Referee #2 Received and published: 22 July 2016

Authors' responses are in blue.

General comments

The authors examine whether using crop rotations to increase temporal biodiversity within an agroecosystem enhances soil biochemical functioning. Specifically, they hypothesised that crop rotations will enhance catabolic diversity (through community-level physiological profiles) and soil function (enzyme activities, soil microbial biomass, potentially mineralizable C and N). Further, they hypothesized that the crop rotation effect would lessen over the growing season. The study used soils from a well-established at the W.K. Kellogg Biological Station (est. 2000).

I find the paper well-structured and easy to follow with important findings that contribute new knowledge on the effect of crop rotation and soil biology and function. The study is quite unique in that there are few management variables (fertilization, pest control etc.) that could confound the effect of crop rotation.

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.

The introduction appears to have an unbalanced focus on CLPP and soil substrate use, while neglecting research gaps/other studies relating to extracellular enzyme activities.

Response: We would agree with this reviewer's assessment, but we have done this intentionally. The CLPP is the most novel aspect of the manuscript. And to our knowledge, no one has done this over multiple dates from the same soils. That being said, we still have added some more in the Introduction and Discussion regarding the extracellular enzymes (L 54-55, 366-368, 503).

The methods appear valid and adequate to test the hypotheses.

Response: Thank you.

Results are well communicated, although supplementary data are disorganised and do not link to the present manuscript. Serious repetition of sentences from L273-285 in L296-308.

Response: We have removed this duplication and link all supplementary data directly to the manuscript.

Discussions and conclusions are well-substantiated by the results, although some aspects relating to cover crops may enhance the discussion further – see specific comments below. Further, there is a lack of discussion around the enzyme activities (as was the case in the introduction).

Response: We have now added more on the importance of the cover crop treatments (L. 378-392) and some more about the extracellular enzymes (L 366-368, 465-478, 503).

There are also a few referencing issues with some references being cited in the text and not listed in the reference list and visa versa.

Response: We have fixed these references, and have thoroughly checked the citations and reference section.

Specific comments

L1 – I don't find any reference to catabolic evenness or diversity in the Abstract; a main component of your first hypothesis.

Response: We have now added catabolic evenness in the abstract (L. 10, 17).

L36 – replace "of" with "on"

Response: This was changed.

L44-51 – The end of this paragraph does not seem to be relevant to identifying gaps in the knowledge around above- and belowground biodiversity relationships (the point raised at the beginning of the paragraph). Perhaps you are expanding on the link to ecosystem function? Then I would suggest a new paragraph dealing with this.

Response: Good suggestion, this was changed to a new paragraph and more details and context about CLPP in agroecosystems (L. 68-78).

L56-67 – Unclear whether "their" refers to "soil microbial functions" or to "crop rotations". Re-structure to make it clearer.

Response: We have replaced "their" with "rotation" to make it clearer (L. 80).

L85 – hyperlink takes you to a page that no longer exists

Response: The hyperlink was changed, and now works. Thank you for checking this.

L86 – Was one crop planted per year, or multiple within one year? I know this might be obvious, but in some rotation systems, there are multiple plantings per year.

Response: For most of the year there was just one crop at a time, but there was actually some overlap at the end of the growing season when red clover was inter-seeded in CSW1 and CSW2 treatments (now clarified in L. 113-115).

L98-99 - When and how were cover crops (in CSW1 and CSW2) planted and was an entire growing season dedicated to this? - i.e. was it a 4-year rotation or a 3-year rotation with cover crop grown in between corn, soy and wheat cropping dates.

Response: See previous response for the answer (L. 113-115). We have also added another supplementary figure for further clarification (Fig. S1).

L146 – Would freezing of the samples for EEA analysis deplete the absolute enzyme activity? – perhaps substantiate this with references to other studies that have done likewise.

Response: Freezing has been shown to slightly decrease absolute activity in some studies (Peoples and Koide 2012), and no effect in others (Lee et al. 2007, Deforest, 2009). While we would like our EEA measurements to be as accurate as possible, we are mostly concerned with relative differences among treatments. Thus, if there were any freezing effects on EEA, we assume any freezing effects on EEAs would be equal across all treatments. We have added a statement in the Methods section (L. 167-169).

DeForest, J.L. 2009. The influcence of time, storage temperature, and substrate age on potential soil enzyme activity in acidic forest soils using MUB-linked substrates and l-DOPA. Soil Biol. & Biochem. 41:1180-1186.

