Received: 5 August 2023 | Accepted: 8 December 2023

DOI: 10.1111/1462-2920.16561

RESEARCH ARTICLE

ENVIRONMENTAL MICROBIOLOGY Applied Microbiology International

Core taxa underpin soil microbial community turnover during secondary succession

Tord Ranheim Sveen[1] | **Maria Viketoft**[1] | **Jan Bengtsson**[1] | **Mohammad Bahram**[1,2]

[1]Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden

[2]Institute of Ecology and Earth Sciences, University of Tartu, Tartu, Estonia

Correspondence
Tord Ranheim Sveen, Department of Ecology, Swedish University of Agricultural Sciences, Ulls Väg 16, 75651 Uppsala, Sweden.
Email: tord.ranheim.sveen@slu.se

Funding information
The Swedish Research Council, Grant/Award Number: 2021-03724; The Swedish University of Agricultural Sciences (early career grant); The Swedish Research Council Formas, Grant/Award Number: 2020-00807

Abstract

Understanding the processes that underpin the community assembly of bacteria is a key challenge in microbial ecology. We studied soil bacterial communities across a large-scale successional gradient of managed and abandoned grasslands paired with mature forest sites to disentangle drivers of community turnover and assembly. Diversity partitioning and phylogenetic null-modelling showed that bacterial communities in grasslands remain compositionally stable following abandonment and secondary succession but they differ markedly from fully afforested sites. Zeta diversity analyses revealed the persistence of core microbial taxa that both reflected and differed from whole-scale community turnover patterns. Differences in soil pH and C:N were the main drivers of community turnover between paired grassland and forest sites and the variability of pH within successional stages was a key factor related to the relative dominance of deterministic assembly processes. Our results indicate that grassland microbiomes could be compositionally resilient to abandonment and secondary succession and that the major changes in microbial communities between grasslands and forests occur fairly late in the succession when trees have established as the dominant vegetation. We also show that core taxa may show contrasting responses to management and abandonment in grasslands.

INTRODUCTION

It is well known that habitats with different types of vegetation cover, climate and management practices harbour distinct microbial communities (Bahram et al., 2018; Hartmann et al., 2015). Understanding how these communities vary across space and time can inform us about the potential mechanisms underlying microbial community assembly and functioning. For example, processes driving turnover between habitats can be attributed to either deterministic or stochastic assembly (Dini-Andreote et al., 2015), where stochastic factors are primarily random dispersal and drift (Ferrenberg et al., 2013; Zhou & Ning, 2017), while deterministic assembly is typically driven by selection imposed by abiotic factors through their impacts on competition and performance (Stegen et al., 2013). Successional gradients resulting from colonisation of novel habitat (primary succession) or through the process of ecosystem recovery following a disturbance (secondary succession), provide unique opportunities to examine patterns of community assembly for a more mechanistic understanding (Fierer et al., 2010; Nemergut et al., 2013). For microbes, the relative influence of stochastic and deterministic assembly processes is strongly mediated by abiotic properties such as pH and Na^+ which exert selection on bacterial community composition (Dini-Andreote et al., 2015; Tripathi et al., 2018). Increasing habitat heterogeneity of the selective environment (i.e., pH) within successional stages is thus expected to lead to community divergence and compositional differences as succession

progresses, due to variable selection dominating assembly (Dini-Andreote et al., 2015). Conversely, if the selective environment within differing successional stages is spatially homogenous, we would instead expect homogenous selection to dominate microbial assembly and lead to community divergence only as selective factors change enough to require significant niche adaptations.

Community turnover (i.e., beta diversity) between distinct stages along a successional gradient may reflect either replacement (spatial turnover) or loss of taxa (nestedness) (Koleff et al., 2003; Legendre, 2014). For spatial turnover, variation in taxonomic composition is caused by the replacement of one or several taxa by other taxa while the overall species richness remains comparable. Conversely, nestedness results from compositional differences driven by the loss of taxa, as richness decreases. Since spatial turnover and nestedness result from antithetic processes, they can be partitioned according to their relative contribution to the total community turnover (Baselga, 2013). So far, however, little is known about the relative contribution of nestedness and spatial turnover of microbial communities along successional gradients. Moreover, because most microbial communities are dominated by low-abundance species or populations (Lynch & Neufeld, 2015), the turnover between two communities is likely to be driven by rare taxa (McGeoch et al., 2019). Conversely, core taxa that occur persistently within a given habitat type are often of disproportionate importance for the maintenance of that system's basic ecosystem properties (Umaña et al., 2017), and could be sensitive indicators of ecosystem changes. In the zeta diversity framework (Hui & McGeoch, 2014; McGeoch et al., 2019), the contribution of common and rare taxa to compositional change can be inferred within or across habitat types through the construction of species decline curves across multiple assemblages (referred to as zeta orders). However, despite the conceptual advances inherent to beta diversity partitioning and the zeta diversity framework, their application to microbial communities during succession remains limited.

Here, we examined bacterial community turnover across a large-scale successional gradient from managed grasslands to forests using beta- and zeta-diversity analyses complemented with models of phylogenetic turnover to study assembly processes. We evaluated abiotic and biotic drivers by examining differences in the selective environment between each set of paired grassland-forest sites in relation to turnover patterns. Our gradient consisted of managed ($n = 49$) and abandoned grassland sites in two stages of succession (recently abandoned, $n = 30$, and late-term successional, $n = 22$), paired with adjacent fully forested sites representing their successional and land-use change endpoint ($n = 104$). We hypothesised that (i) spatial heterogeneity in the selective environment would increase along the successional gradient and that gradual community divergence would be underpinned by spatial turnover (as opposed to nestedness). We further hypothesised that (ii) deterministic assembly processes would shift in their relative influence from homogenous to variable selection. Last, we expected that (iii) zeta diversity analyses would reveal core taxa inherent to each successional stage and that these taxa would exhibit the same divergent turnover patterns as the whole community.

EXPERIMENTAL PROCEDURES

Sampling and design

We selected 101 grassland sites distributed across the whole of Sweden (Figure 1) and categorised them into three differing successional stages (managed, recently abandoned and late-term successional) based on vegetation characteristics (Table S1). We next paired the grassland sites ($n = 93$, see Table S1 for the distribution of paired and unpaired samples across grassland stages) with an adjacent fully afforested site representing the putative end point of land abandonment and succession (Cline & Zak, 2015). We do not have full historical records on the age and management practices of the grassland and forest sites visited, but forest sites in southern Sweden were likely afforested from grassland, heathland, or grazed forest during the 20th century (Cousins et al., 2015). Forests in the central and northern parts of the country were most likely full forests and as such harvested with various intensity from the mid- or late 1800's and onwards. For more detailed discussions on the historical and ongoing afforestation of open landscapes in Sweden, including regional differences, see (Ericsson et al., 2000; Wei et al., 2021). The sites consisted of a circular area of ~200 m^2 and were sampled by taking four cores (diameter = 3 cm, depth = 10 cm) at 2 m distance intervals in every direction from the coordinate indicating the centre of the plot ($n = 16$ subsamples for each site). All sites were sampled between July and September 2020. Subsamples were pooled into a site-specific bulk sample. A subset of the bulk sample was air-dried (<40°C) within 24 h of sampling and stored in a zip-lock plastic bag with silica gel to minimise humidity and prevent the development of moulds during transit (Tedersoo et al., 2014). All equipment was sterilised with 95% ethanol between sites. The air-dried soil was milled to a fine powder for molecular analysis, and the remaining bulk samples were sieved (2 mm) and stored refrigerated (4°C) before further chemical analyses.

