**Micro-environmental variation of soil microbial biodiversity differs across land use types – implications for field sampling designs**

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**Supplementary Information**

**Deterministic and stochastic processes modelling microbial community assembly**

The combination of the β-nearest taxon index (β-NTI) and the Bray-Curtis-based Raup-Crick metric (RCbray) was used to estimate the relative influences of deterministic (homogeneous selection and variable selection) and stochastic (homogenizing dispersal, dispersal limitation, and undominated) processes on community turnover (Stegen et al., 2013). Both indexes were calculated according to Jia et al. (2018) and following the procedure detailed in the R scripts available at https://github.com/Jia-Xiu/Jia\_et\_al\_Microorganisms\_2020. First, the β-NTI was calculated. The β-NTI index compares the observed phylogenetic turnover in ASVs between pairs of communities with a null distribution. The observed phylogenetic turnover between pairs of communities was determined by β-mean nearest taxon distance (β-MNTD) using function ‘comdistnt’ in the R package *picante* (Kembel et al., 2010). The null distribution of phylogenetic turnover was generated by randomly shuffling the ASVs at the tip of the phylogenetic tree 999 times. The β-NTI was measured as the difference between the observed β-MNTD and the mean of the null distribution in units of standard deviation. Second, the RCbray was calculated. The RCbray metric, compares the ASV taxonomic turnover between a pair of communities and the null distribution (Stegen et al., 2013). To create the RCbray metric, the Bray-Curtis dissimilarity between observed communities was calculated using the function ‘vegdist’ from *vegan* R package (Oksanen et al., 2022). Then, the null distribution of the Bray-Curtis dissimilarity between simulated communities were constructed by randomly sampling ASVs 999 times. The deviation between empirically observed Bray-Curtis and the null distribution was then standardized to vary between -1 and +1. To estimate the relative influence of each assembly process the combination of both, the β-NTI and the RCbray metric, was used for each land use by depth (Dini-Andreote et al., 2015; Stegen et al., 2012).

References

Dini-Andreote, F., Stegen, J. C., Van Elsas, J. D., & Salles, J. F. (2015). Disentangling mechanisms that mediate the balance between stochastic and deterministic processes in microbial succession. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(11), E1326–E1332. https://doi.org/10.1073/pnas.1414261112

Jia, X., Dini-Andreote, F., & Falcão Salles, J. (2018). Community Assembly Processes of the Microbial Rare Biosphere. *Trends in Microbiology*, *26*(9), 738–747. https://doi.org/10.1016/j.tim.2018.02.011

Kembel, S. W., Cowan, P. D., Helmus, M. R., Cornwell, W. K., Morlon, H., Ackerly, D. D., Blomberg, S. P., & Webb, C. O. (2010). Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, *26*(11), 1463–1464. https://doi.org/10.1093/bioinformatics/btq166

Oksanen, J., Kindt, G. L., Roeland, S. F., Blanchet, G., Legendre, P., Minchin, P. R., O’Hara, R. B., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., Caceres, M. De, Durand, S., … Weedon, J. (2022). *vegan: Community Ecology Package* (2.6-2). https://github.com/vegandevs/vegan

Stegen, J. C., Lin, X., Fredrickson, J. K., Chen, X., Kennedy, D. W., Murray, C. J., Rockhold, M. L., & Konopka, A. (2013). Quantifying community assembly processes and identifying features that impose them. *The ISME Journal*, *7*(11), 2069–2079. https://doi.org/10.1038/ismej.2013.93

Stegen, J. C., Lin, X., Konopka, A. E., & Fredrickson, J. K. (2012). Stochastic and deterministic assembly processes in subsurface microbial communities. *The ISME Journal*, *6*(9), 1653–1664. https://doi.org/10.1038/ismej.2012.22