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Dear Professor Zhu:

Thank you very much for handling the review of our revised manuscript (ENVINT\_2018\_2311) entitled "High spatiotemporal dynamics of methanogenic microbes drive biogas production of full-scale anaerobic digesters"! We appreciate the insightful comments and suggestions of the three anonymous reviewers. We have carefully considered each point brought up by the reviewers and revised the manuscript to address them.

The revised manuscript is prepared in the Environment International template format. Our point-by-point replies to the reviewers are shown in the next pages.

Best wishes,



Yunfeng Yang, Ph.D., Professor

**Comments from the editors and reviewers:**

**-Reviewer 1**

**The manuscript by Zhang et al claims to report the spatiotemporal dynamics of microbial community in full-scale anaerobic digesters by using high-throughput genomic technologies includes MiSeq sequencing and GeoChip. However, the study about three digesters feeding two different substrates (excess sludge or solid wastes) from three different locations (Beijing, Ningbo and Qingdao) is not enough to call it a spatial scale research. The fours highlights are very common statements and the innovation is not obvious. The overall quality of the work was below the average.**

**Major comments:**

1. **Introduction part, lines 77-78, Have any basis for this hypothesis?**

**Response:** Yes, there are many studies addressing the special diversification of microbial communities in different ecosystems as well as bioreactors (references). Besides, at temporal scale, many recent studies have displayed stable instead of dynamic communities in bioreactors. Based on these studies, we hypothesize that anaerobic communities in full-scale digesters also follow the pattern “spatial difference but temporal stable”.

1. **Methods parts, The aim of the research is to evaluate the dynamics of methanogenic microbes in space and time. Therefore, it would be better to use the same type of substrates. The three digesters chosen in this study not only treat different substrates, but also from different locations. Thus, all feeds should be excess sludge or solid wastes, and geographic differences should be the only variable.**

**In fact, as mentioned above, digesters chosen from only 3 locations could not express the difference in spatial scale very well.**

**Response:** Thank you for addressing this topic. Actually we aim at temporal dynamic patterns at different digesters under different conditions rather than spatiotemporal patterns of communities under similar conditions. The latter one has been addressed in many studies upon lab scale research. We admit that digesters from only 3 locations are not enough to express differences in spatial scale. We have revised our topic to focus on temporal stabilities.

**3. Results part, section 3.1, line 185, how about the biogas production in Beijing digester, why no information supplied in Table S1 about feed and biogas production in Beijing digester, this data is incomplete, please revise it and complete the data.**

**Response:**.

**4. Results part, Section 3.2, lines 227-230, although both Syntrophomonas and Smithella could syntrophically oxidize organic acids, but target organic acids was different, for example, Syntrophomonas is often functioned as syntrophic butyrate-oxidizing bacteria, while Smithella is syntrophic propionate-oxidizing bateria, please give more information about them and their functions.**

**Response:**

**5. Results part, section 3.4, Why the data presented in this paragraph were quite different from Figure 4? The manuscript has not provided the biogas production in Beijing digester, how does MRM analysis conduct? Use the average data from other two digesters? Or just from one digester? You had better clarify it.**

**Response:** We

**6. Discussion part, section 4.1, line 326, author claimed space played a more important role in determining community variation, in fact, feed or substrate, instead of the space should be response for determining community variation. Anaerobic digester was man-made reactors rather than natural products which influenced by local environment and climate, in fact, spatial difference should not be existed, if we feed in the same substrate, operate the same type and scale of digester. Please reevaluate the concept weather suit for anaerobic digester or not.**

**Response:**

hi my name is qiuqiu

**Specific comments**

**1. lines 87, Please give more information about the full scale-anaerobic digesters&#xff0c; including reactor type, working volume, OLR, HRT and etc.**

**Response:** We.

**2. Line 97, How does the WWTP achieve the VFA measurement online?**

**Response:** We.

**3. Line 102, Why not measures the pH value online?**

**4. Line 138, it could be had better to provide a supplemented figure to describe how functional genes work?**

**5. Line 211, I do not understand this statement. What the “one digester” refer to? Line 198, the authors claims 36.938 OTUs were detected, could you definite the value from which digester? These two statements were confusing, please make them clear.**

**6. Lines 247-247, the value of r is small, could it be viewed as have correlations? In addition, Why COD is different?**

**7. Line 307, What the “environmental parameters” refers to, please clarify here.**

**8. Line 333, it should be “a few cores” or “a few core genera”.**

**9. Line 364, could syntrophic community utilize the feed stock directly? Please revise your expression.**

**10. Lines 369-371, the second highlight expressed the same idea with this sentence, does it come from your study or other researcher’s study?**

