

1 Anonymous Referee #1 Received and published: 21 July 2016

2 [Author's responses in blue.](#)

3 I acknowledge the amount of work put into this study by the authors. This is a unique and
4 comprehensive investigation of the effects of a single management practice (rotation) on microbial
5 function, where many studies cannot study individual management decisions due to other, confounding
6 management decisions. The introduction and discussion sections are very clear, with minimal jargon,
7 and the data interpretation is logical. However, some issues need to be addressed (some very minor,
8 others more crucial):

9 [Response: Thank you for this comprehensive and thorough review. We really appreciate the time and
10 effort this reviewer put in on their feedback. We have incorporated nearly all their suggestions and we
11 feel it has greatly improved the manuscript.](#)

12 1. Throughout the manuscript: I noticed several problems with regards to the references, e.g. wrong
13 year of publication reported in the main body of the text (Treseder et al. 2015; Anderson & Domsch,
14 1989), missing citations in the reference section (Ret et al. 2008; Guckert et al., 1996; Paul et al., 1999;
15 Robertson et al., 2000; Schimel et al., 1989; Tibbits et al., 2002; Mou et al., 2008; Frey et al., 1999),
16 references not cited in the main body of the text (Adviento-Borbe et al. 2010; Behnke-Ryser et al., 2012;
17 Berard et al., 2015; Cambardella et al., 1999; Morillas et al., 2015; Plante et al., 2011; Thoms et al., 2010;
18 Trivedi et al., 2015), and authorship misreported (e.g. "McDaniel (2014c)" instead of "McDaniel et al.
19 (2014c)"; "Giller, K. E. N. E." instead of "Giller, Ken E."; "Van Der Putten, W. I. M. H." instead of "Van Der
20 Putten, Wim H."). References were also not in alphabetical order in some instances. Additionally, there
21 were spelling mistakes for some of them (see Doran et al., 2000; Franzluebbers et al., 1995; Hamilton et
22 al., 2015), and incomplete references for Venter et al., 2016. Please make appropriate changes where
23 needed.

24 [Response: Thank you for this thorough reporting of the reference mistakes, we appreciate it! I
25 apologize for you having to spend the time finding these mistakes. I am quite embarrassed by all of
26 these errors, and learned you should not rely on your citation manager program. The references have
27 now been thoroughly checked.](#)

28 2. L. 31: The authors may want to reconsider the statement as species richness in a rotated cropping
29 system is one only if all weeds have been removed from the system, which is theoretically possible but
30 not always the case.

31 [Response: This is true, we have modified the statement to include the consideration of weeds \(L. 34\).
32 Thank you for this observation.](#)

33 3. L. 52-64: The authors introduce CLPP and how it works. Being unfamiliar with these measurements
34 and how to interpret them, I think it would be helpful to other readers like myself if the authors added
35 more information about this technique in the context of agricultural studies. How would a more or less
36 even CLPP profile be interpreted in the context of agricultural soils? Does an even profile generally
37 correlates with more efficient nutrient transfer to the plant or perhaps better C retention? Are there any
38 previous studies that have looked into this that can be referred to here?

39 Response: Good point, we have now illustrated how CLPP data could be useful in an agricultural context
40 (L. 63-78).

41 4. L. 83: 42° 24' N?

42 Response: This has been corrected.

43 5. L. 96 & 98: It is Zea mays and Trifolium pratense. Please correct.

44 Response: These have been corrected.

45 6. L. 101: Where in the field were the soil cores taken during the summer? Were they within or between
46 rows? Likewise, were cores in the spring and autumn timepoints taken where previous rows or
47 interrows had been? The rhizosphere effect can have a large impact on microbial communities and
48 functioning and the summer collection is the only time point in which corn is actively growing. This
49 might change interpretation of the data.

50 Response: We have indicated where the soil cores were collected (between the rows, L. 118).

51 7. L. 114-122: The amount of sample used for specific measurements is omitted and should be included
52 for reproducibility. Also, why specifically using a 50% water-holding capacity?

53 Response: We indicated the amount of soil in the incubation (10 g, L. 137), and that 50% WHC was used
54 because it is near optimal water content for respiration in these soils (L. 138).

55 8. L. 127-129: What is the rationale for measuring PMC more frequently at the beginning of the
56 experiment than the end?

57 Response: The reason for higher frequency measurements were two-fold: 1) reduce CO₂ build-up and
58 lack of O₂ in the jar when respiration rates are extremely high, and 2) to get better resolution of the
59 exponential portion of the CO₂ “decay curve” for modeling C pools (data we did not use in this
60 manuscript). We state this now in the manuscript (L. 146-147).

