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Niche Based or Neutral Processes? Bacterial Community Assembly in Soil Aggregates During Primary and Secondary Succession

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ABSTRACT

Abiotic and biotic filters shape microbial phylogenetic diversity. Here, we evaluated bacterial phylogenetic diversity in micro- and macro-aggregates across primary and secondary soil successions to assess the relative influence of niche-based and neutral processes. We reanalyzed a large amplicon dataset from soil aggregates of contrasting textures (sandy and clay) collected in Belgium and the Czech Republic. Phylogenetic diversity, estimated from the standardized effect size of mean pairwise distance, consistently showed negative values, ranging from −3 to −7, indicating strong phylogenetic clustering. Higher diversity in macro-aggregates compared with micro-aggregates was only observed in sandy soils under secondary succession. No aggregate-size differences were detected in primary succession soils. Over time, bacterial phylogenetic diversity decreased in sandy soils but increased in clay soils during secondary succession, while no temporal trend was observed in sandy soils under primary succession. Together, these findings demonstrate that bacterial community assembly in soil aggregates is primarily driven by niche-based processes, modulated by soil texture, succession stage, and ecosystem age.

1 | Introduction

Soil microbial communities exhibit diverse taxonomic compositions and co-occurrence patterns (Bach et al. 2018; Ge et al. 2021). At fine scales, soil aggregates represent microhabitats that may influence microbial assembly (Dong et al. 2021). Understanding assembly processes is critical, as they underpin plant–soil feedbacks and organic matter turnover during succession (Kandlikar et al. 2019; Kong et al. 2019).

Soil aggregates provide distinct niches defined by oxygen availability, nutrient gradients, and physical constraints (Davinic et al. 2012). These environments may filter microbial taxa via deterministic processes, yet neutral or dispersal processes can also play important roles (Stegen et al. 2013). Aggregate dynamics further complicate community assembly: macro-aggregates may disintegrate into micro-aggregates under fluctuating moisture and temperature, whereas micro-aggregates can re-form larger structures through root entanglement, fungal hyphae, or microbial mucilage (Cates et al. 2022). Disturbances, such as those resetting primary succession, add additional layers of complexity, and their role in driving niche-based versus neutral assembly remains debated (Liao et al. 2016).

Phylogenetic community structure, such as clustering, provides insights into assembly mechanisms (Langenheder et al. 2010). Building on this framework, we evaluated bacterial phylogenetic structure across soil aggregate fractions during primary and secondary succession in sandy and clay soils in Europe (Sun et al. 2022). Specifically, we tested two hypotheses: (H1) bacterial community assembly is niche-based and depends on soil texture and aggregate size; and (H2) niche-based assembly

patterns are more pronounced during secondary than primary succession.

2 | Material and Methods

2.1 | Soil Samples

Soils were collected from seven chronosequences spanning primary and secondary successions in Belgium (Liereman, 51°20′N, 5°0.02′E) and the Czech Republic (Sokolov, 50°14′N, 12°40′E) (Table S1) (Sun et al. 2022). Substrates included: (i) primary succession at Liereman after topsoil removal, (ii) secondary succession on abandoned fields, (iii) primary succession at Sokolov on post-mining overburden, and (iv) secondary succession rehabilitated by alder planting. Samples (0–10 cm depth) were collected with a 5 cm corer; three composites per site, each composed of three subsamples.

2.2 | Soil Fractionation

Briefly, 40 g of freshly sieved (2 mm) soil was saturated with 200 mL distilled water overnight. The floating organic matter was removed twice using a vacuum pump the next morning. The remaining soil was put on a sieve with a mesh size of 250 μm and washed on a shaker until the eluent turned clear. The fractions passing through the 250 μm sieve but retained on a 53 μm sieve were continuously centrifuged and then frozen as micro-aggregates$_{(<250\,\mu m)}$ (Figure S1). The leftover on top was defined as macro-aggregates$_{(>250\,\mu m)}$. An aliquot of the macro-aggregates remaining on the 250 μm sieve was sonicated to break up the macro-aggregates. After sonication, the soil was consecutively wet-sieved through 250 and 53 μm sieves. The fractions passing the 53 μm sieve were defined as micro-aggregates$_{(<53\,\mu m)}$ and the remaining was macro-aggregates$_{(53-250\,\mu m)}$ (Sun et al. 2022).

2.3 | Phylogenetic Structure

Amplicon sequences (16S rRNA gene) from Sun et al. (2022) were reprocessed with QIIME2 (DADA2). After quality filtering (expected error ≤ 1.0), chimera removal (USEARCH v11.0), and exclusion of non-target reads, 10,578,451 reads and 16,184 ASVs were retained.

