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Depth effects on bacterial community assembly processes in paddy soils

Weitao Li [a,c], Yakov Kuzyakov [d,e,f], Yulong Zheng [a,c], Pengfa Li [b], Guilong Li [b], Ming Liu [b,*], Hattan A. Alharbi [g], Zhongpei Li [b,**]

[a] CAS Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun, Mengla, Yunnan, 666303, China
[b] State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, No. 71, East Beijing Road, P.O. box 821, Nanjing, 210008, China
[c] Center of Conservation Biology, Core Botanical Gardens, Chinese Academy of Sciences, Mengla, Yunnan, China
[d] Department of Soil Science of Temperate Ecosystems, Department of Agricultural Soil Science, University of Göttingen, Göttingen, Germany
[e] Institute of Environmental Sciences, Kazan Federal University, 420049, Kazan, Russia
[f] Agro-Technology Institute, RUDN University, Moscow, Russia
[g] Department of Plant Protection, College of Food and Agriculture Sciences, King Saud University, Riyadh, 11451, Saudi Arabia

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ABSTRACT

Bacterial communities in soil play a key role in carbon (C) and nutrient cycling. Unravelling how bacterial community assemble and distribute with soil depth is a prerequisite for understanding microbial functions, nutrient cycling and management. Twenty-six rice fields in a typical red soil area in a wet subtropical climate were sampled in the topsoil (0–10 and 10–20 cm) and subsoil (20–40 cm). Physico-chemical soil properties, quantitative fluorescence PCR and high-throughput sequencing were used to analyse the V4 region of 16S rDNA. The rRNA operon copy number and alpha diversity decreased continuously with soil depth because of reduced access to carbon, energy, oxygen and nutrients. The relative abundance of the dominant phyla Proteobacteria and Actinobacteria decreased with increasing soil depth, whereas the opposite trend was observed for the phylum Nitrospirae. The interaction intensity between taxa increased with depth, as limited carbon and nutrients in the undisturbed subsoil lead to the cooccurrence of taxa with similar ecological niches that cooperated to reduce functional redundancy. The higher modularity of the bacterial network in the topsoil is associated with greater environmental perturbations (flooding, fertilization, etc.) to maintain the robustness of the microbial community. Bacterial community assembly processes were stochastic up to 40 cm, but ecological drift was the predominant process in the topsoil, whereas dispersal limitation was dominant in the subsoil. The contribution of abiotic factors (e.g. nutrient and iron contents) and biotic factors (taxa-taxa interactions) as well as dispersal limitations to bacterial community assembly was depth specific. Concluding, the basic principles of bacterial community assembly were evaluated for the first time for a broad range of paddy soils.

1. Introduction

Similar to plant communities, the pattern of microbial community assembly processes can be predicted by temporal and spatial variations in soil, environmental and management factors (Freedman and Zak, 2015; Evans et al., 2017; Bay et al., 2020; Li et al., 2020). In addition to these factors, the composition of the soil bacterial community depends on depth: in deeper soils, nutrients and carbon are more limited (Kautz et al., 2013) and oligotrophic phyla occur (Elul et al., 2021). Subsoils imply relatively stable environmental conditions and consequently, a more stable species composition compared to the topsoils. The extent of spatial dissimilarity in microbial community composition and diversity at each depth is related to parent material, soil type and fertility, tillage, etc. (Sheng et al., 2015; Sun et al., 2015; Gu et al., 2017). It is not clear how geographic divergence in bacterial community composition depends on soil depth, which processes underlie community assembly, and which are the main factors.

In general, deterministic processes (selection) versus stochastic processes (dispersion and drift) define microbial community assembly (Stegen et al., 2012). The bacterial communities will be assembled by

* Corresponding author.
** Corresponding author.
E-mail addresses: mliu@issas.ac.cn (M. Liu), zhpli@issas.ac.cn (Z. Li).