61 9. L. 133: The acronyms MBC and MBN should be introduced here for later use in the manuscript.

62 Response: We have abbreviated them here (L. 153).

63 10. L. 142: “Soils were for 7 extracellular enzyme activities” should be changed to “8”.

64 Response: This was changed.

65 11. L. 173: Please indicate what sort of transformation was applied to the data (remind the reader in the
66 Figure titles as well).

67 Response: We have now indicated which variables were transformed, and how they were transformed
68 (L.199- 201).

69 12. L. 184: it is conventionally accepted to provide details about the version of R used. Later versions
70 generally have bugs fixed and may explain discrepancies observed by other users (if they were to run
71 the exact same dataset as the authors’).

72 Response: We have now included the version of R we used (v3.0.0, L. 208).

73 13. L. 195: "There were no significant rotation or season effects on total soil C and N". Table 1 says
74 otherwise. Please correct and, if necessary, adapt your interpretation of the data and conclusions.

75 [Response: We have clarified this statement to reflect there are small differences in total soil C and N \(L.
76 221-223\).](#)

77 14. L. 198-212: Clearer language should be used throughout the results section. In several instances the
78 authors average over the rotation treatments or the season treatments without telling the reader,
79 making the percentage increases difficult to interpret (see lines 199, 200, 202, 204, 206-208 [why
80 combining CSW and CS treatments here?]). Furthermore, I find different results for the DOC:DON mean
81 in autumn (i.e. 17.4, five times that of spring and 13 times that of the summer). Report the standard
82 deviation or standard error for these calculations.

83 [Response: Thank you for noticing these, and we appreciate the reviewers comment on clarification. We
84 have reported standard error throughout the section where we mention means, and clarified the
85 language.](#)

86 15. L. 217-218: This is impossible to state without a post-hoc test. Please use the appropriate test and
87 rephrase if necessary.

88 [Response: We have provided the post-hoc test *P* values in the text \(L. 258-261\), and post-hoc test results
89 in Figs 1 & 2.](#)

90 16. L. 221-222: I disagree. These effects seem strongest in the spring and autumn.

91 [Response: This sentence was removed and we only talk about the rotation effect now \(L. 254\).](#)

92 17. L. 228: This is not the p-value reported in the Figure.

93 [Response: The one reported in the figure was correct, we fixed the text to match it \(L. 261\).](#)

94 18. L. 240-241: It should be "25% greater than autumn and 99% greater than spring".

95 [Response: This has been changed.](#)

96 19. L. 256 and 272: I do not understand how the authors obtained these values. Could Table 4 be
97 erroneous?

98 [Response: These are the values we received when calculating catabolic evenness. We double checked
99 our calculations and compared with other studies \(Degens et al. 2000, 2001; Carney & Matson 2005; Sall
100 et al. 2015\). Of course it is dependent on how many substrates you use, but our values are in the range
101 of what has been published in the literature \(from 8 to 24\).](#)

102 [Degens, B. P., Schipper, L. A., Sparling, G. P., & Vojvodic-Vukovic, M. \(2000\). Decreases in
103 organic C reserves in soils can reduce the catabolic diversity of soil microbial communities. *Soil
104 Biology and Biochemistry*, 32\(2\), 189-196.](#)

105 [Degens, B. P., Schipper, L. A., Sparling, G. P., & Duncan, L. C. \(2001\). Is the microbial
106 community in a soil with reduced catabolic diversity less resistant to stress or disturbance?. *Soil
107 Biology and Biochemistry*, 33\(9\), 1143-1153.](#)

108 Carney, K. M., & Matson, P. A. (2005). Plant communities, soil microorganisms, and soil carbon
109 cycling: does altering the world belowground matter to ecosystem functioning? *Ecosystems*, 8(8),
110 928-940.

111 Sall, S. N., Ndour, N. Y. B., Diédhiou-Sall, S., Dick, R., & Chotte, J. L. (2015). Microbial response
112 to salinity stress in a tropical sandy soil amended with native shrub residues or inorganic
113 fertilizer. *Journal of environmental management*, 161, 30-37.

114 20. L. 263: Refer to table 4 instead of Figure S3. 21.

115 Response: This was changed (L. 301) to Fig. S5 and Tables S2 and S3. The MANOVA results were not in
116 Table 4, just the catabolic evenness.

117 L. 274: "Complementary" not "complimentary".

118 Response: This was changed (L. 324).

119 22. L. 279: I agree that there is a positive relationship between CLPP and EEA but I would not say they
120 are "strong".

121 Response: We refer to the relationship as "significant" now (L. 328).

122 23. L.296-307: This is almost the same paragraph as l.273-295. Please remove.