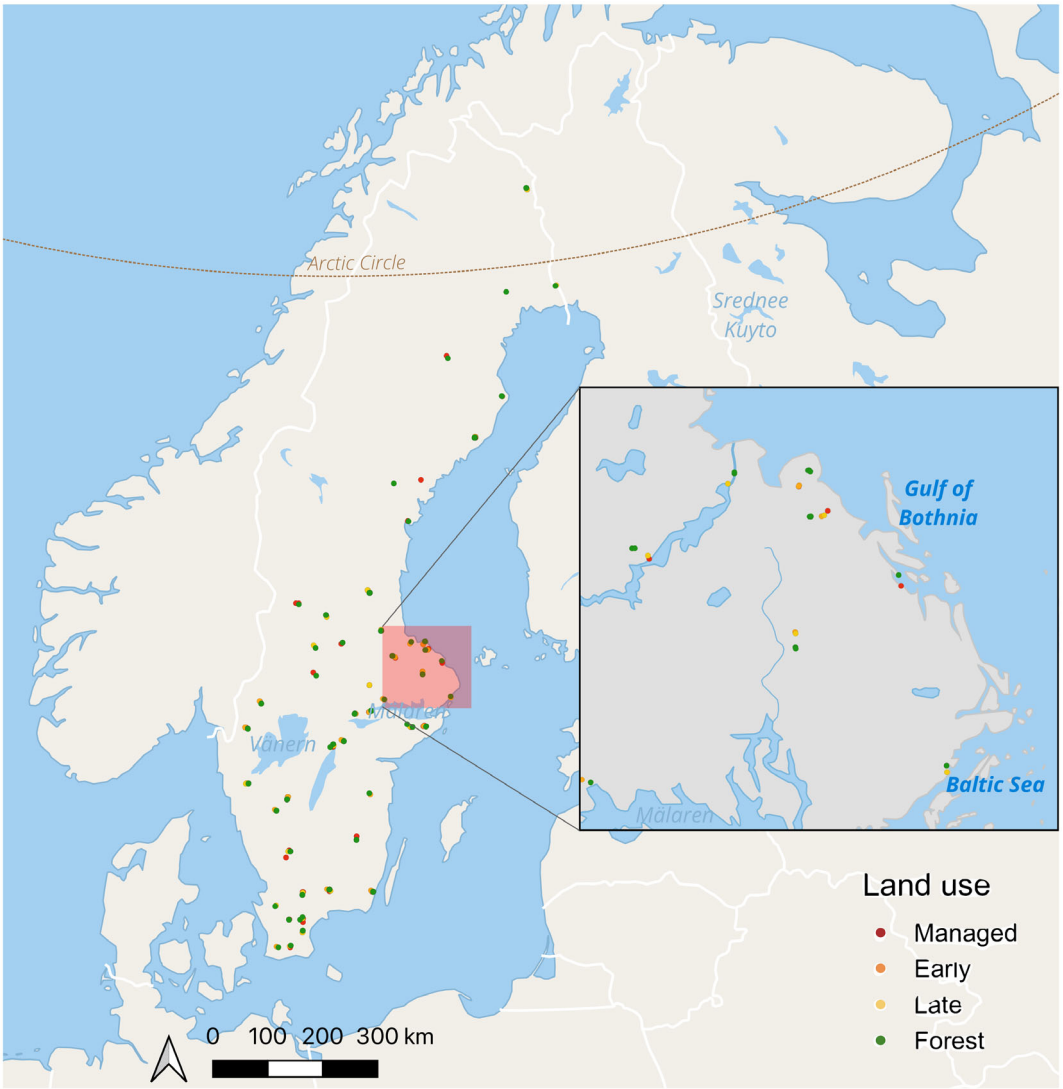

FIGURE 1 Map of sampling sites. Map of sampling sites and their distribution across Sweden. The zoomed-in quadrant shows an example area of paired grassland–forest sites.

Laboratory methods

Soil chemical analyses

Approximately 15 g of the refrigerated soil was used to analyse pH (1:5 soil: water suspension). Available phosphorus (P-AL) and potassium (K-AL) were extracted using ammonium lactate and acetic acid at pH 3.75 (Egnér et al., 1960) and analysed using the stannous chloride-molybdate procedure (P-AL) and inductively coupled plasma atomic emission spectroscopy (ICP-AES), respectively. Exchangeable calcium and magnesium concentrations were measured in ammonium acetate extract (pH = 7.0) using ICP-AES at Agrilab Uppsala, Sweden. Total C and N contents were determined on aliquots (1–20 mg) of air-dried soil using an Elemental Analyser (Euroa EA, Eurovector, Milano, Italy).

DNA extraction, PCR amplification, library preparation and sequencing

DNA was extracted from 200 mg of dried and milled soil samples using the PowerMax Soil DNA Isolation Mini kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's instructions. Extraction occurred within 3 weeks of sampling. We are aware that storage of air-dried samples can lead to compositional changes of bacterial communities compared to fresh or frozen storage, but these are likely to be few and minor in relation to differences between land uses (Lane et al., 2022).

The extracted DNA was quality-checked based on the 260/280 and 260/230 nm wavelength ratios using a NanoDrop™ (Thermo Scientific, Massachusetts, USA) and stored at −20°C until sequencing. For the production of amplicons for sequencing, the universal prokaryote primers 515F and 926R were used to amplify the 16S V4 subregion of the rRNA gene (Walters et al., 2015). DNA samples were amplified using the following conditions in three replicate runs: 95°C for 15 min, followed by 26 cycles of 95°C for 30 s, 50°C for 30 s and 72°C for 1 min with a final extension step at 72°C for 10 min. The 25 μL PCR mix consisted of 18 μL sterilised H_2O, 5 μL 5 × HOT FIREPol Blend MasterMix 0.5 μL of each primer (20 μL), and 1 μL template DNA (final concentration of 400 nM). The amplicons from the replicates were pooled, and purified using a purification kit containing agarose gel (FavourPrep Gel/PCR Purification mini Kit-300 Preps; Favourgen) and shipped for library preparation in the sequencing service facility of the University of Tartu (the Estonian Biocenter). DNA libraries were sequenced on two runs using an Illumina MiSeq platform (2 × 250 bp paired-end chemistry). Blanks containing ddH_2O instead of DNA template were used as negative controls in the library preparation. The raw sequences derived from all soil samples have been deposited at NCBI under accession PRJNA994701.

Bioinformatics

We used the LotuS2 version 2.22 (Özkurt et al., 2022) pipeline to quality-filter, demultiplex and process the filtered reads into operational taxonomic units (OTUs). Chimera detection and removal was done using Uchime (Edgar et al., 2011) with all singletons and sequences shorter than 100 bp discarded. Clustering of sequences was done using a de-novo clustering algorithm in UPARSE (Edgar, 2013) based on a 97% similarity threshold. Taxonomy was assigned against the SILVA database (Quast et al., 2013) (ver. 138.1) for prokaryotic sequences. For inferring a phylogenetic tree from the sequences needed for analysis of assembly processes, multiple sequence alignment of OTUs was done using MAFFT (Katoh & Standley, 2013), and from these a maximum likelihood phylogeny was constructed using fasttree2 (Price et al., 2010). The resulting dataset was manually curated to remove contaminant sequences based on negative controls, and by omitting all OTUs not assigned to Bacteria. This resulted in a total of 13,901 OTUs and 8,893,038 reads across all samples, with a median sample coverage of 0.979.

Statistical analyses

All statistical analyses were conducted using R (ver. 4.1.3) (R Core Team, 2023). In the case of multiple comparisons, test results were adjusted with Benjamin-Hochberg's corrected *p*-values.

Rarefaction and links to vegetation community

All taxa unassigned at the phylum level were omitted from the dataset. We used coverage-based rarefaction to standardise samples to equal completeness (Chao & Jost, 2012) prior to alpha- and beta-diversity analyses, whereas zeta diversity, core microbiome and assembly process analyses were done using raw (i.e., unrarefied) matrices. The coverage depth opted for (Good's coverage = 0.8) was chosen as reflecting a balance between sampling breadth and sequencing depth, and we note beta diversity patterns for many environments including soils are robust even at low sequencing depths after rarefaction (Kennedy et al., 2018). One sample was discarded due to too low sequencing coverage, and a total of 378,840 sequences remained after rarefaction (mean sample depth = 1839 sequences). To test whether bacterial community composition was related to plant community composition along the successional gradient, we first conducted Mantel tests between bacterial and vegetation distances matrices using the *mantel* function in *vegan* (Oksanen et al., 2022). Tests were based on Spearman correlations between Bray–Curtis distance matrices with 9999 permutations. We next extracted the first axes from a PCA comprising the full vegetation community found at each site and used the resulting coordinates as a constraining variable of bacterial community composition in Permanova and RDA analyses.

Species richness, community composition and soil heterogeneity

Taxonomic richness was computed using *Hill* numbers of the order $q = 0$ (Chao et al., 2014) with the *hill_div* function in the *hilldiv* package (Alberdi & Gilbert, 2019). We used pairwise Wilcoxon rank-sum tests to test for differences between successional stages. Compositional differences between successional stages were tested based on Bray-Curtis distance matrices using global permanova tests (9999 permutations). For differences between grasslands and forests, these analyses were done with permutations restricted to paired sites using the *strata* argument of the *adonis2* function in *vegan* (Oksanen et al., 2022). For differences between grassland stages, the same function was applied omitting the *strata* argument. Results were visualised using NMDS plots separated for each set of grassland-forest comparisons. Soil and environmental variables influencing bacterial community composition were extracted using forward selection with the *forward.sel* function in the *adespatial* package (Dray et al., 2023), and fitted to a distance-based redundancy biplot (db-RDA, Bray-Curtis distances) using the *microViz* package (Barnett et al., 2021). We also computed the coefficient of variation (CV) for the two variables explaining most of the community variation (pH and

C:N) and tested for differences in the heterogeneity of these between successional stages using a modified signed-likelihood ratio test (MSLRT) (Krishnamoorthy & Lee, 2014). To assess the contribution of species spatial turnover and nestedness to the community turnover, as well as the influence of variation in taxa abundances, we computed and partitioned dissimilarities using the *betapart* approach (Baselga, 2013, 2017). Spatial turnover and nestedness were computed on presence–absence matrices based on Sørensen dissimilarities, whereas the contribution of balanced or unbalanced abundances to turnover was computed on abundance matrices based on Bray–Curtis dissimilarities. Because the partitioned metrics were quantified between paired sites, we used the absolute difference in soil properties between the same paired sites in linear regressions to analyse the abiotic drivers of the observed patterns.

