berlese-Tullgren funnel methods. What is the justification here, and how do you know that all arthropods were extracted if there wasn't a heat gradient?

We used the modified high-gradient Tullgren funnels (Crossley and Blair, 1991) method to extract all microarthropods. This method uses small light bulbs as a heat source above the funnels and yielded a gradient of temperature and moisture along the extractor to ensure that microarthropods can be collected at the bottom of the extractor. Many reports in the literature (Kardol et al., 2011; Risch et al., 2018; Soong et al., 2016) used this method to study the microarthropod community; Google scholar shows 181 citations of the Crossley and Blair (1991) paper which is a good indication that the method is widely used.

10. Although there is information on the various groups of soil fauna sampled in Table S1, it would have useful to include the average densities and standard errors for each taxonomic unit according to tillage treatment. Soil core extractions will usually capture springtails, mites, and nematodes – which of these groups and functional guilds were most abundant?

The abundance of soil fauna was added in the supplementary file as Table S4.

11. The assumptions of the model are acceptable and similar to previously published

models (e.g., Schwarz et al. 2017, Koltz et al. 2018, Pressler et al. 2018). However, because some of the literature values are from very different regions and study systems, the authors should explain why these parameter values are appropriate for this study. Obviously, it would've been nice to measure and confirm each parameter value, but that is usually impossible to accomplish for these types of models.

The explanation about how the parameter values were chosen was added in the line 283-287.

12. Lines 221-223: I would recommend stating if the N mineralization equations are modified from de Ruiter et al. (1993), and if so, how?

Line 269-273: There was no modification for these equations; in the revision, we clarified that we used the Equations from de Ruiter et al. (1993).

13. Lines 305-314: The Results don't do an adequate job in covering how the food web modeling relates to the crop yield. Maybe I missed this, but is there a way to loosely compare the energetic food web modeling to the crop yield data?

The relations between the mineralized N within the soil food web and the soybean yield was explored by the forward stepwise multiple linear regression (MLR) model. We rewrote the sentences in the line 318-320 and line 388 and 393 to make it clear to reader.

## **Reviewer 2**

1. Overall comments: One year of data is simply not enough to draw the conclusions you have done here. This is a long-term study so why not include historical yield data? There were no crop yield differences and the mineral N concentrations, in 0-15 cm depth, were contrary to expectations. The estimations for N cycling within the soil food web are therefore rendered difficult to interpret or meaningless. If only one year of data is presented, without any data on growing conditions, could it not be simply that N mineralization was controlled more by soil water content and temperature?

Our study was focused on investigating how the whole soil organism communities regulate nutrient cycle impacting crop growth not on the variation in crop yield among different tillage practices. Furthermore, it is impractical to monitor the changes in the whole soil organism communities in every year. Although the samples were only taken within one year during the soybean growing season, the sampling time was 14 years after initiation of long-term (continuous) conservation tillage practice. It is well documented (Six et al., 2004) that soil environment reaches a new stable equilibrium after a long-term (>10 years) application of conservation tillage. Therefore, our samples are representative and can obtain reliable results to understand the relations between mineralized N delivered by soil food web and soybean yield after long-term application of conservation tillage system.

There was indeed no statistical analysis for the soybean yield among different tillage practice, but there was a lower ( $P = 0.027$ ) soil mineral N pool of 0-15 cm in NT than in CT (Table 1). This is the key point and poses the question of why the low soil mineral N pool in NT supports the soybean yield comparable to CT? Therefore, we proposed

that NT has a larger mineralizable N pool that was regulated by soil organisms than CT, and this was supported by the presented results in our study. A concise summary for the relations between soybean yield and soil mineral N pool and mineralizable N pool was added in line 399-410.

We agree that in addition to soil organisms, soil moisture and temperature also influence N mineralization, but the underlying process of N mineralization is primarily governed by the activity of soil organisms which are live in the water film. Additionally, our study was focused on investigating the effect of tillage on soil N mineralized by organisms as opposed to determining absolute quantities of soil N mineralized.

The historical yield variation at the same experimental site has been reported in the studies of Fan et al. (2012) and Zhang et al. (2015).

2. Ln 6, replace "Relationships" with "Relations", and remove "of soil organisms". Use "relation" throughout, where applicable.  
Line 7, 'relationships' was replaced by 'relations', and "of soil organisms" was deleted. In the whole revised manuscript, the 'relationships' was replaced by 'relations'.
3. Ln 7, as it stands, the title is rather broad. I would suggest making it more specific. For example, you could include where the study was conducted as part of the title.  
Line 7, the title has been rewritten.
4. Ln 33, suggest changing "promoting" to "to promote"  
The Abstract has been rewritten, so "promoting" was deleted.
5. Ln 34, again, in the N being mineralized exclusively from soil organisms themselves? You could say "by soil organisms", or just "...N mineralization and soybean ..." because the process is assumed to be biologically driven/mediated.  
Thank you for your suggestion, we changed the preposition from 'of' to 'by' to relate N mineralization and soil organisms.
6. Ln 41, as you plowed to 20 cm but only measured the N release to 15 cm you cannot say "throughout the plow layer". Rephrase.  
'throughout the plow layer' was replaced by 'the entire soil layer (0-15 cm)' in the whole revised manuscript.
7. Ln 44, it is unclear what a "plant channel" is and how it mineralizes N.  
To make it clear to readers, 'plant channel' was replaced by 'root pathway' and its definition was given in the line 295. The role of how soil organisms in root pathway mineralize N was given in the part of introduction (Line 72-80) and in the part of discussion (Line 504-521).
8. Ln 42-46, the logic of this sentence is hard to follow. It is also a run-on sentence. Soybean yield was related to N mineralization, though fungal, plant, and bacterial channels (?) and this somehow demonstrates the role of spatial variability? Remove