Phylogenetic structure was assessed using the standardized effect size (SES) of mean pairwise distance (MPD) (Webb et al. 2008) using the formula:

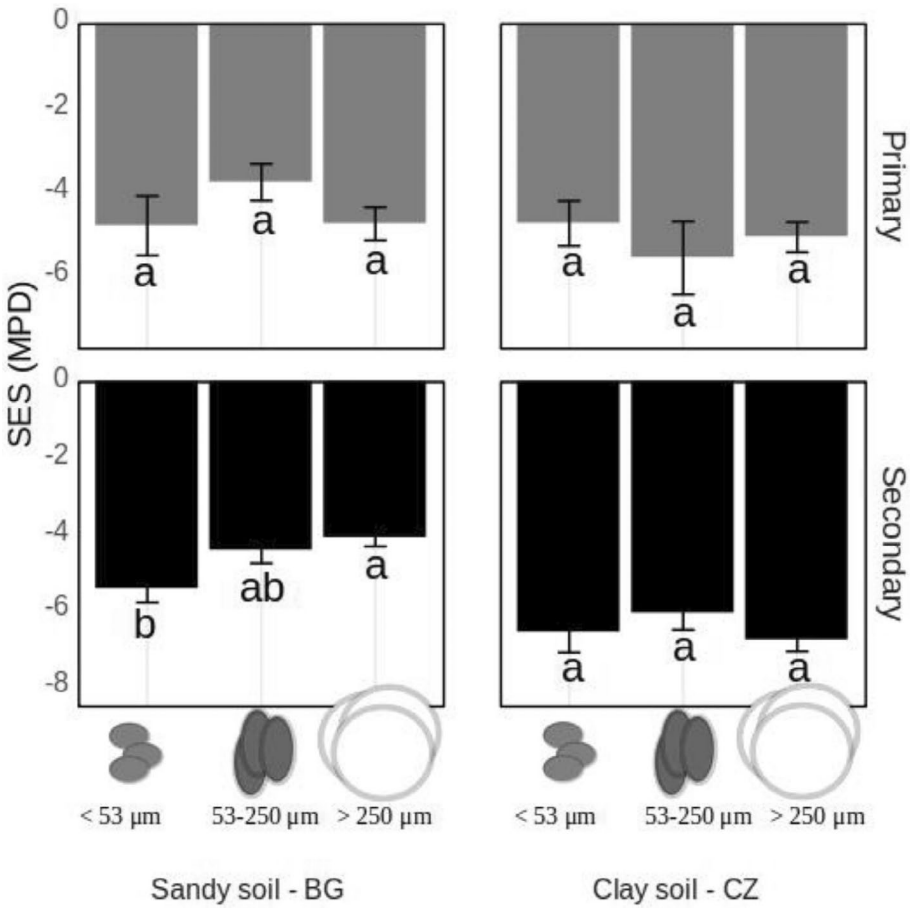

FIGURE 1 | The standardized effect size (SES) of mean pairwise distance (MPD) measuring bacterial phylogenetic community structure in primary and secondary successional soil aggregates with sizes of < 53 μm, 53–250 um, and > 250 um from two chronosequences in Belgium (Liereman) (BG) and the Czech Republic (Sokolov) (CZ). Means and standard errors of means are shown. One-way ANOVA (Tukey, $p < 0.05$) indicates significant differences between treatments. SES(MPD) values ranging from −3 to −7 indicate strong and statistically significant phylogenetic clustering.

$$SES(MPD) = \frac{MPD_{obs} - mean(MPD_{null})}{sd(MPD_{null})}$$

SES > 0 indicates phylogenetic overdispersion (competitive exclusion), SES < 0 indicates clustering (environmental filtering). SES(MPD) value above the threshold of 1.96 (or below −1.96) indicates significant phylogenetic dispersion (or clustering). Analyses were performed in R (MicEco package). Multiple-way ANOVA in respect to dependent variables including location (L), succession (S), age (A), and size, and their interactions on the SES of mean pairwise distance (MPD). One-way ANOVA (Tukey, $p < 0.05$) was conducted to test statistical differences between treatments.

3 | Results and Discussion

Among the related environmental factors, location, succession, and age were the main factors ($p < 0.05$) controlling bacterial phylogenetic community structure. A statistically significant interaction was found between location, age, and aggregate size (Table S2). Our results partly supported H1. Across soils, bacterial communities exhibited strong phylogenetic clustering (negative SES values ranging from −3 to −7; Figure 1), consistent with niche-based assembly. In contrast to agricultural soils where larger aggregate sizes have more phylogenetically clustered bacterial communities compared to small aggregates (Dong et al. 2021), aggregate-size effects in our successional soils were limited and context dependent. Only sandy soils in Belgium under secondary succession showed significantly higher diversity in macro-aggregates (> 250 μm) than in micro-aggregates (< 53 μm). Clay soils in the Czech Republic showed no size-dependent differences.