Zeta diversity and analyses of core taxa within successional stages

While beta diversity partitioning captures spatial turnover and nestedness with compositional change, it is insensitive to occupancy changes in common taxa. The zeta diversity framework instead analyses turnover across multiple sites (referred to as zeta orders), and discriminates diversity patterns across common, intermediate and rare taxa. The steep decline in zeta values, corresponding to the number of shared taxa at *n* zeta orders (i.e., sites) therefore implies turnover driven by rare taxa, whereas a gentler decline implies a pool of core taxa common to all sites shared within a given habitat. Here, we used the zeta diversity framework to assess turnover within successional stages. Zeta values were computed across 20 zeta orders (i.e., 20 sites) using the function Zeta.decline.mc in the *zetadiv* package (Hui & McGeoch, 2014) with 1000 bootstraps. As rarefaction can alter the number and identities of rare and common taxa (Neu et al., 2021), we used raw (i.e., unrarefied) matrices as input for the zeta diversity and core microbiome analyses (see below), but discarded all samples with fewer than 3000 reads ($n = 3$) to account for the worst biases of uneven sequencing depth. Zeta decline curves were also calculated using both raw and normalised zeta values (Jaccard normalisation).

We also computed zeta values across the full set of grasslands ($n = 101$) and forest sites ($n = 104$), respectively. Patterns of zeta decline were evaluated based on their fit to an exponential or power law form with the best fit determined with AIC criteria (Hui & McGeoch, 2014). Taxa retention curves, showing the degree to which common taxa are more likely to be retained across sites, were constructed from the zeta ratio values obtained across the full range of zeta orders (McGeoch et al., 2019).

To test for the presence of core microbiomes, we first confirmed that zeta decline patterns showed evidence of shared taxa within each successional stage across 20 zeta orders. This was interpreted as indicating the presence of core taxa within the successional stages. Next, we derived these putative core microbiomes for further analyses by prevalence filtering based on occupancy thresholds (Custer et al., 2023). For this, we considered taxa occupying a minimum of 80% of the sites within any given successional stage to be part of the core microbiome. Lastly, we tested for differences in the community composition of core microbiomes using the same approach as for the whole community (see above) and performed indicator species analyses to derive indicator taxa for specific or shared successional stages. Indicator analyses were done using the *multipatt* function from the *indicspecies* package (Cáceres & Legendre, 2009), with 10^4 permutations.

Assembly processes

We used the framework developed by Stegen et al. (2013, 2015) to measure phylogenetic turnover between communities. For full details on the framework and its underlying assumptions, see Stegen et al. (2013, 2015). Briefly, phylogenetic turnover is compared to a null distribution generated through randomizations where taxa names and abundances are shuffled across the phylogeny tips. The difference between the observed and mean null value is referred to as the β-nearest taxon index (β-NTI) and placed in units of standard deviation in relation to the null distribution so that ($|\beta\text{-NTI}| > 2$) indicates domination of deterministic assembly either through homogenous ($\beta\text{-NTI} < -2$) or heterogeneous ($\beta\text{-NTI} > +2$) selection. If there is no significant deviation from the null expectation, differences in phylogenetic community composition are assumed to result from dispersal limitation, homogenising dispersal, or random drift, and partitioned accordingly based on Raup-Crick Bray–Curtis dissimilarity (RC_{Bray}). We used the R scripts provided by Barnett et al. (2020) to calculate pairwise β-NTI and null-model generations based on 999 randomizations. Differences in the relative influence of the differing assembly processes were tested using pairwise comparisons of proportions. Phylogenetic null-model analyses assume that closely related taxa are also ecologically similar, that is, they share the same ecological niches (Stegen et al., 2013). To confirm the validity of this assumption, we tested the presence of phylogenetic signal with pH and C:N (these being the selective variables explaining most of bacterial community composition) using Mantel correlograms as implemented in *vegan* (Oksanen et al., 2022) with the codes provided by Barnett et al. (2020). We found a significant positive correlation between phylogenetic distance and

the difference in optimum soil property (i.e., OTU niche preference) across a range of phylogenetic distance classes (Figure S1). Lastly, we analysed the influence of pH on phylogenetic turnover (i.e., β-NTI) through Mantel tests (Spearman correlation, 9999 permutations) across all successional stages combined and separately, and analysed differences in the relationship between pH and β-NTI for each successional stage by comparing their slopes of linear regression with the *lstrends* function in the *emmeans* package.

RESULTS

Microbial communities in grasslands are compositionally stable and differ from forest sites

Beta diversity analyses did not show any differences between the three grassland successional stages (unrestricted Permanova, $p > 0.05$; Table S2), contrary to our hypothesis of a gradual divergence. Differences in taxonomic richness followed a similar pattern with no evident differences between the stages (Wilcoxon, $p > 0.05$), but markedly higher richness in all grassland communities than in forest sites (Figure S2; Wilcoxon, $p < 0.05$). By contrast, when comparing bacterial community composition between grasslands and their paired forest sites, we found that the grasslands differed markedly from forests for every grassland successional stage (Permanova with strata restricted to paired sites, $p = 0.0001$) (Figure 2B–D). The results reflected the overall small differences in soil edaphic properties between grassland stages but large differences between grasslands and forests (Table S3), but contrasted with the differences observed in the community composition of plants between grasslands stages (db-MANOVA, $p < 0.05$; Table S1). As expected, pH was the main variable explaining community composition (Permanova, $R^2 = 0.22$, $p < 0.001$) with C:N, total N, Mg, mean annual precipitation and mean annual temperature together with the first PCA axis of plant community composition explaining an additional 4.6% of variation (Figure 2E). Plant community composition was further only weakly related to bacterial community composition across the whole successional gradient (Mantel test, $r = 0.035$, $p = 0.036$).

Beta diversity between grasslands and forests dominated by spatial turnover

In line with our first hypothesis, species spatial turnover contributed more to total grassland-forest beta diversity than nestedness, in line with our first hypothesis, but we found little variation across the different grassland stages (Figure 3A). The nestedness contribution to beta diversity was higher in recently abandoned grasslands than in late-term successional grasslands (Figure 3A; Wilcoxon, $p = 0.02$). These patterns were reflected also in analyses of the abundance variation, with overall few differences in the contribution of balanced variation to Bray–Curtis dissimilarities between grassland stages (Figure S3A). Analyses on raw (i.e., unrarefied) matrices produced the same patterns of beta diversity partitioning (Figure S4A,B). Differences in soil pH and C:N between the paired grassland-forest sites were the main drivers of the overall community dissimilarity, explaining 46% and 39% of the variation in the Sørensen dissimilarity, respectively (Figure 3B,C). Similar values were obtained when regressing the soil variables against Bray–Curtis dissimilarities (Figure S3B,C). When examining the partitioned components of beta diversity separately, we found that the soil properties better fitted the spatial turnover than the nestedness component (Figure 3D–G), indicating that differences in soil pH and C:N between grasslands and forests drive species spatial turnover more than nestedness in the community turnover. Similarly, the contribution of balanced variation in the abundances to Bray-Curtis dissimilarities between the paired sites was driven by pH and C:N, whereas differences in abundances of bacterial taxa (i.e., abundance gradients) were more related to differences in pH only (Figure S3D–G). By contrast, differences in community composition between sites were not related to the geographic distance separating them (Figure S5A–D).

