Short Communication

Microbial community assembly in soil aggregates: A dynamic interplay of stochastic and deterministic processes

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A B S T R A C T

Understanding how distinct processes operate in mediating community assembly is a long-standing theme in (microbial) ecology. Particularly in soil microbial communities, we still lack a fundamental appreciation of how assembly processes structure communities at the fine-scale level of soil aggregates. In this study, samples from a long-term agricultural field subjected to different fertilization regimes were used to quantify the relative influences of stochastic and deterministic processes on soil bacterial community assembly. First, we found bacterial communities to be more phylogenetically clustered in larger soil aggregates comparatively to small aggregates (Spearman's $r = -0.366$, $P < 0.05$). Second, we found the overall relative influence of selection to gradually decrease with an increase of aggregate size (Mantel $r = 0.161$, $P < 0.01$). By partitioning aggregate sizes and fertilization regimes, we found that sites subjected to fertilization (including chemical, organic, and bio-organic fertilizers) displayed a stronger relaxation of selection and an increased influence of stochasticity with an increase in aggregate size; a pattern not significantly observed at the control (unfertilized) treatment. Collectively, our results highlight the importance of evaluating community assembly at the fine-scale levels of soil aggregates and illustrate how regional level disturbances (i.e., agricultural management) exert an influence on the dynamic interplay of stochastic and deterministic processes.

1. Introduction

Disentangling the relative influences of ecological processes mediating the assembly of microbial communities is a major challenge in microbial ecology (Nemergut et al., 2013). Currently, it is broadly recognized that local community assemblages are influenced by two classes of ecological processes – deterministic and stochastic processes (Stegen et al., 2012; Tripathi et al., 2018). The niche-based theory states that deterministic process is mediated by differences in species traits, interspecies interactions (e.g., competition, predation, mutualisms, and trade-offs), and environmental filtering (e.g., pH, temperature, salt, and moisture) (Tripathi et al., 2018; Chesson, 2000). On the other hand, the neutral theory suggests that community assembly can be seen by a dynamic balance of stochastic processes, i.e., random birth and death events, dispersal and diversification (Hubbell, 2001; Chave, 2004;

Linquist et al., 2015). Recently, it has been broadly recognized that both deterministic and stochastic processes simultaneously mediate the assembly of local communities. The challenge relies on advancing knowledge on the mechanisms underpinning their relative influences structuring communities at distinct spatiotemporal scales and across divergent systems (Zhou, 2017). In particular, spatial and temporal scales have been shown to directly reflect on the outcome of how these distinct processes operate, the so-called 'scale-dependence' (Dini-Andreote et al., 2015; Chase et al., 2018).

Soils are highly heterogeneous and encompass diverse spatially structured micro-habitats that are defined at the scale of soil aggregates and pore structure. Resource availability (Briar et al., 2011; Chenu and Stotzky, 2002) and physicochemical conditions (Vos et al., 2013) are known to vary with aggregate size and to support different microbial communities (Bach et al., 2018; Trivedi et al., 2017). Several studies

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have addressed the extent to which deterministic and stochastic processes influence the assembly of bacterial communities in soils (Huber et al., 2020; Tripathi et al., 2018); however, we still lack a detailed assessment of whether these processes interplay at the fine-scale level of soil aggregates. Besides, at a regional scale, it has been shown that different fertilization regimes exert a major influence on the stability and distribution of soil aggregates (Li et al., 2019). Thus, in this study, we sought to investigate the balance between stochastic and deterministic processes structuring soil 'aggregate-level' bacterial communities, and examined whether distinct fertilization regimes influence the dynamics by which these processes operate. We hypothesized that fertilization treatments will result in a different interplay of stochastic and deterministic processes between soil aggregates due to the different stability of soil aggregates. We also expected that the input of chemical fertilizer will cause more stochasticity because of the overall disturbance to bacterial community, while organic fertilization would induce more determinism because of the selection of nutrient resources.