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deterministic processes such as habitat specialization (Lozupone and Knight, 2007), but also stochastic processes may be important (Zhou and Ning, 2017). Studies based on quantifying phylogenetic turnover as the deviation from a null expectation suggested that subsurface microbial community is assembled by domination of deterministic processes (Stegen et al., 2012; Wang et al., 2013). Stochastic processes, however, refer to probabilistic colonization, random extinction, and random dispersal events (Chase and Myers, 2011). The balance between deterministic and stochastic processes could be mediated by pH, redox potential, salinity, temperature, etc. (Tripathi et al., 2018; Zhang et al., 2019; Jiao and Lu, 2020). Stegen et al. (2012) suggested that deterministic processes could be maximized towards extreme environmental variation compared to stochastic factors. Homogeneous selection was common for bacterial community formation influenced by pH and land use (Barnett et al., 2020).

With increasing soil depth, the contributions of dispersion limitation to microbial community assembly increased compared to environmental filtering (Chen et al., 2020; Li et al., 2020). Dispersion is an important factor affecting the natural bacterial community composition (Albright and Martiny, 2018). In addition to environmental filtering, interactions between groups of the microbiome contribute to assembly processes (Sun et al., 2018; Cosetta and Wolfe, 2019). There is a gap in understanding i) how the intensities of biotic interactions change with soil depth, ii) how these changes affect bacterial community assembly, and iii) how environmental factors affect biotic interactions and indirectly influence bacterial community assembly. These unknowns are especially prominent regarding the bacterial community assembly in paddy soils.

Rice paddy cultivation mainly occurs in the wet subtropics, with high temperature and rainfall, and paddy fields are widespread on various soil parent materials (Liu et al., 2021). Most rice roots are located in the topsoil (Ap, 0–20 cm depth). However, the subsoil (below 20 cm depth) can be an important C sink and key to root and crop growth (Kautz et al., 2013). Unlike topsoil, which is more exposed to alternating drying and flooding, ploughing, fertilization, planting, etc., the subsoil physico-chemical properties are more related to the parent material. The confined and homogeneous subsoil combined with lower nutrient availability recruits bacteria with similar physiological ecotypes, resulting in more intense taxa-taxa interactions, whereas species dispersal is more limited. There is a gap in knowledge of the spatial distribution pattern of the subsurface bacterial communities and their assembly processes. We hypothesized that 1) the dissimilarity in the bacterial community increases due to variation in soil properties and dispersal limitation; 2) bacterial community assembly is more stochastic in the topsoil due to greater exposure to anthropogenic perturbations and environmental fluctuations, while by contrast in the subsoil nutrient-limitations leads to increased associations between bacterial species.

2. Materials and methods

2.1. Soil sampling

Soils were sampled in late December 2017 from typical areas of red paddy soils (Hydrargic Anthrosols) in Yujiang County, Jiangxi Province, China (Fig. S1, Table S1). The paddy fields were ploughed to a depth of 10–17 cm and agronomic practices such as irrigation and fertilization were almost the same in the fields in recent years. Fertilizer applications were about 300 kg N ha^{-1} yr^{-1}, 66 kg P ha^{-1} yr^{-1} and 250 kg K ha^{-1} yr^{-1}, coupled with complete return of straw to the field. The cropping system is double cropping of rice (*Oryza sativa* L.) (i.e., early and late rice). We randomly selected 26 sites considering the parent material and cropping background. A well-managed plot was selected at each site, where five 40 cm deep soil cores (diameter 6 cm) were randomly taken from the bulk soil and divided into three depth intervals: 0–10 cm, 10–20 cm and 20–40 cm. At the end, 78 soil samples (26 sites × three soil depths) were taken for subsequent analysis. The samples were refrigerated at 4 °C and transported to the Soil Biochemistry Laboratory in Nanjing, Institute of Soil Science. The subsamples for physical and chemical properties were air-dried, ground, and sieved through a 2-mm sieve. Subsamples for microbial properties were stored at −40 °C.

2.2. Analysis of soil physical and chemical properties

Soil chemical properties were measured using routine methods described by Lu (1999). Soil pH was determined using a pH metre (FE30, Mettler-Toledo, CH) with a 1:2.5 soil-water suspension. Soil organic C (SOC) was determined by $K_2Cr_2O_7$–H_2SO_4 oxidation. Total and available N were measured as Kjeldahl-N; total P and available P were determined by HF–$HClO_4$ digestion and sodium bicarbonate extraction (molybdenum blue method), respectively; total K and exchangeable K were determined by HF–$HClO_4$ digestion and ammonium acetate extraction (flame photometer), respectively. The hydrometer method was used to analyse the soil texture.