Core microbiomes reflect whole-community turnover

All land uses showed similar patterns of rapid community turnover over the first ~5 zeta orders and reached asymptotic levels around zeta orders ~10 (Figure 4A). The patterns of zeta decline fitted a power law considerably better than the exponential form for all land uses (Table S5), indicating that community assembly is predominantly deterministic since communities with clear niche differentiation are expected to show a power-law form of zeta diversity decline (Hui & McGeoch, 2014). Taxa retention curves, constructed from plotting zeta ratios against zeta orders, also approached asymptotic levels of around 95% retention after ~15 zeta orders (Figure 4B), indicating the presence of a core set of taxa in line with our third hypothesis. When merging all grasslands to a single category, zeta decline curves for these and all forest sites (104 zeta orders), we again found the same asymptotic patterns and retention curves (Figure S6C,D), indicating that core taxa in these habitats represent generalists common to all grasslands independent of succession. Similarly, when looking at the asymptotic normalised (Jaccard) zeta

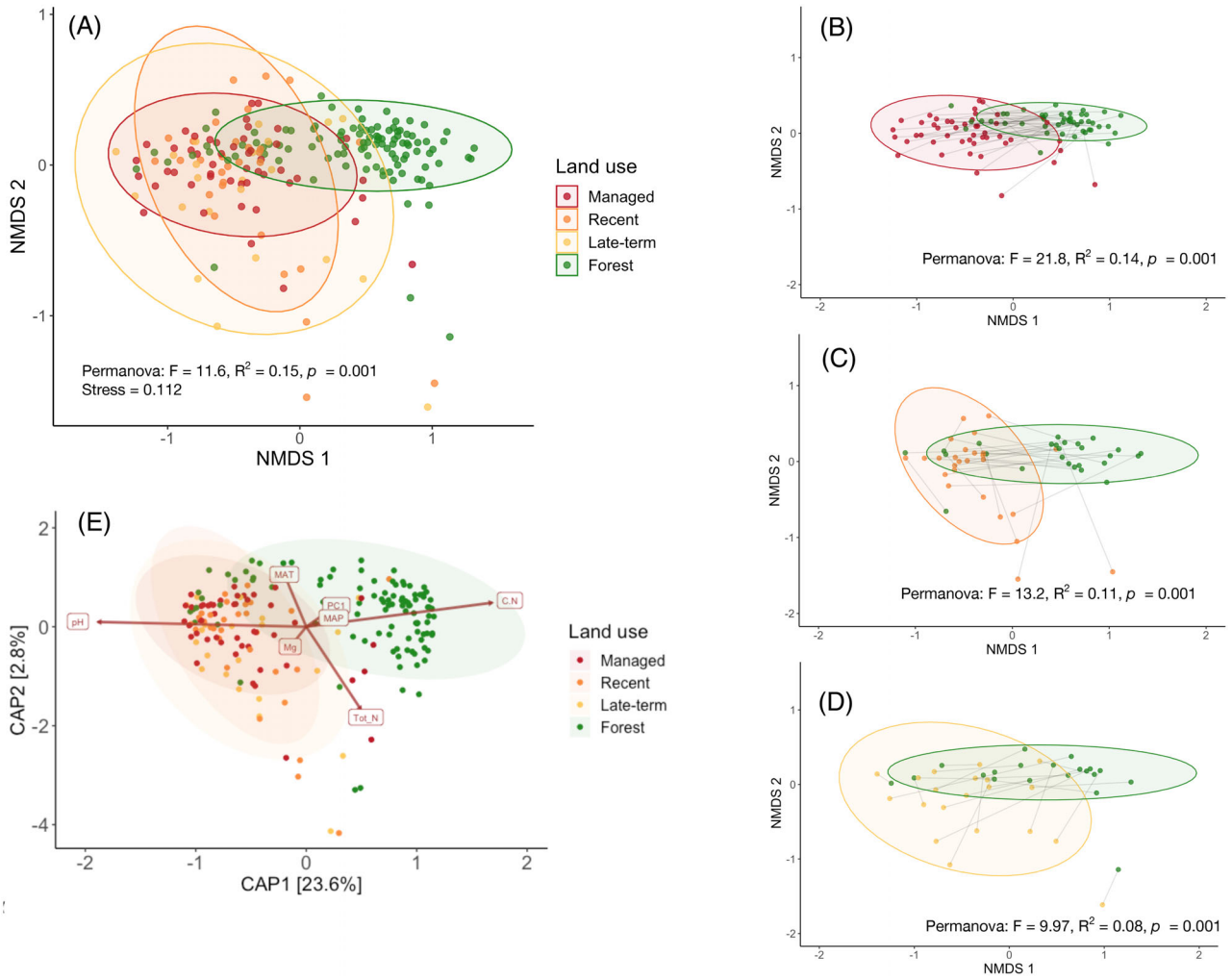

FIGURE 2 Community composition across the successional gradient. NMDS plots showing differences in community composition between (A) all grassland and forest sites based on unpaired permanova tests (See Table S1 for the number of sites in each successional category), (B) managed ($n = 47$), (C) recently abandoned ($n = 26$), and (D) late-term successional grasslands ($n = 20$) displayed along with their respective paired forest sites and test results based on permanova tests accounting for the paired structure. All permanova tests were run with 9,999 permutations with the strata term restricted to within grassland-forest pairs for paired comparisons. The NMDS projections for paired comparisons are based on the whole-community analysis in A). (E) Distance-based redundancy biplot (db-RDA) of all grassland and forest sites along with significantly fitted soil and climatic variables after forward selection ($p < 0.05$).

values at higher orders, we found that 0.7–1.3% of OTUs were shared across 20 sites depending on the land-use category (Figure S6A,B and Table S4).

We next examined the core microbiomes further by occupancy filtering retaining only the microbes present at 80% or more in sites within the different successional stages, and tested if they reflected the same patterns of turnover as the entire microbial communities. Occupancy filtering resulted in a total of 917 OTUs, with most taxa found in managed grasslands and fewest in late-stage successional grasslands (Table S5). We again found a clear separation between grassland and forest core microbiomes (Figure 4C), reflecting the whole-community differences found previously (Figure 2A) but also a difference between the core microbiomes in managed compared to abandoned grasslands (permanova, $p < 0.05$; Table S6). In light of these differences, we next examined the core taxa in each successional stage using indicator species analyses. In total, 745 out of the 917 (81%) identified core taxa were classified as indicator taxa for any or a combination of the successional stages. Fully afforested sites had the highest proportion of specialist indicator taxa (i.e., taxa not shared with any other successional stage), amounting to 45% of all OTUs singled out as indicator taxa (Figure 4D; Table S5). By contrast, the late-term successional stage had the fewest overall number of indicator taxa and the lowest proportion of specialist indicator taxa (Figure 4D; Table S5). The three grassland successional stages shared 183 indicator taxa between them, reflecting a high degree of compositional stability among the grassland core

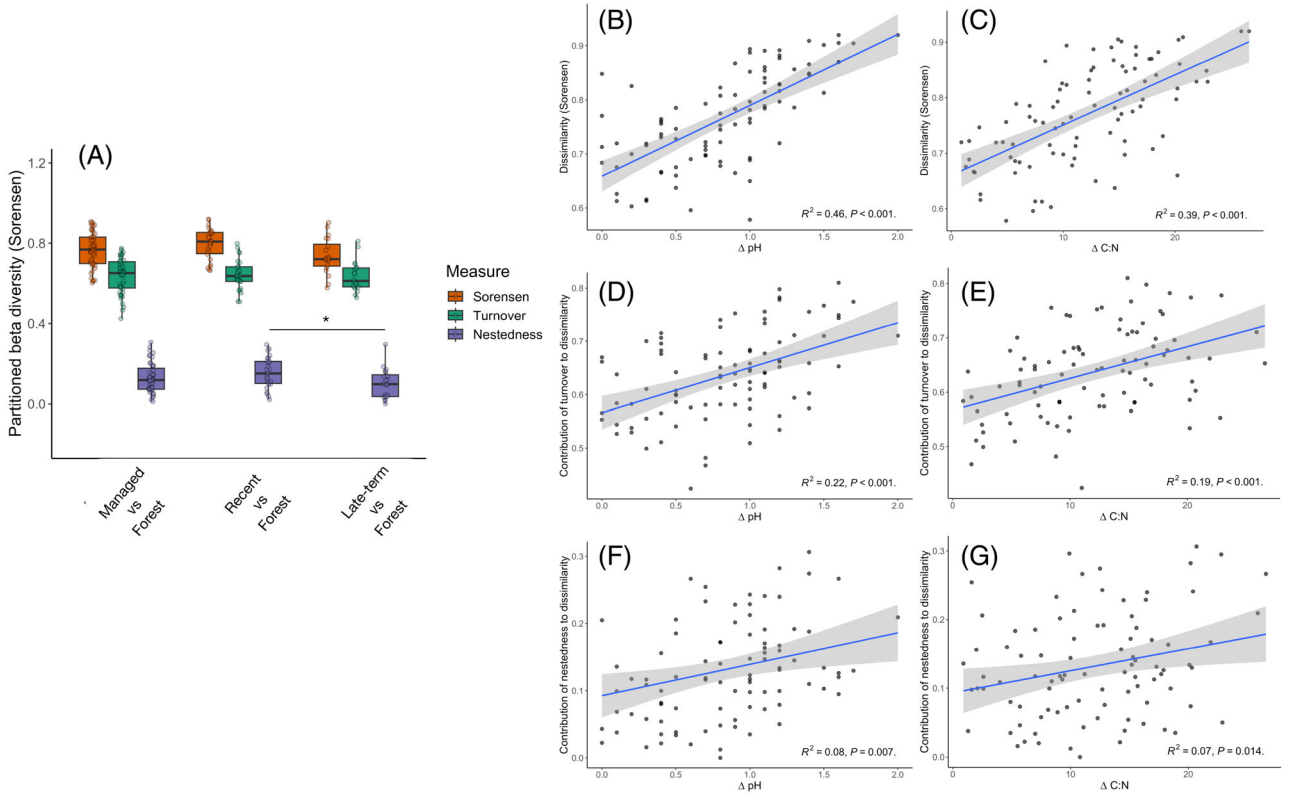

FIGURE 3 Beta diversity components and their abiotic drivers. Boxplots showing (A) beta diversity based on Sørensen dissimilarity and its compartmented turnover and nestedness fractions between paired grassland-forest sites along the differing grassland successional stages. The asterisk (*) denotes a significant difference ($p < 0.05$) in nestedness between successional stages of grasslands when compared to their respective paired forest sites. Tests are based on pairwise Wilcoxon tests. Scatter plots displaying the relationship between absolute differences in pH and C:N and (C,D) overall Sørensen dissimilarity, (E,F) turnover, and (G,H) nestedness, for each pair of grassland-forest sites.

microbiomes, although managed grasslands also had a high proportion of stage-specific indicator taxa (15%; Table S5).