To study the impact of distinct fertilization treatments on the processes structuring bacterial communities at distinct soil aggregate sizes, we examined datasets of samples collected from a long-term agricultural experimental field in Nanjing, China (Dong et al., 2020). The experimental sites have been subjected to different types of fertilization treatments (including a control site, not fertilized) for seven consecutive seasons. Soil samples were separated into four classes (based on size) of soil aggregates using a wet-sieving method (Dong et al., 2020). Bacterial community structure at each class of soil aggregate per sample was determined by profiling the bacterial 16S rRNA gene using an Illumina Miseq platform. The relative influences of stochastic and deterministic processes were quantified using a phylogenetic null modelling analysis (Stegen et al., 2012; Dini-Andreote et al., 2015).

2. Methods

2.1. Datasets

We examined datasets of samples collected from a long-term agricultural experimental field in Nanjing, China (Dong et al., 2020). These field sites were located at a subtropical monsoon climate with an average annual temperature of 17 °C (5.5–38.5 °C). The soil pH values ranged from 7.21 to 7.64. These field sites were regularly irrigated during tomato cultivation. Soil samples were collected at triplicated plots across the following treatments: no fertilizer input (CK), chemical fertilization (CF), organic fertilization (OF), and bio-organic fertilization (BF). Chemical fertilization consisted of predefined amounts of urea (225 kg N/ha), calcium superphosphate (65 kg P/ha), and potassium sulphate (150 kg K/ha) amendments. The organic fertilizer (i.e., compost) was obtained by the fermentative process of a mixture of rapeseed meal and chicken manure. The bio-organic fertilizer was produced by adding *Bacillus amyloliquefaciens* T-5 to the organic fertilizer before the fermentative process (Tan et al., 2013). In every season, fields were tilled before the planting of tomato.

Soils were sampled 1 month after tomato harvesting at the end of the 7th season. Nine parts of soil cores (7.4 cm diameter and 10 cm deep) were collected from each field plot and mixed as one composite sample. All soil samples were transported to the laboratory in aluminum boxes with ice and stored at 4 °C. Samples were processed by separating the soil aggregates into 4 size classes: large macro-aggregates >2 mm (LMa); medium macro-aggregates 1–2 mm (MMa); small macro-aggregates 0.25–1 mm (SMa), and micro-aggregates <0.25 mm (Mi); using specific modifications of the wet-sieving method. In brief, soils were not air-dried before sieving to minimize the impact of this disturbance on microbial communities (Elliott, 1986; Davinic et al., 2012). A total of 100 g of soil were put on a column of sieves and immersed in sterilized water for 5 min. The sieves were gently shaken by hand at a frequency of ca. 50 times per minute. Each aggregate fraction remaining on the respective sieve meshes was stored at −80 °C for DNA extraction. Soil DNA was

extracted from 0.25 g of dry-weight soil using the PowerSoil DNA Isolation Kit (Mobio Laboratories Inc., Carlsbad, USA). The bacterial community composition at each class of soil aggregate per sample was determined using an Illumina MiSeq platform (i.e., the bacterial 16S rRNA gene V4 region, primer set 520F-802R (Claesson et al., 2009)) at Personal Biotechnology Co., Ltd. (Shanghai, China). For additional details on the field experiment, sample collection and processing, and the Illumina Miseq sequencing, see Dong et al. (2020).

2.2. Data analysis

Raw paired-end reads were processed using QIIME2-2019.7 (Bolyen et al., 2019) with the "dada2 denoise-paired" function to determine amplicon sequence variants (ASV). A total of 1,074,416 high-quality and non-chimeric sequences were obtained from a total of 48 samples, with a median of 22,930 (range from 11,028 to 30,263), clustered into 9759 bacterial ASVs.

Sequences of each ASVs were aligned and a phylogenetic tree was constructed using the "align-to-tree-mafft-fasttree" function in QIIME2. To test for phylogenetic signal, soil aggregate sizes optima for each ASVs (mean diameter) were calculated according to Stegen et al. (2012), as follows: the relative abundance-weighted mean value of each ASV was calculated across soil aggregates sizes taking into account the soil pH value (i.e., ASV relative abundance × pH value). Further, Euclidean distances were calculated and the Mantel correlograms tests were performed to measure the correlation coefficients between differences in soil aggregates size optima and the phylogenetic distances. The significance of these correlations was determined using 999 permutations with Bonferroni correction.