2.3. Illumina sequencing analysis of 16S rRNA gene amplicons

Total genomic DNA was extracted from 0.5 g of fresh soil samples using a FastDNA™SPIN kit (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's protocol. DNA concentration and purity were monitored on 1% agarose gels. Depending on the concentration, DNA was diluted to 1 ng μl^{-1} with sterile water. The V4 region of the 16S rRNA gene was PCR-amplified using primers 515F and 806R with the barcode, and sequencing was performed on an Illumina HiSeq platform, generating 250 bp paired-end reads. The HiSeq sequences were deposited in GenBank with the BioProject accession number PRJNA726344.

Paired-end reads from the original DNA fragments were merged

Glossary box

Microbial community assembly: The sum of all processes involved in shaping microbial community composition (Vellend, 2010).

Dispersal: Movement of organisms across space (Vellend, 2010).

Drift: Stochastic variation in the relative abundance of species in a community over time (Vellend, 2010).

Environmental filtering: Direct and indirect impacts of abiotic factors that follow geographic patterns and restrict the distributions of organisms (Nemergut et al., 2013).

Networks: Mathematical representations of communities, in which nodes represent

individual taxa and edges represent observed correlations in abundances among taxa, from which interactions may be inferred (Barberan et al., 2012).

Modularity: Tightly knit groups in a network (Newman, 2006).

using FLASH (Magoc and Salzberg, 2011) and then assigned to each sample using unique barcodes. Sequences were analysed using the Quantitative Insights Into Microbial Ecology (QIIME) software package (Caporaso et al., 2010), and in-house Perl scripts were used to analyse alpha (within samples) and beta (between samples) diversity. First, the reads were filtered using QIIME quality filters. Then, we used pick_de_novo_otus.py to select operational taxonomic units (OTUs) by creating the OTU table. Sequences with ≥97% similarity were assigned to the same OTUs. We chose a representative sequence for each OTU and with reference to a subset of the SILVA 119 database (http://www.arb-silva.de/download/archive/qiime/) to annotate the taxonomic information for each representative sequence (7.2% unclassified at the level phylum). After removing singletons and resampling with 19666 sequences per sample, 12520 OTUs were obtained for all 78 soil samples (26 sites × three soil depths) and used in downstream analysis. Shannon index was calculated using the alpha_diversity.py script in QIIME.

The Shannon index was used to estimate the alpha diversity of the bacterial community within each soil sample. Using QIIME we calculated the Bray-Curtis measures of bacterial community dissimilarity with soil depth and visualized the results using nonmetric multidimensional scaling (NMDS) analysis. To obtain robust test results on the beta diversity of differences between soil layers, a significance test was performed using statistical analysis methods, including ANOSIM, MRPP and Adonis.

2.4. Quantitative PCR

The 16S rRNA gene was analysed by high-throughput qPCR on an ABI ViiA 7 Real Time qPCR platform (Applied Biosystems) according to the procedures described by Schmittgen et al. (2008). There were 78 DNA samples in total, and each DNA sample was amplified by PCR in triplicate.

2.5. Cooccurrence network analysis

A taxon-taxon cooccurrence network was constructed to examine associations between taxa within each soil depth. It was derived using the Spearman correlation matrix created with the 'WGCNA' package (Langfelder and Horvath, 2012). We selected phyla with a mean relative abundance greater than 1% within each soil layer, and OTUs with relative abundances greater than 0.01% were selected for Spearman correlation calculation. All *p*-values were adjusted using the false discovery rate (FDR) control procedure of Benjamini and Hochberg (Benjamini et al., 2006). The cut-off for FDR-adjusted *p*-values was set to 0.001. The cut-off for correlation coefficients was set to 0.72 using random matrix theory-based methods (Luo et al., 2006). Finally, the modified random matrix was used to construct the network. It was visualized using Gephi (http://gephi.github.io/). A total of 78 samples were divided into three groups along the soil depth gradients. Network properties were calculated using the 'igraph' package (http://igraph.org) in R (ver. 3.6.2). Taxon-environment networks were calculated using the same procedure.