Assembly processes reflect heterogeneity of soil pH within successional stages

Phylogenetic null-modelling showed significant variations in mean β-NTI values between successional stages (Figure 5A), with deterministic assembly processes (|β-NTI| > 2) generally higher than stochastic assembly and differing significantly in relative influence across the different successional stages (Figure 5A). Notably, Homogenous selection was the dominant deterministic assembly processes, whereas homogenising dispersal dominated among stochastic processes (Figure 5A). As expected, differences in pH between sites was the best predictor of assembly (Mantel test, $r = 0.536$, $p < 0.001$; Figure S7A). Differences in C:N were also positively correlated with β-NTI (Mantel test, $r = 0.288$, $p < 0.001$; Figure S7B), but weaker so than pH. We did not find the expected shift from homogenous to variable selection with increasing succession. Instead, variable selection was highest in late-successional grasslands and lowest in recently abandoned grasslands (proportion test, $p < 0.001$), with managed grasslands and forest sites exhibiting similar overall profiles (Figure 5A). The relative influence of homogenous and variable selection correlated well with pH variability within the successional stages. Recently, abandoned grasslands had lower pH variability (MSLRT, $p < 0.001$; Figure 5B) than all other stages, and also a significantly weaker relationship between pH and β-NTI (Figure 5C–F; Table S7). Conversely, increased pH variability between sites resulted in stronger relationships between pH and assembly, and to higher relative influence of variable selection (Figure 5; Table S7).

DISCUSSION

In this study, we examined soil bacterial community turnover and assembly across a large-scale successional gradient comprising managed and abandoned grasslands paired with fully afforested sites. Contrary to our initial expectations, our results do not support a gradual community divergence along the gradient of different grassland successional stages. Instead,

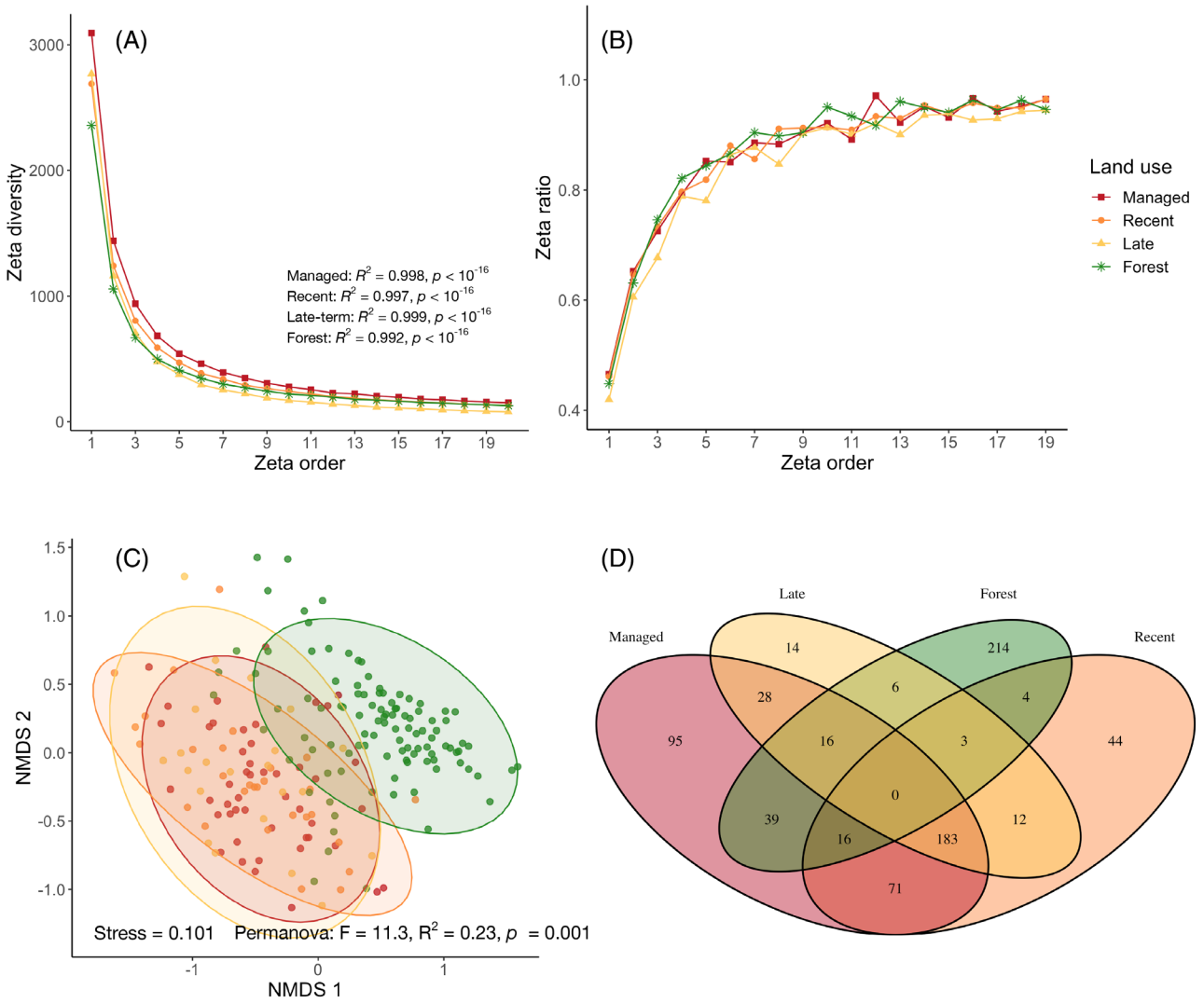

FIGURE 4 Patterns of zeta decline and community turnover of core taxa. Patterns of zeta diversity of the four successional stages across 20 assemblages showing (A) zeta decline curves with power law model fits, and (B) species retention curves showing the ratio of retained taxa between between each successive pair of zeta orders. A ratio of 0 indicates no retained taxa (complete turnover), and a ratio of 1 indicates full taxa retention (zero turnover). (C) NMDS plot showing differences in the composition of core microbiomes of the differing successional stages. Permanova tests for differences in composition were run with 9999 permutations. Results of pairwise comparisons can be found in Table S6. (D) Venn diagram displaying the number of stage-specific and shared indicator species. Only OTUs with a *p*-value <0.05 based on 9999 permutations are included in the analysis.

bacterial communities appeared to be compositionally stable across all grassland stages independent of successional stage and differed only in relation to their paired forest reference sites (Figure 2A). While these results contrast to other observations of clear successional patterns in soil microbial turnover during secondary succession (Cline & Zak, 2015; Gellie et al., 2017), they nonetheless concur with a meta-analysis of microbial development patterns during secondary succession globally (Zhou et al., 2017). The rate of community change between stages in a secondary successional system is related to the severity of the initial disturbance (Kearns & Shade, 2018) and factors such as dormancy, priority effects and the development of soil edaphic properties influencing both the trajectory and speed of microbial succession (Debray et al., 2022; Dini-Andreote et al., 2015). Successional trajectories are likely linked to the biotic and abiotic legacies of cultivation and management (Cramer et al., 2008), and a plausible explanation for the high compositional stability of microbes found here is that semi-natural grasslands represent a low-intensive form of agricultural management rather far from the blank slate environments created during, for example, forest fires (Ferrenberg et al., 2013). Interestingly, a recent study found high levels of soil microbial resistance in the context of reintroduced grazing on abandoned alpine meadows (Vidal et al., 2020). This indicates that microbial communities may be compositionally stable to both abandonment and subsequent re-grazing. In this context,