To evaluate the community phylogenetic community structure, we calculated the standardized effect size measure of the mean nearest taxon distance (SES.MNTD) using the "ses.mntd" function (null.mode = "taxa.labes", runs = 999) in the "picante" package in R 3.6.1. Lower values of SES.MNTD indicate phylogenetic clustering, while higher values indicate phylogenetic overdispersion. To evaluate the relationship between the soil aggregate size and SES.MNTD, we applied Spearman's correlation test using the "cor.test" function of "stats" package in R 3.6.1. The pairwise phylogenetic turnover between communities was calculated by the mean nearest taxon distance metric (βMNTD) using the "comdistnt" function (abundance.weighted = TRUE) in "picante". The difference between observed βMNTD and the mean of the null distribution was measured in units of standard deviations (of the null distribution), using the convention to β-Nearest Taxon Index (βNTI) (Stegen et al., 2013). To assess the relative influences of stochastic and deterministic processes, we tested for all possible pairwise comparisons between βNTI values within each soil aggregate size. βNTI values (which are derived from pairwise comparisons) were regressed against Euclidean distance matrices of soil aggregates sizes. To evaluate the relationship between phylogenetic turnover and soil aggregates, we performed Mantel tests using 999 permutations. To calculate the relative influences of stochastic and deterministic processes, pairwise comparisons of βNTI values between the same soil aggregate class were extracted. In brief, βNTI < −2 or >+2 indicates that βMNTDobs deviates from the mean βMNTD$_{null}$ by more than two standard deviations. Thus, the model considers βNTI < −2 or >+2 to indicate significantly less than or greater than expected phylogenetic turnover, respectively, for a given pairwise comparison. In this case, βNTI < −2 indicates the dominance of deterministic processes and low turnover (i.e., homogeneous selection); βNTI > 2 indicates the dominance of deterministic processes and high turnover (i.e., variable selection); and −2 < βNTI <2 indicates the lack of deviation and the dominance of stochastic processes (Stegen et al., 2012; Dini-Andreote et al., 2015). In addition, we calculated the Bray–Curtis-based Raup–Crick metric (RCbray) as described by Stegen et al. (2013, 2015) to further partition the relative influences of non-selection processes, i.e., dispersal limitation (RC$_{bray}$ > 0.95), homogenizing dispersal (RC$_{bray}$ < −0.95) and undominated processes (−0.95 >

RC$_{bray}$ > 0.95). Dispersal limitation constrains the movement of species and led to higher levels of community dissimilarity; on the contrary, homogenizing dispersal, defined as high levels of species movement, led to a decrease in community dissimilarities (Zhou, 2017).

3. Results

Significant phylogenetic signals were found across short phylogenetic distances in all datasets (Supplementary Fig. S1). Therefore, we calculated the standardized effect sizes of the mean nearest taxon distance (SES.MNTD) and the β-nearest taxon indexes (βNTI), as both of these metrics are indicated when significant phylogenetic signals occur at short phylogenetic distances (Stegen et al., 2012; Tripathi et al., 2018). Taking all samples together, values of SES.MNTD were found to be negatively correlated with soil aggregate sizes ($r = -0.366$, $P < 0.05$, Spearman's correlation test, Fig. 1). This result shows that larger aggregate sizes have more phylogenetically clustered bacterial communities comparatively to small aggregates.

We further evaluated the relationship between βNTI values and aggregate sizes in order to quantify the relative influences of stochastic and deterministic processes mediating community assembly. Pairwise comparisons of βNTI values between all samples were significantly correlated with the difference in aggregate sizes (Mantel test, $r = 0.161$, $P < 0.01$) (Fig. 2a). When calculating βNTI values in samples partitioned by the different fertilization treatments (Fig. 2b), pairwise comparisons of βNTI in CF, OF and BF were all significantly correlated ($P < 0.05$) with aggregate sizes. Interestingly this pattern was not found to be significant in the 'control' (CK) treatment ($P = 0.085$). Collectively, these results indicate an overall effect of fertilization regimes on the dynamic interplay of stochastic and deterministic processes operating on bacterial community assembly at the fine-scale levels of soil aggregates.

