Vertical and temporal variations of soil bacterial and archaeal communities in maize-soybean rotation agroecosystem

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In this paper, the authors examined in depth the possible vertical and temporal patterns of soil bacterial and archaeal communities in maize-soybean rotation agroecosystem. In particular, the authors pointed out that the bacterial and archaeal communities are impacted and shaped by the soil depth. Conversely, significant temporal patterns were not observed for both communities. The authors also focused on the rare taxa, identifying the rare bacterial community as the key determinants of the soil fertility index.

I find interesting the general concept of the work that incorporates temporal variation, in addition to vertical variation, as a putative determinant in influencing the distribution of soil microbes. However, I have some comments/suggestions on some points that I believe the authors should clarify and/or improved.

- 1) Soil characteristics (as TN, TC, etc.) obtained by the physiochemical analysis are not reported as supplementary results, as well as the methodologies are not explained in Materials and Methods. I think that these results, if available, should be reported as supplementary results. Otherwise, the authors should mention it only in the results sections as "data are not reported". More important, are these parameters measured for the different sampling times? I think the authors should report this information.
- 2) The analysis was conducted in a maize-soybean rotation agro-ecosystem over one year. However, it seems to me that the agricultural context in which this analysis is carried out is little emphasized. For example, in the introduction, as well as in the discussion, the authors do not explicit/clarify why it is important to understand if (and eventually how) the soil depth influences the diversity and composition of soil microbial communities in agricultural soils especially when a crop rotation is performed. The deeper soils are important for crop yield, especially for cereal crops as maize. Therefore, the authors should explain how the information on the vertical patterns can help the sustainability of agricultural systems. Moreover, the soil prokaryotic community compositions are not linked to the agricultural context and the crops present in the different sampling times.
- 3) The authors should discuss more deeply why they did not detect a temporal variation in this particular crop rotation system. In particular, by Random forest analysis, the authors found the Total Nitrogen as one of the key determinants for bacterial richness and evenness. It is well known that the use of the leguminous plant in a crop rotation is crucial for increasing the total nitrogen in the soil. Concurrently, they found that the bacterial richness did not show temporal variations after the rotation with soybean, which certainly increased the Nitrogen content. I think that the authors should examine indepth this point. Also, in the discussion section, the authors stated that "The temporal variations of the soil microbial community were attributable to two factors: the shift of soil nutrients and/or the plant growth stage (Yang, 2017)" (line 261-263) and justify their results saying that the soil samples were collected from only two plant individuals. What about the shift of soil nutrients? Again, did the authors verified if soil nutrients contents and, in general, soil properties were different among the sampling times? If not, I think that the phrase "The minimal impact may also be due to no apparent difference in soil environmental conditions among sampling times" (line 265-266) should be removed.
- <u>4) In general, the impression is that the authors glossed over the results, which are sometimes roughly reported. Moreover, It is often not clear to which figures (and to which panels of the figures) the authors were referring. Line 172-174 for example.</u>
- 5) The authors have confused Figure 3 with Figure 4.
- 6) <u>I suggest the author using the Tax4fun software package</u>, which can predict the functional profiles of bacterial and archaeal communities. This might shed a light on the functionality of rare bacterial and archaeal communities the authors found, then greatly improved the paper.