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Interactive comment on “Soil microbial biomass and function are altered by 12 years of crop rotation” by M. D. McDaniel and A. S. Grandy

Anonymous Referee #2

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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

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etc.) that could confound the effect of crop rotation.

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.

The methods appear valid and adequate to test the hypotheses.

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.

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).

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.

Specific comments

L1 – I don't find any reference to catabolic evenness or diversity in the Abstract; a main component of your first hypothesis. L36 – replace "of" with "on" 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. L56-67 – Unclear whether "their" refers to "soil microbial functions" or to "crop rotations". Re-structure to make it clearer. L85 – hyperlink takes you to a page that no longer exists 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. 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

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crop grown in between corn, soy and wheat cropping dates. 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. L165 – Why are only two readings taken and why after only 6 hours? Does the CO₂ efflux plateau within 6h? L241 – “season had no...” the first half of this sentence is a bit clumsy and difficult to understand. Perhaps re-word it. 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. L276 – remove “quite” 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. L296-308 – all this is a repetition of L273-285. Remove either section and simplify as suggested above. 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. 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). 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. L342 – what are the units for “0.1”? 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). L416-419 – This is a confusing sentence, please re-word. L427-431 – I do not understand this logic. Do you mean to say that using CLPP as a

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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. 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. 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.

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