We found homogeneous selection (βNTI<−2) to be the dominant process structuring bacterial communities across all soil aggregate sizes. Interestingly, the relative influence of homogeneous selection was found to gradually decrease (i.e., 'selection relaxation') with an increase in aggregate size (Mantel $r = 0.161$, $P < 0.01$; MMa fraction = 93.9%, SMa = 81.8, Mi = 78.8%, and LMa = 71.2%) (Fig. 3). The relative influence of stochasticity was mostly associated with dispersal limitation, also displaying some variation according to aggregate size (Fig. 3). Last, the fertilization treatment also exerted an impact on the dynamics by which these assembly processes operate (Supplementary Materials Fig. S2). In brief, homogeneous selection appeared to exert a higher influence in sites subjected to organic fertilization (i.e., 95.5% and 90.9% at OF and BF, respectively); whereas stochastic processes (mostly due to dispersal

limitation) exerted a relatively higher influence in sites subjected to chemical fertilization (13.6% at CF).

4. Discussion

In the present study, we initially showed that larger soil aggregates support more phylogenetic clustered bacterial communities (Fig. 1). This finding is somehow counter-intuitive, given that greater surface area is expected to support more diverse (and thus, phylogenetically 'dispersed') community types. Second, we found the interplay of deterministic and stochastic processes to differ in structuring bacterial communities in soil aggregates of distinct sizes. This finding provides new insight into the scale dependency of how these mechanisms operate, as supported by previous studies in microbiology and macroorganism ecology (Dini-Andreote et al., 2015; Chase et al., 2018). Besides, taken together these results open up new avenues to investigate whether similar patterns may occur across distinct soil types and at different systems (agricultural and natural). Third, we showed that fertilization treatments also exerted an influence on how dynamically these processes operate. Interestingly, we found that pairwise comparisons of βNTI significantly correlated with changes in soil aggregate sizes across all fertilization treatments (CF, OF, BF); however, this correlation was not found to be significant in the control (CK, not fertilized) treatment (Fig. 2). This indicates that fertilization significantly and differentially affects the balance between community assembly processes and that such effects occur at different relative influences at soil aggregates of different sizes. Our findings also integrate knowledge from previous studies showing that soil microbial community structures differ across soil aggregates collected within the same soil site, and that differences can be found across sites subjected to distinct nutrient amendments (Wang et al., 2017). Worth mentioning, similar findings were also reported by the short- and long-term application of organic fertilizer, in which microbial communities showed different responses according to soil aggregate sizes in sites subjected to fertilization (Li et al., 2019). Thus, the results obtained in our study advance knowledge on explaining these differences by showing that these observed differences in community structure are modulated by a dynamic interplay of ecological assembly processes.

We found the relative influence of deterministic processes (i.e., homogeneous selection) to largely modulate the structure of bacterial communities in soil aggregates of all sizes (Fig. 3). Most interestingly, we identify a relaxation of selection as aggregate sizes increase (from MMa 1–2 mm to LMa >2 mm). This may likely support the idea that small aggregates are under stronger selective pressure and might be less subjected to stochastic processes caused by either random dispersal or ecological/environmental disturbance. Another line of reasoning would argue that these differences might also be a result of differences in aggregate physicochemical properties, such as the availability of nutrients and pH (Wang et al., 2015).

To a lowered extent, stochastic processes also exerted an influence on bacterial community assembly. This was found to increase in importance at larger aggregate sizes and at sites subjected to fertilization treatments. By partitioning the influence of non-selective processes, we found dispersal limitation to be the major mechanism accounting for stochasticity. This might likely be linked to the fact that aggregates may work as 'incubators' of soil microbial communities (sensu Rillig et al., 2017; Vos et al., 2013). The effect of fertilization can be explained by envisioning fertilization amendment as a 'disturbance' to the system, which temporarily disrupts environmental selection, and, as such, increase stochasticity. This is supported by the fact a that lower influence of stochasticity was quantified in the control (CK, not fertilized) treatment.

