Revision Notes

Blue text = authors' reply. Line numbers mentioned here correspond to those in the revised manuscript.

Anonymous Referee #2

General comments:

Soil enzymes play an important role in maintaining ecosystem quality, functional diversity, and nutrient cycling. The authors investigated the spatial variations in soil enzyme activities and their stoichiometry in soil profile of agroecosystem along precipitation gradient. They highlight the importance of enzyme activities stoichiometry for indication the nutrient limitation for microbe. The results are meaningful for comprehensive understanding of soil enzymes in agroecosystem responding to future climate change. The manuscript topic is timely with this work likely being of interested to a fairly wide readership. Further, the structure, figures and table of the manuscript are clear. However, the authors should highlight the novel of the paper and following issues should be fixed.

Thank you very much for the kind words, and we are very glad that you are interested in our topic. Our novelty is utilizing enzymatic stoichiometry to assess microbial nutrient limitations in the topsoil and subsoil of agroecosystems along the precipitation gradient and to determine the drivers, filling a gap in climate change on soil profiles of agroecosystems. We further clearly emphasize the novelty of this study in lines 59-63, 89-92, 108-120 and 420-429. See below for how we have carefully revised the manuscript in response to your comments.

1. Please revise the hypothesis, e.g. the second hypothesis, the driving factors change with an increase in depth, the driving factors for what?
   We are grateful for the suggestion. The second hypothesis is that the driving factors of microbial nutrient limitation change with an increase in soil depth (lines 117-118).

2. Materials and methods section: the MAP data is important, how did you get these data?
   Meteorological data that included monthly observations of the precipitation and temperature from 7 meteorological stations in Jilin Province were collected from the National Meteorological Information Center (http://data.cma.cn/) for the period from 1971 to 2017 (see lines 128-130).

3. Line 152, and 157, the full names of SM and TP should be given when they first appeared.
   I'm sorry the acronym is not explicit. "SM" means soil moisture, and "TP" means total phosphorus (see lines 164 and 171).

4. Line 158, the company information of continuous flow analyzer should be given.
   The company information of continuous flow analyzer is SKALAR SAN++,

Netherlands. We have added it in line 173.

5. Line 166, The methodology of enzymes should be revised as The methodology of enzymes activity measurement
   Thanks. The methodology of enzymes activity measurement in Supplementary figure 1 has been modified into the manuscript based on the first referee's comments, so this sentence has been removed.

6. Line 213, 3.1. Soil properties and their stoichiometry should be revised as Soil properties and nutrient stoichiometry.
   We have revised it in line 237.

7. Line 216-218, soil moisture and nutrients (TC, TN, and TP) were positively correlated with precipitation, but this strong correlation was not observed in the subsoil for TC, TC was positively correlated with precipitation in topsoil but not subsoil?
   Yes, our results show that the total carbon was positively correlated with precipitation in topsoil (0-10 cm, 10-20 cm), while this relationship was not observed in subsoil (20-30 cm, 30-40 cm, 40-50 cm). This may be due to the fact that soil carbon is stable and immediately below the plough layers, where plough pan formation typically occurs in tillage in the subsoil, considerable changes in the soil total carbon are unlikely to occur due to the persistence of compacted layers.

8. Line 232, enzyme nutrient stoichiometries?
   It has been revised to the enzymatic C:N and N:P ratios in line 260.

9. Line 294, P-acquiring enzyme was influenced by soil pH and moisture, the authors should give the reason.
   We gave the reason by supplementing the analytical methods and research evidence. A perpendicular projection of P-acquiring enzyme onto the line overlaying the environmental variable arrow revealed that P-acquiring enzyme was influenced by soil pH and moisture (Fig. 3). P is predominately derived from mineral aerosol deposition and weathering, and it is highly dependent on pH (Mahowald et al., 2008; Thingstad et al., 2005). High soil moisture and low soil pH increase P-acquiring enzyme activity, which facilitates P solubilization (Collavino et al., 2010; Xu et al., 2020b). Please see lines 333-338.

10. In the discussion section, the author should compare their results with the two hypothesis.
    Many thanks for your suggestion. Our findings are in support of our hypothesis that microbial nutrient limitations were stronger in the subsoil than in the topsoil along the precipitation gradient. Also, we show that the driving factors of microbial nutrient limitation are different in topsoil and subsoil (see lines 360-362).

11. Line 373-376, these two sentences seems to not relate to the conclusion, the authors should summarize your findings, discuss the implications or give the future research directions in the conclusion.

    We greatly appreciate your suggestion. We reorganized the Conclusion and deleted these two sentences. We observed an increase in microbial C and P limitation along the precipitation gradient in agroecosystems. Furthermore, our study showed stronger microbial C and P limitation in the subsoil compared to the topsoil. Given that the high sensitivity of microbial nutrient limitation was observed in the subsoil, our results suggest that the impact of precipitation on microorganisms may be underestimated if only the topsoil is assessed, especially in agroecosystems. Our study also provides insights to elucidate the differentiation in microbial nutrient limitation mechanisms among soil profiles, generating realistic predictions of how agroecosystems will respond to ongoing climate changes. Future research will incorporate deep tillage to maintain microbial nutrient balance in the subsoil to meet production goals and protect vital life-support systems in the context of climate change. Please see lines 420-429.