123 Response: The first paragraph was deleted, and left in the "Relationships..." section.

124 24. L. 339-359: The authors discuss how drying and wetting impacted their results in the summer
125 treatment. Why was this date chosen as a sampling date? Was the rain just bad luck? The fact that other
126 timepoints were during dry periods may confound the comparison between summer and other seasons.
127 The study may have benefited from another summer timepoint taken when a wetting period had not
128 occurred for comparison. The author's comments on this would be appreciated.

129 Response: This reviewer is correct. In a sense, it was just "bad luck." Apriori planning dictated our
130 sampling time, but this is of course confounded with climate conditions. And dry periods are common in
131 the summer at our research location. It would be preferable to have more than 3 samples collected
132 over the year, but because many of the methods here are very labor intensive (i.e. CLPP and enzymes)
133 we were limited to three sampling events. That being said, however, we are the first to our knowledge
134 to have run community-level physiological profiles on the same soils on 3 dates (one of which includes
135 this unique dry-wet event).

136 25. L. 347: It should be "Table 1", not "Table 2".

137 Response: We have changed this (L. 487).

138 26. L. 365: Are the authors sure about these values? I was unable to find the same results.

139 Response: Thank you, we had incorrect values here and have changed these to the correct values (L.
140 505)

141 27. L. 376: The authors refer to microbial biomass C:N as having a season × rotation interaction.
142 However, Table 4 does not show this interaction as statistically significant. Is the data in the table
143 incorrect or is the text wrong? In the case that microbial biomass C:N does not have this interaction

144 does the following interpretation (line 377-378) that these interactions are “indicative of the enhanced
145 ability of soil microbes under diverse rotation to process, provision, and retain N” still hold?

146 **Response: Yes, it should still hold. Our MBC:MBN values did show significant interaction (Fig. 2). We**
147 **also added some further interpretation (and evidence) for how these interactions might be indicators of**
148 **this (L. 351-377).**

149 28. L. 376-378: Regardless of what measurement showed season × rotation interaction, the
150 interpretation that these interactions serve as evidence for enhanced N cycling and retention in diverse
151 systems could use strengthening. What specific functions of NAG and PER indicate that they can
152 improve N cycling, and why is an interaction between season × rotation meaningful? If microbial
153 biomass C:N does show an interaction, how specifically does this serve as evidence for enhanced N
154 provisioning? As it stands, these lines seem like a very important argument in the manuscript with little
155 discussion to strengthen it.

156 **Response: While we do not have any direct measurements, such as tracer ¹⁵N data, we do have some**
157 **evidence for this. Probably the strongest evidence is the correlation between potentially mineralizable**
158 **net N (PMN) and yield. We have provided some elaboration on this, further support for enhanced N**
159 **cycling, and we reworded as more speculative (L. 351-377).**

160 29. L. 405: Repetition of “studies”.

161 **Response: The second “studies” has been removed.**

162 30. L. 471-472: The authors may want to reconsider the strength of their statement. There is no proof
163 rotation facilitate microbes in supplying more N to crops; there is only more potentially mineralizable N
164 thanks to diversification (and the word “potentially” is important in this context).

165 **Response: We now state this more speculatively (L. 523-524). While we do not have direct evidence**
166 **that microbes are supplying more soil N to the crops, we have some strong inferential evidence in the**
167 **relationship between yield and potentially mineralizable N (Fig. S8).**

168 31. Tables: 1-page Tables would be much more convenient to read and the number in parentheses/use
169 of bold text/colors need to be explained in the title. Additionally, Tables S2, S3, and S4 are not reported
170 in the text.

171 **Response: We have made the tables 1-page, and added further explanation to the captions. Also we**
172 **now refer to all Tables in the text, except for Table S4, which we removed.**

173 32. Figures: I would have liked to find post-hoc analyses for each panel in Figure 1 and 2. The statistics
174 used for Figure 2 need to be briefly explained in the title, as well as what the error bars represent in
175 Figure S2. The legend for Figure S3 is too vague; what are the lines and icons representing? Finally, there
176 is an overall problem with Figure numbering in the Supplementary Material.

177 **Response: We have put the post-hoc analyses for the crop rotation in Figs. 1 and 2 – but only for the**
178 **crop rotation factor. We feel this is the factor of most importance, and discussing the season post-hoc**
179 **results in the text of the manuscript is sufficient. Sometimes figures can become too busy with letters**
180 **indicating more than one factor at a time. We have also further explained the statistics in both Fig. 1**
181 **and 2. Furthermore we have explained that the error bars are the errors associated with the PC loading**

182 values in Fig. S2. Also, we have fixed the supplemental figure number, thanks for noticing these things
183 in the supplemental figures.