Despite we could not technically determine potential differences in physicochemical properties within and across distinct soil aggregate sizes, it is tempting to speculate that these observed dynamic interplay of ecological processes are mechanistically underpinned by differences
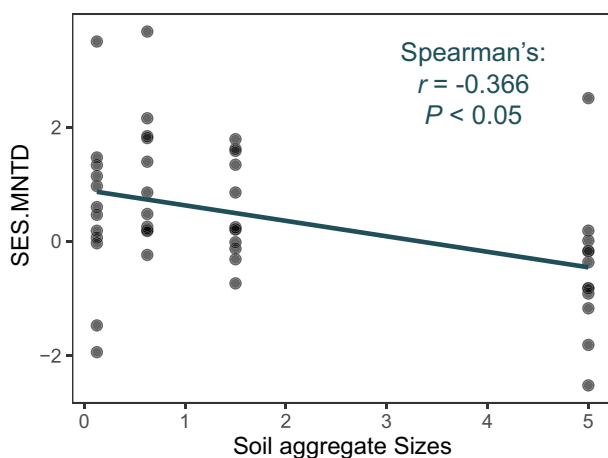

Fig. 1. Relationship between soil aggregate sizes and standardized effect size measures of the mean nearest taxon distance (SES.MNTD). Low and high values of SES.MNTD indicate phylogenetic clustering and overdispersion, respectively.
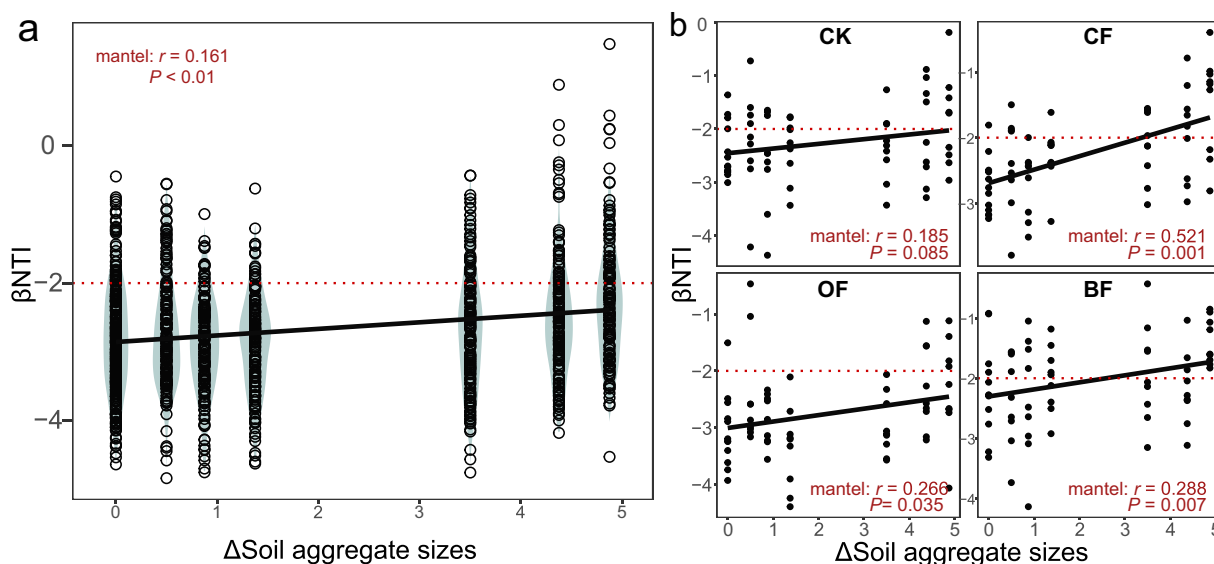
Fig. 2. Relationship between βNTI values and differences in soil aggregate sizes. (a) Correlational analysis based on the entire dataset, (b) correlational analyses per each independent fertilization treatment. Statistics are provided as inset panels.
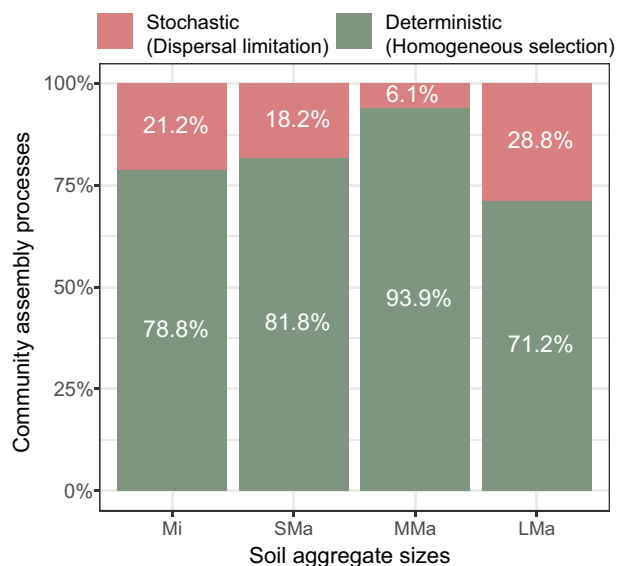