Niche-based assembly was more pronounced during secondary than primary succession, supporting H2. Soils under secondary succession likely supported denser root systems and higher biological activity, enhancing selective filtering processes. Similar effects of plant inputs on microbial assembly via soil aggregation (Jiang et al. 2011) and pore structure have been reported (Zhou et al. 2013).

In sandy soils, phylogenetic diversity of year 1 was higher than that of year 2 or 3, while in clay soils phylogenetic diversity of year 1 was lower than that of year 2 or 3 under secondary succession (Figure 2). No statistical differences in phylogenetic diversities were found in sandy soils under primary succession. The phylogenetic diversity of year 1 was lower than that of year 2 in clay soil under primary succession. The phylogenetic diversity in the first year was often found to be different from the second year, which may indicate its sensitive response to the environmental disturbance. The niche-based processes in soil aggregates also depend on soil texture and the succession stages. Together, these results highlight the role of soil texture, succession stage, and succession age in shaping bacterial community assembly. Our re-analysis emphasizes that deterministic, niche-based processes dominate microbial assembly in soil aggregates under natural succession.

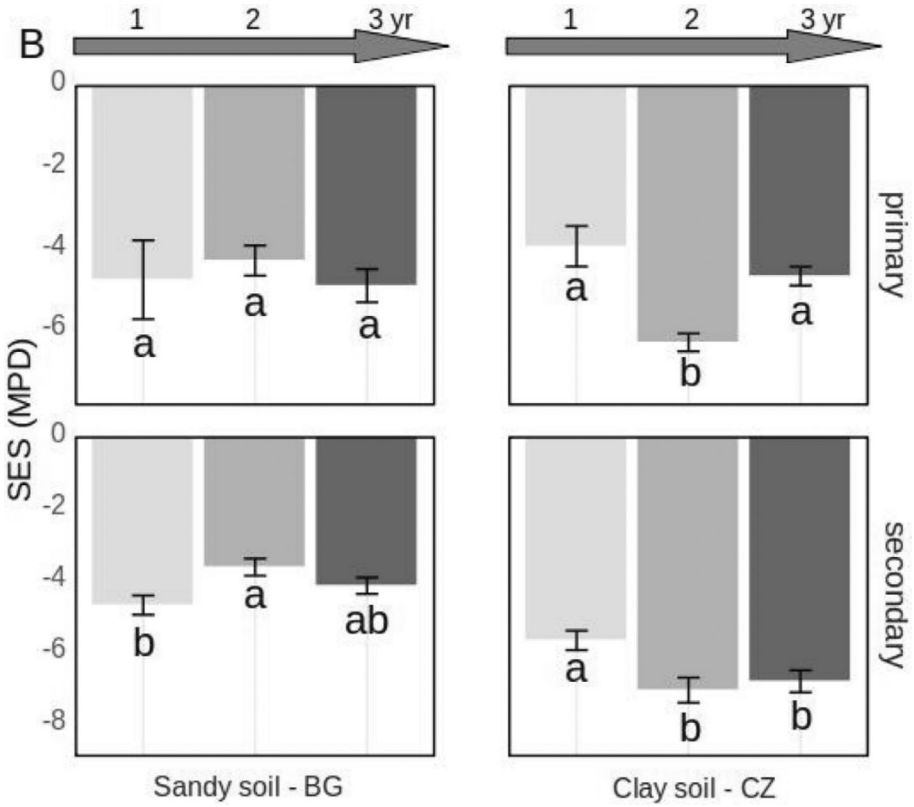

FIGURE 2 | The standardized effect size (SES) of mean pairwise distance (MPD) measuring bacterial phylogenetic community structure in primary and secondary successional soil aggregates ranging 1–3 years along two chronosequences in Belgium (Liereman) (BG) and the Czech Republic (Sokolov) (CZ). Means and standard errors of means are shown. One-way ANOVA (Tukey, $p < 0.05$) indicates significant differences between treatments.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** General characteristics of two sampling sites from two chronosequences in Belgium (Liereman) and the Czech Republic (Sokolov). **Table S2:** Results of four-way ANOVA of the effects of location (L), succession (S), age (A), and size (SI), and their interactions on the standardized effect size (SES) of mean pairwise distance (MPD). F and p values (<0.05, in bold) are indicated. **Figure S1:** Procedures for the separation of micro-aggregates($<250\,\mu m$) and macro-aggregates($>250\,\mu m$) by wet sieving with a $250\,\mu m$ sieve. Macro-aggregates ($>250\,\mu m$) were subjected to sonication and then separated to micro-aggregates ($<53\,\mu m$) and macro-aggregates ($>53\,\mu m$) by wet sieving with a $53\,\mu m$ sieve.