Lee, Y.B., Lorenz, N., Dick, L.K., Dick, R.P. 2007. Cold storage and pretreatment incubation effects on soil microbial properties Soil Sci. Soc. Am. J. 71:1299-1305.

Peoples, M.S., Koide, R.T. 2012. Cosiderations in the storage of soil samples for enzyme activity analysis. Appl. Soil Ecol. 62:98-102.

L165 – Why are only two readings taken and why after only 6 hours? Does the CO2 efflux plateau within 6h?

Response: The 6 h incubation of the plates is directly from the MicroResp™ manual. But this time, we believed, is based in several papers, one of which is one by Anderson and Domsch (1985). This paper shows that CO_2 is stable in response to glucose for 6 h, but then substrate exhaustion or other factors begin to cause erratic respiration rates at 8-13 h.

Anderson, T.-H., Domsch, K.H. 1985. Maintenance carbon requirements of actively-metabolizing microbial populations under *in situ* conditions.

L241 – "season had no. . .." the first half of this sentence is a bit clumsy and difficult to understand. Perhaps re-word it.

Response: We have reworded this sentence (L. 279-280).

L273-285 – I don't see how the correlation between EEA and CLPP contributes to the overall thesis of the study. These results do corroborate each other and evidence the reliability of the CLPP and EEA data, but, in my opinion do not warrant such a long paragraph in the Results, especially since there is no follow-up discussion points in the Discussion section. I would advise simplifying or leaving this out.

Response: We agree here, and have now reduced this paragraph (L. 323-336) and deleted the duplicated paragraph.

L276 – remove "quite"

Response: This was removed.

L278 – The Fig. S4 does not relate to Nag amine. I think the order of supplementary figures is incorrect and does not correspond to the manuscript. Please check this throughout.

Response: We have corrected the order of the supplemental figures.

L296-308 – all this is a repetition of L273-285. Remove either section and simplify as suggested above.

Response: We removed the duplicate paragraph.

L309 – I do not see any discussion around cover crops and how they affect soil biochemical responses relative to non-cover crop treatments. Increases in soil biochemical functioning may not be a result of

plant species diversity per se - rather, cover crops alter soil physical characteristics (e.g. soil moisture through covering soil in between cash crops) which drive changes in biochemical processes. I would suggest clarifying the definition of cover crops in the methods and expanding on their relative effects on soil physico-chemical characteristics in the discussion.

Response: We have added a paragraph discussing the importance of cover crops (L. 378-392).

L321 – I do not see any direct reference to the second hypothesis here. It will make it easier for the reader to follow if this is done (as you have done in L393).

Response: We now directly refer to the second hypothesis (L. 451-453).

L336 – I would advise some discussion (also in relation to the cover crops) about legumes and soil N. Increases in microbial biomass may not be driving increased N, rather key-stone microbial species (rhizobia) may be responsible.

Response: We added some discussion on keystone species, such as legumes (L. 387-389).

L342 – what are the units for "0.1"?

Response: We now have put units after "0.1", "m^3 m^{-3}" (L. 482).

L372 – Again, I would suggest making the link to the original hypothesis more explicit (partly done in L396 but would suggest doing this earlier as well).

Response: We now directly refer to the second hypothesis (L. 451-453).

L416-419 – This is a confusing sentence, please re-word.

Response: We have rephrased this sentence (L. 416-419).

L427-431 – I do not understand this logic. Do you mean to say that using CLPP as a measure of catabolic evenness in bacterial-dominated soils may not adequately reflect the true microbial catabolic diversity because (1) bacteria are generalists and use all substrates evenly, and (2) fungi tend to be excluded through disturbing the soil? If so please re-structure this or explain what you are attempting to say.

Response: We have removed this portion from the discussion because we felt it was not adding much and to accommodate adding the suggestions from the reviewers.

L431-433 – How does this support the previous statements? 16S rRNA diversity would not necessarily correspond to catabolic evenness, so cannot be used to firmly support your findings.

Response: We have rephrased these sentences as to indicate this is more speculative (L. 421-423)

Table 1. – Include full stop. To which variables do the units apply to? (e.g. does mg.kg- 1 apply to C:N ratio?) - make clearer please. Give full descriptions of crop rotation abbreviations in the title.

Response: We have referred to the crop rotation abbreviations in Table 1, and then refer to Table 1 in the subsequent tables.