Fig. 3. Relative influences of distinct community assembly processes structuring aggregate-level bacterial communities. Mi: microaggregate, <0.25 mm; SMa: small-macroaggregate, 0.25–1 mm; MMa: middle-macroaggregate, 1–2 mm; LMa: large-macroaggregate, >2 mm.

in aggregates' physical (e.g. pore space, O_2 tension, volatiles) (Rillig et al., 2017) and chemical properties (e.g., water content, organic matter, pH) (Bronick and Lal, 2005; Six and Paustian, 2014; Wang et al., 2015). These physicochemical gradients within aggregates will likely provide different niches for distinct microbial populations to establish, thus resulting in a strong deterministic (selection) assembly. However, a more holistic view would also consider that at least part of these populations will likely fluctuate over time due to random stochastic processes of dispersal and drift, both of which will be affected by aggregate size. Together, these nicely illustrate and explain our findings in this study.

The further development of technology to detect soil physical and chemical properties in situ at the level of soil aggregates, in particular the determination of pH and oxygen gradients, as well as the composition of organic matter fractions will likely assist a better explanation of

the extent of which stochastic and deterministic processes operate. Overall, our study corroborates the overarching idea that processes and mechanisms operating on soil microbiome assembly are dynamic and scale-dependent (Dini-Andreote et al., 2015). The last holds true even when the spatial scale is miniaturized at the level of differences in soil aggregate sizes. We advocate that proper management of soil biodiversity (i.e., applied soil ecology) can more effectively be achieved in light of a better understanding of how ecological processes operates in a system, and by identifying the mechanisms modulating their relative influences.

This study highlights the importance of evaluating community assembly at the fine-scale levels of soil aggregates. Bacterial communities are more phylogenetically clustered in larger soil aggregates. The heterogeneity of soil aggregates significantly mediated the balance of stochastic and deterministic processes in fertilized fields, resulting in a dominated deterministic assembly process in soil aggregates. These deterministic processes all belong to homogeneous selection, and have different contributions among soil aggregates. Regional level disturbance (fertilization in this study) also showed influence on the dynamic interplay of stochastic and deterministic processes.

CRediT authorship contribution statement

MHD, RL, GAK, FDA, and QRS designed the study. MHD, XHD, NZ and HJL performed all the experiments. MHD, XHD and FDA analyzed the data. MHD, FDA and RL wrote the manuscript. FDA, GAK, RL and QRS participated in the discussion and editing of the manuscript. All authors approved the final version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2021.103911.