"demonstrating the role of spatial variability of soil organisms in linking N mineralization to plant growth".

These sentences were rewritten in the line 50-54.

9. Ln 47, the sentence would need to define what "energy channel" you are referring to. Be specific.

The sentence was reworked in the line 49.

10. Ln 51, change "optimal" to "maximum". Optimal is a subjective term and you have not defined it here.

Abstract has been rewritten, so "optimal" was deleted.

11. Ln 60, include citation(s) that state this.

The citation was added in Line 66.

12. Ln 61-63, you have omitted portions of the soil N cycle. Simply because N recovery in plants from fertilizer application is not 100% doesn't mean that all is lost. Immobilization could be a factor as well. Or that pools of inorganic N in soil build up. So, include all possible loss pathways in your statement.

The possible loss pathways of inorganic N were added in line 69.

13. Ln 63-66: How does one exploit the role of soil organisms? Make your meaning explicit.

These sentences have been reworked in the line 70-71.

14. Ln 73: In what form is the excreted N? Is it plant available?

Line 80: the sentence was replaced by 'the excess N is excreted into the soil ammonium (NH<sub>4</sub><sup>+</sup>) pool' to make it clear to readers.

15. Ln 79: What accounts for the rest of the N mineralized? Don't leave the reader wondering here.

Line 82: It is impossible to include every trophic group in the soil to simulate the N mineralization, and thus the simulated N amount released from the predation of soil organisms only accounts for 30%-80% of the annual N mineralization. Therefore, we attributed the remainder of the mineralized N to the uninvestigated trophic groups. This was clarified in the revision.

16. Ln 80-82: I think that this statement is too bold. It would seem that your citation refers to the second part of the sentence, i.e., conservation tillage can promote richness and abundance of soil organisms. You need to cite the source of your statement that conservation tillage is "one of the most efficient practices to maintain optimal productivity". Presumably you mean agricultural practices. In what way would it be efficient? No-till systems can take several years to "settle", so chemical fertilizers are likely more efficient in that sense. Also, what is "optimal"? Maximum?

These sentences were rewritten in the line 90-99.

17. Ln 97, please clarify what you mean by "benefit". Who or what benefits here? The producer? But in what sense?  
The sentence was rewritten in the line 93-94.
18. Ln 99, what changes are you referring to? Be specific and start the sentence with the subject.  
These sentences were rewritten in the line 103-107. So, 'changes' was deleted.
19. Ln 102-104: I would expect mention of soybean from the title of the manuscript. In the title you say "soybean yield", so is it just one crop or all?  
We changed 'plant yield' to 'soybean yield' in the title (Line 8) and in the objective (Line 119-121).
20. Ln 108, specify the time frame? Over a rotation? Over the entire length of the study? Season? Or from week to week?  
The time frame was added in the 'Title' (Line 8), 'Abstract' (Line 36), 'Introduction' (Line 128) and 'Methods' (Line 176) to make it clear for readers.
21. Ln 109, I think that you should be precise about what the "key role" is that you are looking at. How will you judge if a set of organisms plays a "key role" at one depth and not at another?  
The hypotheses were rewritten in the line 121-125. So, the 'key role' was deleted.
22. Ln 119: Missing article at the beginning of this sentence.  
Agreed, the article was missing. We added 'The' at the beginning of the sentence in line 149 so it now reads: "The long-term tillage experiment was established ...."
23. Ln 124: Please choose one system of spelling and be consistent. Here you mix British and American.  
Line 155: 'mouldboard plowing' was replaced by 'moldboard plowing' and consistent throughout the whole revised manuscript.
24. Ln 126: Could you please state what the common practice would be in the study area? How common is seeding maize and soybean into ridges?  
The detailed information about the local common tillage practice was added in the part of instruction in the line 108-112. Traditional practice is to create ridges to promote soil warming, and seed maize and soybean into the ridges.
25. Ln 124-128, since tillage systems are the focus of your research you should refer to the specific instruments that were used. Make and model. As you would for analytical equipment. This is needed to judge the relative intensities of the systems. The reader likely won't know what a "modified lister and scrubber" looks like or who intense the soil modifications would be.



The model and the produced company of no-till planter was added in Line 160. The other instruments that used in different tillage practices had no specific model and company.