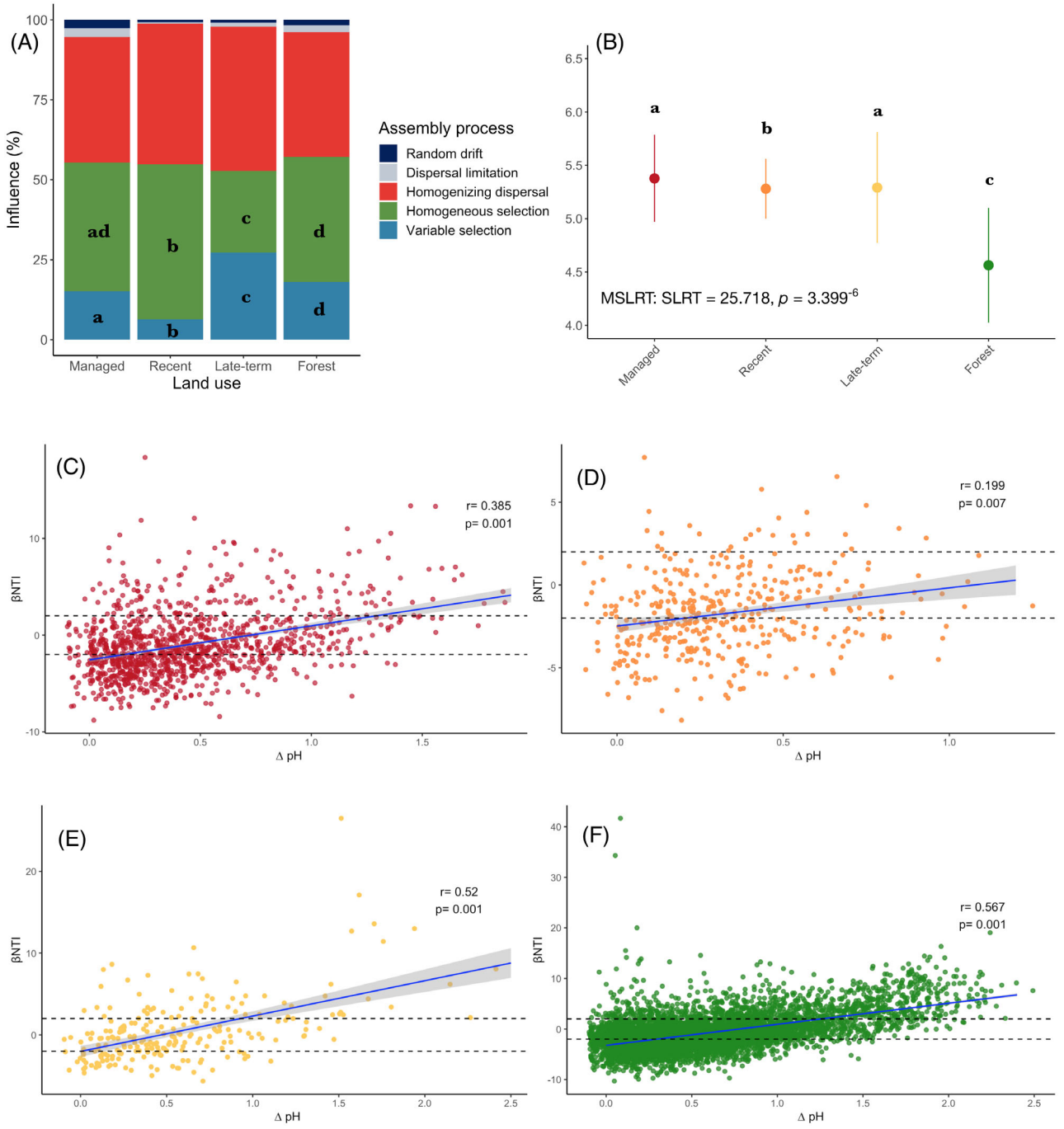

FIGURE 5 Relative influence of assembly processes and their link to pH variability. Barplot showing (A) the relative influence of deterministic (homogeneous selection, variable selection) and stochastic (random drift, dispersal limitation, and homogenising dispersal) assembly processes within each successional stage. Significant differences between successional stages ($p < 0.05$) based on pairwise comparisons of proportions. (B) Mean and standard deviation of soil pH along the successional gradient, along with letters denoting differences in their coefficient of variation (CV) based on modified signed-likelihood ratio tests (MSLRT) for equality of CV's. Relationship between differences in pH and β-NTI values for sites within (C) managed grasslands, (D) recently abandoned grasslands, (E) late-term successional grasslands and (F) forests. Test values derive from Mantel tests with 9999 permutations where *r* indicates Pearson correlation coefficient.

interesting questions can be posed as to the overall resistance and resilience of grassland microbiomes under shifting land-use intensities that contrast with the idea of a global trajectory of grassland degradation (Bardgett et al., 2021).

Soil pH was the main determining abiotic variable structuring bacterial communities (Figure 2E) and the main variable underpinning phylogenetic turnover (Figure S7). Contrary to our expectation of a gradual increase in the within-stage pH heterogeneity, we found

that land abandonment initially led to the opposite pattern of less variation in soil pH between the sites in recently abandoned grasslands, only to increase considerably in the next successional stage (Figure 5B). As expected, this was followed by a concomitant shift in the relative influence of deterministic assembly from homogenous to variable selection between early and late-term successional stages (Figure 5A). In the two-stage conceptual model proposed by Dini-Andreote et al. (2015), the authors suggest that homogenous selection results either due to spatially homogenous environmental filtering within each successional stage or because of increasingly extreme filtering in later stages. The latter scenario certainly applies to our results, with the more acidic forest soils exerting a homogenous filtering of bacterial communities to diverge from those in grasslands (Figure 2). However, in line with the former scenario, the higher pH heterogeneity and relative influence of variable selection in late-stage compared to early-stage grasslands should also translate into higher niche differentiation and community turnover, something we did not find.

The whole-community compositional stability of grassland communities was reflected also at the core taxa level, with marked differences between grassland and forest core microbiomes (Figure 4C). Forests had the highest proportion of specialist indicator taxa (45% or 214 taxa) of all successional stages, whereas 183 indicator taxa were shared between all three stages of grasslands (Figure 4D). Core microbiomes can be characterised by bacterial taxa that are highly enriched across certain environments or host plant species, as defined through prevalence or occupancy thresholds (Custer et al., 2023), and can be of disproportionate relevance for host fitness or ecosystem function (D Ainsworth et al., 2015; Toju et al., 2018). Based on our results, the asymptotic convergence of zeta decline and taxa retention curves for all land-use categories into core sets of taxa shared across multiple sites provides compelling complementary evidence for core microbiomes in both grasslands and forests, that generally reflect the overall community turnover patterns. That is, core taxa occupy a broad range of niches that remain relatively stable within grasslands despite vegetation succession. Conversely, non-core taxa with rapid turnover within the differing successional stages are likely to represent taxa with high habitat specificity (Jiao & Lu, 2020). Interestingly, the composition of core taxa in managed grasslands differed from those found in abandoned grasslands (Figure 4C) and managed grasslands had more OTUs classified as indicator species, and a higher proportion of stage-specific indicator taxa than the two abandoned grassland successional stages (Figure 4D; Table S4). Overall, bacteria are emerging as indicators of perturbations and land-use change (Gschwend et al., 2021; Hermans et al., 2016), especially when perturbations cause substantial variation in physicochemical properties. With the improved capacity to describe and characterise unculturable microbes and their life history strategies in detail, the current rather blunt classifications surrounding bacterial habitat preferences could also give way to more nuanced views at higher taxonomic resolution (Stone et al., 2023). It is further likely that transient and core taxa within the same community exhibit differing assembly patterns (Jiao & Lu, 2020), and a further question to pursue is whether and if so what abiotic drivers differentially shape generalist and specialist taxa in the context of succession.

Little is known about the relative contributions of species spatial turnover and nestedness to the turnover of microbial communities during secondary succession. We found that most of the turnover between grasslands and forests was due to species spatial turnover rather than nestedness (Figure 3A), in line with our first hypothesis. However, we also found that species spatial turnover between grasslands and forests was higher in recently abandoned fields compared to late-stage successional fields despite similar levels of taxonomic richness (Figure S2). These patterns were reproduced using raw (i.e., not rarefied) data, and therefore independent of rarefaction (Figure S4A,B). Dynamics of species spatial turnover and nestedness play a fundamental role in plant successional theory, and recent evidence suggests that the proportion of nestedness in plant community turnover increases with successional age in old-field systems (Ladouceur et al., 2023). However, the relative dominance of spatial turnover or nestedness is related to the number of rare taxa in the communities compared (Shade et al., 2013), with species spatial turnover increasing in importance with decreasing number of rare taxa. As our partitioning analyses were based on the turnover of whole communities, the dominance of spatial turnover likely reflected a species spatial turnover of transient taxa between the communities. Similarly to the overall community composition (Figure 2E), soil pH and C:N were the main factors underpinning increased turnover and species spatial turnover between paired sites (Figure 3C–F).