References

Bach, E.M., Williams, R.J., Hargreaves, S.K., Yang, F., Hofmockel, K.S., 2018. Greatest soil microbial diversity found in micro-habitats. Soil Biol. Biochem. 118, 217–226. https://doi.org/10.1016/j.soilbio.2017.12.018.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Cope, E.K., Da Silva, R., Diener, C., Dorrestein, P.C., Douglas, G.M., Durall, D.M., Duvallet, C., Edwardson, C.F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J.M., Gibbons, S.M., Gibson, D.L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G.A., Janssen, S., Jarmusch, A.K., Jiang, L., Kaehler, B.D., Kang, K. Bin, Keefe, C.R., Keim, P., Kelley, S.T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langille, M.G. I., Lee, J., Ley, R., Liu, Y.-X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B.D., McDonald, D., McIver, L.J., Melnik, A.V., Metcalf, J.L., Morgan, S.C., Morton, J.T., Naimey, A.T., Navas-Molina, J.A., Nothias, L.F., Orchanian, S.B., Pearson, T., Peoples, S.L., Petras, D., Preuss, M.L., Pruesse, E., Rasmussen, L.B., Rivers, A., Robeson, M.S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S.J., Spear, J.R., Swafford, A.D., Thompson, L.R., Torres, P.J., Trinh, P., Tripathi, A., Turnbaugh, P.J., Ul-Hasan, S., van der Hooft, J.J.J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., von Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K.C., Williamson, C.H.D., Willis, A.D., Xu, Z.Z., Zaneveld, J.R., Zhang, Y., Zhu, Q., Knight, R., Caporaso, J.G., 2019. Author correction: reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37, 1091. https://doi.org/10.1038/s41587-019-0252-6.

Briar, S.S., Fonte, S.J., Park, I., Six, J., Scow, K., Ferris, H., 2011. The distribution of nematodes and soil microbial communities across soil aggregate fractions and farm management systems. Soil Biol. Biochem. 43, 905–914. https://doi.org/10.1016/j.soilbio.2010.12.017.

Bronick, C.J., Lal, R., 2005. Soil structure and management: a review. Geoderma 124, 3–22. https://doi.org/10.1016/j.geoderma.2004.03.005.

Chase, J.M., McGill, B.J., McGlinn, D.J., May, F., Blowes, S.A., Xiao, X., Knight, T.M., Purschke, O., Gotelli, N.J., 2018. Embracing scale-dependence to achieve a deeper understanding of biodiversity and its change across communities. Ecol. Lett. 21 (11), 1737–1751.

Chave, J., 2004. Neutral theory and community ecology. Ecol. Lett. 7, 241–253. https://doi.org/10.1111/j.1461-0248.2003.00566.x.

Chenu, C., Stotzky, G., 2002. Interactions between microorganisms and soil particles: an overview. In: Huang, P.M., Bollag, J.M., Senesi, N. (Eds.), 2002. Interactions Between Soil Particles and Microorganisms. Impact on the Terrestrial Ecosystem. John Wiley and Sons, USA, pp. 3–40.

Chesson, P., 2000. Mechanisms of maintenance of species diversity. Annu. Rev. Ecol. Syst. 31, 343–366. https://doi.org/10.1146/annurev.ecolsys.31.1.343.

Claesson, M.J., O'Sullivan, O., Wang, Q., Nikkilä, J., Marchesi, J.R., Smidt, H., de Vos, W. M., Ross, R.P., O'Toole, P.W., 2009. Comparative analysis of pyrosequencing and a phylogenetic microarray for exploring microbial community structures in the human distal intestine. PLoS One 4. https://doi.org/10.1371/journal.pone.0006669.

Davinic, M., Fultz, L.M., Acosta-Martinez, V., Calderón, F.J., Cox, S.B., Dowd, S.E., Allen, V.G., Zak, J.C., Moore-Kucera, J., 2012. Pyrosequencing and mid-infrared spectroscopy reveal distinct aggregate stratification of soil bacterial communities and organic matter composition. Soil Biol. Biochem. 46, 63–72. https://doi.org/10.1016/j.soilbio.2011.11.012.

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. Proc. Natl. Acad. Sci. U. S. A. 112, E1326–E1332. https://doi.org/10.1073/pnas.1414261112.

Dong, M., Zhao, M., Shen, Z., Deng, X., Ou, Y., Tao, C., Liu, H., Li, R., Shen, Q., 2020. Biofertilizer application triggered microbial assembly in microaggregates associated with tomato bacterial wilt suppression. Biol. Fertil. Soils 56, 551–563.

Elliott, E.T., 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. Soil Sci. Soc. Am. J. 50, 627–633.

Hubbell, S.P., 2001. The Unified Neutral Theory of Biodiversity and Biogeography. Princeton University Press, Princeton.