**11. Lines 375-378, what is the correlation between these two sentences?**

**Figures and Tables**

**1. Table 1, pH in Ningbo, P=0.075, which is larger than 0.05, it shouldn’t be bold.**

**2. Figure 2, in figure legend, “the node size is proportion to its degree”, what does the degree mean? Does it mean the relative abundance of specific genera? If yes, why the node sizes of Methanosaeta and Methanolinea were larger than Smithella?**

**3. Figure 4, as mentioned before, how do you analyze and get this figure without the biogas production data from Beijing digester. In addition, what is the linkage between fermentation bacteria/carbon degradation genes with biogas production? Could calculate the contributions?**

**4. Figure S3b, it showed that average signal intensity of methanogenic functional genes varied in three digesters, does it mean the dominant pathway of methane production varied? Please give more explanation.**

**5. Table S1, the data was incomplete, please complete them.**

**6. Table S3 and line 163, MRPP or Mrpp? Please make it constant.**

**Referee #2 (Comments to the Author):**

**The research paper of “Microbial functional traits are sensitive indicators of mild disturbance by lamb grazing” focused on an interesting topic of “mild environment disturbance” (here, lamb grazing) on the microbial taxonomic/function traits, the authors showed clearly results of disturbance of lamb grazing on functional traits but not taxonomic community in three conditions (non, aeolian soil erosion and deposition).**

**However, I have some major points I need to propose:**

**1) The authors chosen a pairwise comparison of grazing or not on taxonomic community and function genes under three environment conditions (aeolian soil erosion, deposition and CK). I am curious about effects of the simulated aeolian soil erosion or deposition on the soil microbial community or functions, as the simulated aeolian soil erosion or deposition in this case is also environment disturbance, are they wild disturbance or strong disturbance? And how they (simulated aeolian soil erosion and deposition) affect the taxonomic community and functions.**

**Response:** We thanks the anonymous reviewer for the encouragement! Both simulated aeolian soil erosion and deposition are strong disturbance. Aeolian erosion increased soil temperature for 0.80oC at the marginally significant level (*P*=0.07), but decreased soil total organic carbon (TOC) from 15.80 to 14.10 g/kg soil (*P*=0.08) and plant coverage from 53.29% to 36.38% (*P*=0.06). Soil deposition increased (*P*=0.03) soil dissolved organic carbon (DOC) from 74.13 to 123.46 mg/kg soil, which was consistent with expected outcome of soil deposition. The functional process of soil respiration was also sensitive to environmental disturbance, as it was decreased by aeolian erosion but increased by soil deposition (*P*<0.05). Consistent with those changes, soil microbial communities were altered (*P*<0.039) by both soil erosion and deposition, with dramatic increase in *Cyanobacteria* related to increased stability in soil aggregates. *amyA* genes encoding α-amylases were specifically increased (*P*=0.01) by soil deposition and positively correlated (*P*=0.02) to DOC, which likely explained changes in DOC. A large number of microbial functional genes associated with carbon, nitrogen, phosphorus and potassium cycling were decreased by both erosion and deposition, probably arising from acceleration of organic matter mineralization.

Since our manuscript focus on mild disturbance, we have excluded the results of strong disturbance in this manuscript. We have chosen to present the results in a separate manuscript, which has recently been published ([Ma et al 2017](#_ENREF_2)).

**2) One more important issue is the term of taxonomic community or microbial species in the main conclusion. The authors highlighted that lamb grazing on functional traits but not taxonomic community. As I found the authors only focused on bacteria based on 16S rRNA not whole community (such as soil virus and eukaryta), while the function gene based on whole soil genes by GeoChip, right? Thus, I prefer to use the bacterial community instead of taxonomic community in the main conclusion. As we don’t know whether soil other groups changed or not.**

**Response:** We have followed the suggestion to replace the term of taxonomic community or microbial species with bacterial communities or bacterial species throughout the manuscript.

**Some other issues:**

**1) In abstract, author described that “Microbial taxonomy remains unchanged, along with no changes in vegetation and soil variables.” Is it “remains unchanged” or “changed not significantly”? In addition, this sentence is not completed.**

**Response:** "Changed not significantly" is more proper. We have revised the sentence in lines 28-31 as “No significant change (P>0.05) was observed in any vegetation and soil variables. Nonetheless, hundreds of microbial functional gene families, but not bacterial taxonomy, were significantly (P<0.05) shifted”.