Finally, our study suggests that the major changes in microbial communities after grassland abandonment and succession to forests may occur rather late in the successional trajectory. This highlights the need to follow succession from grasslands to forests for a long time or to design studies that explicitly include stages with young and mid-aged forests. However, such sites are rare in the Swedish monitoring programmes, since it is a common practice among landowners to plant forest trees like Norway spruce or birch once a grassland has been abandoned, effectively short-cutting the natural succession pattern. Hence another approach to studying the transition from grassland to forest microbial communities may be needed, following individual abandoned grassland sites for a long time, that is, decades or even longer.

CONCLUSION

In this study, we examined the patterns of bacterial community composition and assembly in a large-scale secondary successional gradient using three complementary perspectives on assembly and turnover: partitioned beta diversity, zeta decline and the relative influence of assembly processes. Contrary to our hypotheses, we found that community composition remained compositionally stable across grasslands independent of the successional stage and that these patterns were underpinned by persistently occurring core taxa and indicator species. Similarly, deterministic assembly patterns were marginally higher than stochastic processes at all stages of the gradient, with differences in homogenous and variable selection reflecting the degree of pH variability. We also found that spatial turnover was the main process driving pairwise community turnover between grassland and forests. Further research is needed to unravel the link between core microbiomes and whole-community composition in natural transient systems, as well as the assembly processes that shape them. Similarly, additional analyses are required to elucidate the genotypic and phenotypic characteristics of core indicator taxa to differing successional stages, including their link to ecosystem functioning and plant–soil feedback.

AUTHOR CONTRIBUTIONS

Tord Ranheim Sveen: Conceptualization; writing – original draft; writing – review and editing; formal analysis. **Maria Viketoft:** Conceptualization; methodology; writing – review and editing; supervision. **Jan Bengtsson:** Conceptualization; writing – review and editing; supervision. **Mohammad Bahram:** Conceptualization; methodology; writing – review and editing; supervision; funding acquisition.

ACKNOWLEDGEMENTS

This work was supported by the Swedish University of Agricultural Sciences (early career grant), the Swedish Research Council Formas (Grant 2020–00807) and the Swedish Research Council (VR; Grant 2021–03724). We thank Anders Glimskär and Bertil Westerlund at SLU for their help with acquiring site information and metadata from the Remiil and National Forest Inventory environmental monitoring programmes. We also thank Sara Cousins, Alistair Auffret, Erik Öckinger, Eva Gustavsson and Emilia Hannula for valuable advice, ideas and background information.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in the NCBI Sequence Read Archive (SRA) under BioProject reference number PRJNA994701: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA994701.

ORCID

Tord Ranheim Sveen https://orcid.org/0000-0002-7019-3560
Maria Viketoft https://orcid.org/0000-0001-5289-4649
Jan Bengtsson https://orcid.org/0000-0002-8303-8818
Mohammad Bahram https://orcid.org/0000-0002-9539-3307

REFERENCES

Alberdi, A. & Gilbert, M.T.P. (2019) hilldiv: an R package for the integral analysis of diversity based on Hill numbers. *bioRxiv*, 545665. Available from: https://doi.org/10.1101/545665

Bahram, M., Hildebrand, F., Forslund, S.K., Anderson, J.L., Soudzilovskaia, N.A., Bodegom, P.M. et al. (2018) Structure and function of the global topsoil microbiome. *Nature*, 560, 233–237. Available from: https://doi.org/10.1038/s41586-018-0386-6

Bardgett, R.D., Bullock, J.M., Lavorel, S., Manning, P., Schaffner, U., Ostle, N. et al. (2021) Combatting global grassland degradation. *Nature Reviews Earth & Environment*, 2, 720–735. Available from: https://doi.org/10.1038/s43017-021-00207-2

Barnett, D.J.M., Arts, I.C.W. & Penders, J. (2021) microViz: an R package for microbiome data visualization and statistics. *Journal of Open Source Software*, 6, 3201. Available from: https://doi.org/10.21105/joss.03201

Barnett, S.E., Youngblut, N.D. & Buckley, D.H. (2020) Soil characteristics and land-use drive bacterial community assembly patterns. *FEMS Microbiology Ecology*, 96, fiz194. Available from: https://doi.org/10.1093/femsec/fiz194

Baselga, A. (2013) Separating the two components of abundance-based dissimilarity: balanced changes in abundance vs. abundance gradients. *Methods in Ecology and Evolution*, 4, 552–557. Available from: https://doi.org/10.1111/2041-210X.12029

Baselga, A. (2017) Partitioning abundance-based multiple-site dissimilarity into components: balanced variation in abundance and abundance gradients. *Methods in Ecology and Evolution*, 8, 799–808. Available from: https://doi.org/10.1111/2041-210X.12693

Cáceres, M.D. & Legendre, P. (2009) Associations between species and groups of sites: indices and statistical inference. *Ecology*, 90, 3566–3574. Available from: https://doi.org/10.1890/08-1823.1

Chao, A., Gotelli, N.J., Hsieh, T.C., Sander, E.L., Ma, K.H., Colwell, R.K. et al. (2014) Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. *Ecological Monographs*, 84, 45–67. Available from: https://doi.org/10.1890/13-0133.1

Chao, A. & Jost, L. (2012) Coverage-based rarefaction and extrapolation: standardizing samples by completeness rather than size. *Ecology*, 93, 2533–2547. Available from: https://doi.org/10.1890/11-1952.1

Cline, L.C. & Zak, D.R. (2015) Soil microbial communities are shaped by plant-driven changes in resource availability during secondary succession. *Ecology*, 96, 3374–3385. Available from: https://doi.org/10.1890/15-0184.1

Cousins, S.A.O., Auffret, A.G., Lindgren, J. & Tränk, L. (2015) Regional-scale land-cover change during the 20th century and its consequences for biodiversity. *AMBIO*, 44, 17–27. Available from: https://doi.org/10.1007/s13280-014-0585-9

Cramer, V.A., Hobbs, R.J. & Standish, R.J. (2008) What's new about old fields? Land abandonment and ecosystem assembly. *Trends*

in Ecology & Evolution, 23, 104–112. Available from: https://doi.org/10.1016/j.tree.2007.10.005

Custer, G.F., Gans, M., van Diepen, L.T.A., Dini-Andreote, F. & Buerkle, C.A. (2023) Comparative analysis of Core microbiome assignments: implications for ecological synthesis. *mSystems*, 8, e01066-22. Available from: https://doi.org/10.1128/msystems.01066-22

D Ainsworth, T., Krause, L., Bridge, T., Torda, G., Raina, J.-B., Zakrzewski, M. et al. (2015) The coral core microbiome identifies rare bacterial taxa as ubiquitous endosymbionts. *The ISME Journal*, 9, 2261–2274. Available from: https://doi.org/10.1038/ismej.2015.39

Debray, R., Herbert, R.A., Jaffe, A.L., Crits-Christoph, A., Power, M.E. & Koskella, B. (2022) Priority effects in microbiome assembly. *Nature Reviews Microbiology*, 20, 109–121. Available from: https://doi.org/10.1038/s41579-021-00604-w

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*, 112, E1326–E1332. Available from: https://doi.org/10.1073/pnas.1414261112

Dray, S., Bauman, D., Blanchet, G., Borcard, D., Clappe, S., Guénard, G., et al. (2023). *adespatial: Multivariate multiscale spatial analysis*.

Edgar, R.C. (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature Methods*, 10, 996–998. Available from: https://doi.org/10.1038/nmeth.2604

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C. & Knight, R. (2011) UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*, 27, 2194–2200. Available from: https://doi.org/10.1093/bioinformatics/btr381

Egnér, H., Riehm, H. & Domingo, W.R. (1960) Untersuchungen uber die chemische Bodenanalyse als Grundlage fur die Beurteilung des Nährstoffzustandes der Böden. II. Chemische Extraktionsmethoden zur Phosphor- und Kaliumbestimmung. *Kungliga Lantbrukshögskolans Annaler*, 26, 199–215.