Huber, P., Metz, S., Unrein, F., Mayora, G., Sarmento, H., Devercelli, M., 2020. Environmental heterogeneity determines the ecological processes that govern bacterial metacommunity assembly in a floodplain river system. ISME J. 14 (12), 2951–2966. https://doi.org/10.1038/s41396-020-0723-2.

Li, F., Qiu, P., Shen, B., Shen, Q., 2019. Soil aggregate size modifies the impacts of fertilization on microbial communities. Geoderma 343, 205–214. https://doi.org/10.1016/j.geoderma.2019.02.039.

Linquist, S., Cottenie, K., Elliott, T.A., Saylor, B., Kremer, S.C., Gregory, T.R., 2015. Applying ecological models to communities of genetic elements: the case of neutral theory. Mol. Ecol. 24, 3232–3242. https://doi.org/10.1111/mec.13219.

Nemergut, D.R., Schmidt, S.K., Fukami, T., O'Neill, S.P., Bilinski, T.M., Stanish, L.F., Knelman, J.E., Darcy, J.L., Lynch, R.C., Wickey, P., Ferrenberg, S., 2013. Patterns and processes of microbial community assembly. Microbiol. Mol. Biol. Rev. 77, 342–356. https://doi.org/10.1128/mmbr.00051-12.

Rillig, M.C., Muller, L.A.H., Lehmann, A., 2017. Soil aggregates as massively concurrent evolutionary incubators. ISME J. 11, 1943–1948. https://doi.org/10.1038/ismej.2017.56.

Six, J., Paustian, K., 2014. Aggregate-associated soil organic matter as an ecosystem property and a measurement tool. Soil Biol. Biochem. 68, A4. https://doi.org/10.1016/j.soilbio.2013.06.014.

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

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. ISME J. 7, 2069–2079. https://doi.org/10.1038/ismej.2013.93.

Stegen, J.C., Lin, X., Fredrickson, J.K., Konopka, A.E., 2015. Estimating and mapping ecological processes influencing microbial community assembly. Front. Microbiol. 6, 370.

Tan, S., Yang, C., Mei, X., Shen, S., Raza, W., Shen, Q., Xu, Y., 2013. The effect of organic acids from tomato root exudates on rhizosphere coloni- zation of *Bacillus amyloliquefaciens* T-5. Appl. Soil Ecol. 64, 15–22. https://doi.org/10.1016/j.apsoil.2012.10.011.

Tripathi, B.M., Stegen, J.C., Kim, M., Dong, K., Adams, J.M., Lee, Y.K., 2018. Soil pH mediates the balance between stochastic and deterministic assembly of bacteria. ISME J. 12, 1072–1083. https://doi.org/10.1038/s41396-018-0082-4.

Trivedi, P., Delgado-Baquerizo, M., Jeffries, T.C., Trivedi, C., Anderson, I.C., Lai, K., McNee, M., Flower, K., Pal Singh, B., Minkey, D., Singh, B.K., 2017. Soil aggregation and associated microbial communities modify the impact of agricultural management on carbon content. Environ. Microbiol. 19, 3070–3086. https://doi.org/10.1111/1462-2920.13779.

Vos, M., Wolf, A.B., Jennings, S.J., Kowalchuk, G.A., 2013. Micro-scale determinants of bacterial diversity in soil. FEMS Microbiol. Rev. 37, 936–954. https://doi.org/10.1111/1574-6976.12023.

Wang, R., Dungait, J.A.J., Creamer, C.A., Cai, J., Li, B., Xu, Z., Zhang, Y., Ma, Y., Jiang, Y., 2015. Carbon and nitrogen dynamics in soil aggregates under long-term nitrogen and water addition in a temperate steppe. Soil Sci. Soc. Am. J. 79, 527–535. https://doi.org/10.2136/sssaj2014.09.0351.

Wang, R., Dorodnikov, M., Dijkstra, F.A., Yang, S., Xu, Z., Li, H., Jiang, Y., 2017. Sensitivities to nitrogen and water addition vary among microbial groups within soil aggregates in a semiarid grassland. Biol. Fertil. Soils 53, 129–140. https://doi.org/10.1007/s00374-016-1165-x.

Zhou, J., 2017. Stochastic community assembly : does it. Microbiol. Mol. Biol. Rev. 81, 1–32.