**2) Not clear about the introduction in Line 38-47, I cannot get the clear main question of “mild environment disturbance” from this part, and not sure the two reference supported the issue.**

**Response:** Reviewer #1 has raised a similar criticism and offered specific instruction how to improve it. We have revised lines 38-47 accordingly as “Earth’s environment is continuously changing, owing to a variety of natural and anthropogenic events. A recent literature survey of 310 experimental and 68 observational investigations across a variety of habitats showed that taxonomic and functional compositions of microbiome were generally sensitive to environmental disturbances in the majority of those studies ([Shade et al 2012](#_ENREF_5)). Closer examination showed that the observed shifts were usually associated with notable changes in environmental variables. However, many disturbances, e.g. introduction of low concentrations of persistent organic pollutants, are mild but may have detectable consequences in the long term ([Chang et al 2009](#_ENREF_1), [Nadal et al 2011](#_ENREF_3)). Those important disturbances are seldom examined by scientific communities because of limited time and the challenge to quantify significant effects of mild disturbances”. We have also done a more extensive literature search for appropriate references. We hope that the first paragraph is better now.

**3) Explain why you choose the three condition including the simulated aeolian soil erosion and deposition? And how will they influence the conclusions?**

**Response:** The inclusion of simulated aeolian soil erosion and deposition is based on the natural environment of our study site. The Inner Mongolian grasslands are vast wind-swept areas, which provide the largest usable wind power capacity in Asia. A downside of strong wind is substantial aeolian soil erosion and deposition. Therefore, including simulated aeolian soil erosion and deposition is not only useful in addressing fundamental scientific questions, but also helpful for evaluating ecological consequences of major environmental disturbance in the Inner Mongolian grasslands. This explanation is included in lines 50-57 of the revised manuscript.

Changes of specific functional taxa or genes vary by soil conditions, which is easily understandable. However, there is a common phenomenon of microbial functional traits as sensitive indicators of mild disturbance by lamb grazing, suggesting that the major conclusion is generalizable. This finding is significant, since describing how microorganisms respond to environmental drivers is often based on taxonomy in the past. However, moving away from taxonomy towards functional traits can be particularly informative since traits determine population demography and ecosystem processes. Therefore, it is a central topic in microbial ecology to understand and predict how taxonomy and functional traits of microbial communities respond to environmental disturbances. The observation highlighted herein, showing a high level of sensitivity with respect to functional traits (functionally categorized taxa or genes) in differentiating mild environmental disturbance, suggests that the key level at which to address bacterial responses may not be “species” (by means of rRNA taxonomy), but rather the more functional level of genes. This explanation is included in lines 32-36 of the revised manuscript.

**4) Line 58-64 can be showed in the supplemental materials, as well as line 65-68.**

**Response:** If we completely move lines 58-68 to the supplemental materials, there will be no information about the experimental setup, results of environmental variables and overall sequencing data. So we have decided to substantially shortened the sentences, while providing detailed information in the supplemental materials. The revised sentences in lines 58-64 is shown as “Here, we examine the effects of light-intensity lamb grazing under three conditions, alone or in combination with simulated aeolian soil erosion and deposition (Figure S1). We examined a total of 15 plant and soil variables and found that no variable was significantly changed by grazing (P>0.05, Table S1). High-throughput sequencing of PCR amplicons of the 16S rRNA gene were carried out to examine bacterial taxonomy in soil samples, resulting in 17,111 sequences per sample affiliated with 474 genera (See Supporting Information for details)”.

**5) Line 74 “resistance indices”?**

**Response:** Resistance indices quantify the extent of changes caused by disturbance ([Orwin and Wardle 2004](#_ENREF_4)). As explained in the supplemental materials, this index of each OTU has a range of -1 and 1, with a value of 1 showing that the disturbance had no effect (maximal resistance), and -1 showing the strongest effects (least resistance).

To provide an explanation of resistance indices, we have revised lines 69-73 as “To identify OTUs that were relatively more sensitive to grazing than others, the resistance index of each OTU was calculated to quantify the extent of changes caused by disturbance (See Supporting Information for details). A subset of genera (less than 13% of total genera), which were predominantly rare taxa, had low resistance index values (Figure S2a)”.

**References**

Chang H, Wan Y, Hu J (2009). Determination and source apportionment of five classes of steroid hormones in urban rivers. *Environmental science & technology* **43:** 7691-7698.

Ma XY, Zhao CC, Gao Y, Liu B, Wang TX, Yuan T *et al* (2017). Divergent taxonomic and functional responses of microbial communities to field simulation of aeolian soil erosion and deposition. *Mol Ecol* **26:** 4186-4196.

Nadal M, Schuhmacher M, Domingo JL (2011). Long-term environmental monitoring of persistent organic pollutants and metals in a chemical/petrochemical area: human health risks. *Environ Pollut* **159:** 1769-1777.

Orwin KH, Wardle DA (2004). New indices for quantifying the resistance and resilience of soil biota to exogenous disturbances. *Soil Biology and Biochemistry* **36:** 1907-1912.

Shade A, Peter H, Allison SD, Baho D, Berga M, Buergmann H *et al* (2012). Fundamentals of Microbial Community Resistance and Resilience. *Frontiers in Microbiology* **3:** 417.