Ericsson, S., Östlund, L. & Axelsson, A.-L. (2000) A forest of grazing and logging: deforestation and reforestation history of a boreal landscape in Central Sweden. *New Forests*, 19, 227–240. Available from: https://doi.org/10.1023/A:1006673312465

Ferrenberg, S., O'Neill, S.P., Knelman, J.E., Todd, B., Duggan, S., Bradley, D. et al. (2013) Changes in assembly processes in soil bacterial communities following a wildfire disturbance. *The ISME Journal*, 7, 1102–1111. Available from: https://doi.org/10.1038/ismej.2013.11

Fierer, N., Nemergut, D., Knight, R. & Craine, J.M. (2010) Changes through time: integrating microorganisms into the study of succession. *Research in Microbiology*, 161, 635–642. Available from: https://doi.org/10.1016/j.resmic.2010.06.002

Gellie, N.J.C., Mills, J.G., Breed, M.F. & Lowe, A.J. (2017) Revegetation rewilds the soil bacterial microbiome of an old field. *Molecular Ecology*, 26, 2895–2904. Available from: https://doi.org/10.1111/mec.14081

Gschwend, F., Hartmann, M., Mayerhofer, J., Hug, A.-S., Enkerli, J., Gubler, A. et al. (2021) Site and land-use associations of soil bacteria and fungi define core and indicative taxa. *FEMS Microbiology Ecology*, 97, fiab165. Available from: https://doi.org/10.1093/femsec/fiab165

Hartmann, M., Frey, B., Mayer, J., Mäder, P. & Widmer, F. (2015) Distinct soil microbial diversity under long-term organic and conventional farming. *The ISME Journal*, 9, 1177–1194. Available from: https://doi.org/10.1038/ismej.2014.210

Hermans, S.M., Buckley, H.L., Case, B.S., Curran-Cournane, F., Taylor, M. & Lear, G. (2016) Bacteria as emerging indicators of soil condition. *Applied and Environmental Microbiology*, 83, e02826-16. Available from: https://doi.org/10.1128/AEM.02826-16

Hui, C. & McGeoch, M.A. (2014) Zeta diversity as a concept and metric that unifies incidence-based biodiversity patterns. *The American Naturalist*, 184, 684–694. Available from: https://doi.org/10.1086/678125

Jiao, S. & Lu, Y. (2020) Soil pH and temperature regulate assembly processes of abundant and rare bacterial communities in agricultural ecosystems. *Environmental Microbiology*, 22, 1052–1065. Available from: https://doi.org/10.1111/1462-2920.14815

Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, 30, 772–780. Available from: https://doi.org/10.1093/molbev/mst010

Kearns, P.J. & Shade, A. (2018) Trait-based patterns of microbial dynamics in dormancy potential and heterotrophic strategy: case studies of resource-based and post-press succession. *The ISME Journal*, 12, 2575–2581. Available from: https://doi.org/10.1038/s41396-018-0194-x

Kennedy, P.G., Cline, L.C. & Song, Z. (2018) Probing promise versus performance in longer read fungal metabarcoding. *The New Phytologist*, 217, 973–976.

Koleff, P., Gaston, K.J. & Lennon, J.J. (2003) Measuring beta diversity for presence–absence data. *Journal of Animal Ecology*, 72, 367–382. Available from: https://doi.org/10.1046/j.1365-2656.2003.00710.x

Krishnamoorthy, K. & Lee, M. (2014) Improved tests for the equality of normal coefficients of variation. *Computational Statistics*, 29, 215–232. Available from: https://doi.org/10.1007/s00180-013-0445-2

Ladouceur, E., Isbell, F., Clark, A.T., Harpole, W.S., Reich, P.B., Tilman, G.D. et al. (2023) The recovery of plant community composition following passive restoration across spatial scales. *Journal of Ecology*, 111, 814–829. Available from: https://doi.org/10.1111/1365-2745.14063

Lane, J.M., Delavaux, C.S., Van Koppen, L., Lu, P., Cade-Menun, B.J., Tremblay, J. et al. (2022) Soil sample storage conditions impact extracellular enzyme activity and bacterial amplicon diversity metrics in a semi-arid ecosystem. *Soil Biology and Biochemistry*, 175, 108858. Available from: https://doi.org/10.1016/j.soilbio.2022.108858

Legendre, P. (2014) Interpreting the replacement and richness difference components of beta diversity. *Global Ecology and Biogeography*, 23, 1324–1334. Available from: https://doi.org/10.1111/geb.12207

Lynch, M.D.J. & Neufeld, J.D. (2015) Ecology and exploration of the rare biosphere. *Nature Reviews Microbiology*, 13, 217–229. Available from: https://doi.org/10.1038/nrmicro3400

McGeoch, M.A., Latombe, G., Andrew, N.R., Nakagawa, S., Nipperess, D.A., Roigé, M. et al. (2019) Measuring continuous compositional change using decline and decay in zeta diversity. *Ecology*, 100, e02832. Available from: https://doi.org/10.1002/ecy.2832

Nemergut, D.R., Schmidt, S.K., Fukami, T., O'Neill, S.P., Bilinski, T.M., Stanish, L.F. et al. (2013) Patterns and processes of microbial community assembly. *Microbiology and Molecular Biology Reviews*, 77, 342–356. Available from: https://doi.org/10.1128/MMBR.00051-12

Neu, A.T., Allen, E.E. & Roy, K. (2021) Defining and quantifying the core microbiome: challenges and prospects. *Proceedings of the National Academy of Sciences*, 118, e2104429118. Available from: https://doi.org/10.1073/pnas.2104429118

Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., et al. (2022). *vegan: Community Ecology Package*.

Özkurt, E., Fritscher, J., Soranzo, N., Ng, D.Y.K., Davey, R.P., Bahram, M. et al. (2022) LotuS2: An ultrafast and highly accurate tool for amplicon sequencing analysis. *Microbiome*, 10, 176. https://doi.org/10.1186/s40168-022-01365-1

Price, M.N., Dehal, P.S. & Arkin, A.P. (2010) FastTree 2–approximately maximum-likelihood trees for large alignments.

PLOS One, 5, e9490. Available from: https://doi.org/10.1371/journal.pone.0009490

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P. et al. (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, 41, D590–D596. Available from: https://doi.org/10.1093/nar/gks1219

R Core Team. (2023) *R: a language environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Shade, A., Gregory Caporaso, J., Handelsman, J., Knight, R. & Fierer, N. (2013) A meta-analysis of changes in bacterial and archaeal communities with time. *The ISME Journal*, 7, 1493–1506. Available from: https://doi.org/10.1038/ismej.2013.54

Stegen, J.C., Lin, X., Fredrickson, J.K., Chen, X., Kennedy, D.W., Murray, C.J. et al. (2013) Quantifying community assembly processes and identifying features that impose them. *The ISME Journal*, 7, 2069–2079. Available from: 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. *Frontiers in Microbiology*, 6, 370.

Stone, B.W.G., Dijkstra, P., Finley, B.K., Fitzpatrick, R., Foley, M.M., Hayer, M. et al. (2023) Life history strategies among soil bacteria—dichotomy for few, continuum for many. *The ISME Journal*, 17, 611–619. Available from: https://doi.org/10.1038/s41396-022-01354-0

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R. et al. (2014) Global diversity and geography of soil fungi. *Science*, 346, 1256688. Available from: https://doi.org/10.1126/science.1256688

Toju, H., Peay, K.G., Yamamichi, M., Narisawa, K., Hiruma, K., Naito, K. et al. (2018) Core microbiomes for sustainable agroecosystems. *Nature Plants*, 4, 247–257. Available from: https://doi.org/10.1038/s41477-018-0139-4

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. *The ISME Journal*, 12, 1072–1083. Available from: https://doi.org/10.1038/s41396-018-0082-4

Umaña, M.N., Zhang, C., Cao, M., Lin, L. & Swenson, N.G. (2017) A core-transient framework for trait-based community ecology: an example from a tropical tree seedling community. *Ecology Letters*, 20, 619–628. Available from: https://doi.org/10.1111/ele.12760

Vidal, A., Schucknecht, A., Toechterle, P., Linares, D.R.A., Garcia-Franco, N., von Heßberg, A. et al. (2020) High resistance of soils to short-term re-grazing in a long-term abandoned alpine pasture. *Agriculture, Ecosystems & Environment*, 300, 107008. Available from: https://doi.org/10.1016/j.agee.2020.107008

Walters, W., Hyde, E.R., Berg-Lyons, D., Ackermann, G., Humphrey, G., Parada, A. et al. (2015). Improved bacterial 16S rRNA gene (V4 and V4-5) and fungal internal transcribed spacer marker gene primers for microbial community surveys. *Msystems*, 1(1), e00009–15.

Wei, X., Widgren, M., Li, B., Ye, Y., Fang, X., Zhang, C. et al. (2021) Dataset of 1 km cropland cover from 1690 to 1999 in Scandinavia. *Earth System Science Data*, 13, 3035–3056. Available from: https://doi.org/10.5194/essd-13-3035-2021

Zhou, J. & Ning, D. (2017) Stochastic community assembly: does it matter in microbial ecology? *Microbiology and Molecular Biology Reviews*, 81, e00002-17. Available from: https://doi.org/10.1128/MMBR.00002-17

Zhou, Z., Wang, C., Jiang, L. & Luo, Y. (2017) Trends in soil microbial communities during secondary succession. *Soil Biology and Biochemistry*, 115, 92–99. Available from: https://doi.org/10.1016/j.soilbio.2017.08.014

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Sveen, T.R., Viketoft, M., Bengtsson, J. & Bahram, M. (2024) Core taxa underpin soil microbial community turnover during secondary succession. *Environmental Microbiology*, 26(1), e16561. Available from: https://doi.org/10.1111/1462-2920.16561