Soil-2016-39 Review 3 comments

Authors' responses are in blue.

General comments:

The article has investigated the effects of temporal plant diversification by rotating crop on soil microbial functions and activities, while many other studies focus on the above-ground spatial biodiversity on soil microbial functions. This study has comprehensively examined a suite of indexes of soil microbial activities (e.g., MBC, MBN, PMC, and PMN) and functions (e.g., EEA and CLPP) over one growing season. The introduction is clear, the methods are easy to follow although they need clarification, and the data interpretation is generally logic. But a couple of issues need to be addressed as follows:

Response: Thank you for this thorough review and kind comments. We really appreciate the time and effort this reviewer put in on their feedback. We have incorporated nearly all their suggestions and we feel it has greatly improved the manuscript.

Majors:

1) The main goal of this study was to investigate whether crop rotation can enhance soil microbial biomass and functions. Since the study has examine microbial biomass and functions (e.g., PMC, PMN, and EEA) over one growing season (i.e., in spring, summer, and fall), how confidently we can attribute enhanced microbial activities and functions to crop rotation rather than seasonality or their interactions? Can we confidently say that crop rotation is the main reason for changes in soil microbial functions and activities?

   Response: This is an interesting point. We agree here with this reviewer, that in order to have a better understanding (and more confidence) of the season versus crop rotation effects we would need more than one year of data. However, due to the large amount of data collected (or "comprehensive" as this reviewer put it) we were limited to three sampling points. We strategically sampled during times when we would expect there would be differences in these soil microbial responses to look at how season might influence the crop rotation effect on soil microbial biomass and functioning. We are the first, to our knowledge, to have published the catabolic response (or community-level physiological profile, CLPP) on more than one date.

2) Were the crop rotation effects on microbial activities and functions general or unique, considering that all the measurements were for soils that have experienced an extreme drought the year before sampling? What the results will be if the sampling took place in a normal year? Particularly, the authors have discussed that the drying-rewetting effects on soil microbial community in the discussion (L339-359).

   Response: We show that this sampling period is very distinct in ways other than high microbial biomass, which is often found at the peak of the growing season (Wardle 2003; Hargreaves & Hofmockel 2013). Since we only sampled once in the summer, we do not know what a "normal" year would look like. Due to the good amount of evidence at hand (L. 485-492, L. 500-504), we

attributed the large differences in the summer to this drying-wetting event. Although, without sampling more times during the summer we do not know for sure.

Hargreaves, S. K., and Hofmockel, K. S.: Physiological shifts in the microbial community drive changes in enzyme activity in a perennial agroecosystem, Biogeochem., 117, 67–79, 2013.

Wardle, D. A., Yeates, G. W., Williamson, W., and Bonner, K. I.: The response of a three trophic level soil food web to the identity and diversity of plant species and functional groups, Oikos, 102, 45–56, 2003.

Minors:

1) Problems with the reference order in the main text: L27-28, L41-42, L50-51, L120, L157, 380, 381,385-386, 398-399, 401-402, 423

   Response: We preferred to cite papers in the manuscript text chronologically. SOIL leaves the in-text citation order up to the authors. "In terms of in-text citations, the order can be based on relevance, as well as chronological or alphabetical listing, depending on the author's preference." See http://www.soil-journal.net/for_authors/manuscript_preparation.html

2) L54-58, it is better to introduce the rationale of CLPP method and substrates used in this method, especially for readers who are not familiar with them. More references are needed about how this method works and how widely it has been used in studies to examine soil microbial catabolic functions.

   Response: We now have added more details about the CLPP method and its context in agroecosystems (L. 68-78)

3) Soil sampling issue. I am confused whether or not the measured soils were sampled under the same crop (maize) in all the three seasons. According the statement in L99-101, it is not the case. Soil sampling took place in April, July, and November, while corn was planted in June and before June some plots may be planted with other crops. Please clarify.

   Response: We now refer to the soils being collected during the same crop phase or year (L. 9, 84, 340) since they technically were not "under" corn during all seasons.

4) PMC and PMN measurements. How long did the incubation last? It is 6 months in line 122, but it is 120 days in line 129. How was PMC calculated according to cumulative respiration? In addition, how many times were the inorganic N extraction conducted to assess PMN during the entire period of incubation? And how was PMN calculated and what is the reference for it? I cannot find it (L117- 131).

Response: It was 120 days, or 4 months. We have changed "6" to "4" (L. 139-148). We further clarified how the cumulative PMC and PMN were calculated (L. 148-151, 192-193).

5) L198, 375 please use the multiple symbol instead of "X" letter.

Response: We have replaced all letter "X's" with multiplication signs (×).

6) Line 2013 should it be "P" rather than "Ps"?

Response: This was changed.

7) L210-211, please clarify the relation between cumulative CO2 respiration and PMC. Otherwise, the statement in these lines is not true.

Response: We removed reference of cumulative respiration and only refer to it as PMC, and they are the same thing.

8) L228-230, the text "increased crop diversity decreasing the qCO2 by 16, 40, 28% in CSW, CSW1, and CSW2…' does not match the results in Fig. 2. In CSW, qCO2 was not always decreased compared to mC (i.e., the control) according to Fig. 2 in spring, summer, and fall. Moreover, is the difference statistically significant?

Response: These were changed, and the significant post-hoc results are displayed in Fig. 2.

9) L291 "negative" should be "negatively"

Response: This was changed.

10) In the discussion, I would like to read the discussion of "crop diversity and soil microbial functions" before "seasonal dynamics and N limitation", as the former is the focus of this study, and they directly answer the two questions asked in the introduction.

Response: We changed the order of these sections.

11) L448-453 the statement does not match Figure. S4. We cannot tell which data are from the less and which are from the more crop rotations from this Figure. Please clarify.

Response: We added the letter 'd' to 'Fig. S5' to signify that this is the panel we were explaining, and hopefully clarify this statement (L. 442).

12) The statements in L399-400 and L411-412 are not clear to me. Which tables or figures can show the results? And what are the indexes of microbial catabolic evenness?

Response: Thank you for noticing this. We added 'Table 4' after these statements - from where we are drawing on these data (L 399,412).

13) L471-472 as a main finding of this study, it should be discussed in details but it was not. Please specify or remove it.

Response: We now explain this idea further, and earlier in the Discussion (L. 350-376).

Figures and tables

Tables 1 and 2: it is easy to read when all the contents of a table are in one page.

Response: We now have all the components of tables on one page.

Table 3 caption. I do not understand why adding Fig. 5 at the end? What does it mean? This is no Fig. 5 in the manuscript.

Response: This was supposed to be 'Fig. 4'. It has now been changed.

Fig. 1: as mentioned before, cumulative respiration is different from potential mineralizable C, please clarify the relation between them.

Response: We have changed this to PMC.

Figs. 1 and 2: since seasonality has strong effects on soil microbial activity and functions, we need to know how crop rotation effects in each season. It is better to add the multiple comparison of soil microbial activity in each season in these two figures.

Response: We have now shown overall crop rotation post hoc tests in these figures. Because season and rotation did not interact, we felt it was improper to analyze each date individually. Although, we can understand this reviewer's perspective. Sometimes when one factor is dominating ANOVAs it might be better to analyze treatment effects within each date individually. We wanted to highlight interactions, also the direction of treatment trends is rather consistent among each season.