- 26.** Ln128- 132, Please provide an explanation as to why residues were replaced. Is this meant to mimic the amount of residue left in such systems under commercial circumstances? Are you not biasing the results by not incorporating the residues in the CT? The lack of inputs in the plough layer would presumably have an influence on the soil biology.

Preventing the water and wind erosion in winter and early spring is the reason for why residues were replaced on the soil surface after fall moldboard plowing in CT, and this reason was added in the line 160-162. Additionally, these residues laid on the soil surface in CT plots were mixed with the plow layer in the following spring tillage, so there was no bias caused by unmixed residues. This was clarified in the revision (line 164-166).

- 27.** Ln 135-136, please provide all relevant information on experimental design here so the reader doesn't have to refer to a separate article. This information is important to the current work.

Detailed information about the experimental design was added in the line 152-154, line 160-162 and line 164-172.

- 28.** Ln 137, specify what years the data were taken in.

The specific sampling year was given in the line 176.

- 29.** Ln 152, you should also include weather and climate data. How representative of the historical precedent were the times when you measured?

The mean precipitation during the growing season of 2015 and of the past 10 years (2004-2014) were added in the line 176-179.

- 30.** Ln 149-152, specify in what years you measured crop yield.

The specific year that measured crop yield was added in the line 194.

- 31.** Ln 217-219, provide a justification as to why the assumption of equilibrium is valid. If C:N shifts, SOM buildings or decreases then it would seem that this assumption is not valid?

The mass-balance assumption of soil food web energetic model approach, that is the energy flowing into the biomass of a group is equal to the energy flowing out through natural death and predation, is the basal principal and necessary requirement for simulation the mineralized N delivered by soil organisms; the assumption is widely used in this type of research. This assumption is based on the biomass, metabolic constant, and allometric growth of soil organisms and not based on the soil environment variance (Barnes et al., 2018). So, this assumption can be applied in a range of soil environments that have different C:N ratios and SOM contents.

- 32.** Ln 246, stating it in this way would seem to say that you transformed the data prior to testing for assumptions. This would be an incorrect approach.  
These sentences were rewritten in the line 309-311 to make it clear that the data were first checked for normality and homogeneity of variances, and if necessary, transformed to meet the underlying assumptions for ANOVA.
- 33.** Ln 255, please explain what you mean by "channel".  
The sentence was reworked in the line 319 to make it clear to reader.
- 34.** Ln 265-266, please properly cite all R packages used.  
The R packages that used in this study were carefully checked and presented in the line 328-330.
- 35.** Ln 270-275, please specify the form of N that you are referring to. nitrate, ammonium, or total?  
In this study, soil mineral N was obtained by the sum of  $\text{NO}_3^-$  and  $\text{NH}_4^+$ . This was clarified in the Methods section in line 200.
- 36.** Ln 271, This statement would appear to incorrect according to Table 1.  $\text{RT} > \text{CT}$  but NT is not different than either, at 0-5 cm.  
These sentences were rewritten in the line 335-338.
- 37.** Ln 272, it is probably not appropriate to call the 5-15 cm depth the "deep layer" as you have measured N content at an even deeper layer.  
Line 336, 'deeper layer' was deleted.
- 38.** Ln 273, this trend is not significant, so in fact, your analysis shows that  $\text{CT} > \text{RT} = \text{NT}$ .  
Although there was no significant difference between RT and NT, the magnitude of soil mineral N value of 0-15 cm was decreased in the order of  $\text{CT} (21.68) > \text{RT} (20.71) > \text{NT} (18.98)$  (Table 1).
- 39.** Ln 323, a description of how this was done should be in the Materials and Methods section.  
The sensitivity analysis was added in the part of statistical analyses in the line 302-308 of the Methods section.
- 40.** Ln 367-369, Rephrase this statement, it is misleading.  $\text{CT} > \text{NT}$ , but RT was not different than either.  
These sentences were rewritten in the line 402-403. Additionally, the order described the magnitude of soil mineral N and soybean yield and did not refer to the results of statistical analysis.
- 41.** Ln 369-370, Again, it is misleading to insinuate there were differences when in fact,

Table 1 states that there were no significant differences in crop yield.

As statement in the above, the order described the magnitude of soybean yield and did not refer to the results of statistical analysis.

42. Ln 451, it would be more appropriate to say that "we estimate" since you did not measure this directly.

Conclusion was rewritten in the line 524-535, so 'showed' was deleted.

43. Ln 456, never were the mineral nitrate concentrations higher in NT than CT, nor was crop yield higher in NT than CT, so this statement is misleading. Please re-phrase.

These sentences were removed in the revised manuscript as the part of conclusion was rewritten.

44. Table 1, when were these soil samples taken? At the end of the season? If there were no yield differences, in one year at least, but there was a difference in mineral N left after harvest (?).

The detailed information about sampled taken time was added in the line 176.

45. Ln 635, please remind the reader where these data came from.

These data were calculated using the equations according to de Ruiter et al. (1993) and detailed calculation process was presented in line 247-299.

46. Ln 637, please rephrase without using repetitive terminology.

These terms were reworked at the bottom of Table S7 as the Table 3 was moved to the supplementary file according to the suggestion of reviewer 1.

## References

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