2.6. Bacterial community assembly analysis

The relative importance of deterministic processes and stochastic processes in bacterial community assembly was evaluated using null model-based methods. These methods were conducted following the analytical framework analyses of standard phylogenetic beta diversity and taxonomic beta diversity (Stegen et al., 2013). First, a null distribution of the β-mean-nearest taxon distance (βMNTD) was generated among samples within each soil depth by re-dominating the taxa labels of the phylogenetic tree 999 times. Then, the β-nearest taxon index (βNTI) was calculated by comparing the difference between the observed βMNTD values and the mean of the null distribution of βMNTD normalized by its standard deviation. A βNTI value < -2 means significantly lower than the expected phylogenetic turnover rate, whereas a βNTI value > 2 indicates significantly higher than the expected phylogenetic turnover rate. When $|\beta NTI| < 2$, it means that this is not the determining process. It should be dispersion-limited (very low dispersion rates), homogeneously dispersed (very high dispersion rates), or not dominated by a single dominant process (i.e., it is "undominated"). To disentangle these cases, a further calculation based on the Bray-Curtis-based Raup-Crick metric (RCbray) as described by Stegen et al. (2013) on the relative contribution to the assembly process with $|\beta NTI| < 2$. The relative contribution of dispersion constraints was estimated in terms of percentages of paired comparisons with $|\beta NTI| < 2$ and RCbray > 0.95. The contribution of relative homogenized dispersion was estimated in terms of percentages of paired comparisons with $|\beta NTI| < 2$ and RCbray < -0.95. In contrast, not belonging to any of these categories suggested that neither single process dominated community assembly (Stegen et al., 2013).

2.7. Data analysis

To assess the effect of depth, we performed one-way repeated measures ANOVA on soil properties, 16S gene copies and relative abundance of phyla using the General Linear Model option in IBMSPSS (version 22.0, Chicago, IL, USA). The Bonferroni's post-hoc test was used to compare the three depths. Multiple bacterial genera were compared between soil depths by pairwise tests in STAMP (Parks et al., 2014).

The slopes of the distance-decay relationships of the microbial community were calculated at three soil depths: 0–10 cm, 10–20 cm, and 20–40 cm. The slope coefficient at each depth was calculated based on the equation below:

$$\mathrm{Ln}\ (S) = \mathrm{Ln}\ (a) + z \times \mathrm{Ln}\ (G/E)$$

where S is the community dissimilarity, G/E is the geographic distance or environmental dissimilarity, a is an intercept parameter and z is the slope coefficient of the distance-decay curve (Martiny et al., 2011). The geographic distance was calculated with the R package 'geosphere' (Hijmans, 2019).

Piecewise SEM was used to evaluate the effects of environmental factors, spatial distance, taxa associations and their indirect effects on betaNTI. Piecewise SEM was performed using the 'piecewise SEM' package in R (ver. 3.6.2).

To examine the relative importance of biotic associations and habitat filtering in determining phylogenetic turnover within each soil layer, variance partitioning of phylogenetic beta diversity was manipulated based on multiple regression of a distance matrix (Swenson, 2014). BetaNTI was used as the dependent variable, and the independent variables included environmental and biotic variables, with the calculation of Euclidean distance. The environmental variables included physicochemical soil properties. The distance matrix of biotic variables was calculated based on the taxa associations in each soil sample. Piecewise SEM was tested to confirm that the model structure was robust, and a separate piecewise SEM analysis was performed for each soil layer.

3. Results

3.1. Soil nutrients and texture depending on depth

Soil organic carbon (SOC) and nutrients decreased dramatically with depth; SOC was 2.6 times higher in the top 10 cm than in the subsoil, while available phosphorus and available nitrogen were 4.4 times and 2.2 times higher, respectively (Fig. 1). The C:N, C:P and N:P ratios were higher in the top 10 cm than in 20–40 cm ($p < 0.05$). Flooding and fertilization practices during the rice growing season led to the downward migration of iron and a decrease in pH (Fig. 1). The soil texture of top 10 cm had a slightly higher silt content and a lower clay content compared to 20–40 cm ($p < 0.05$).

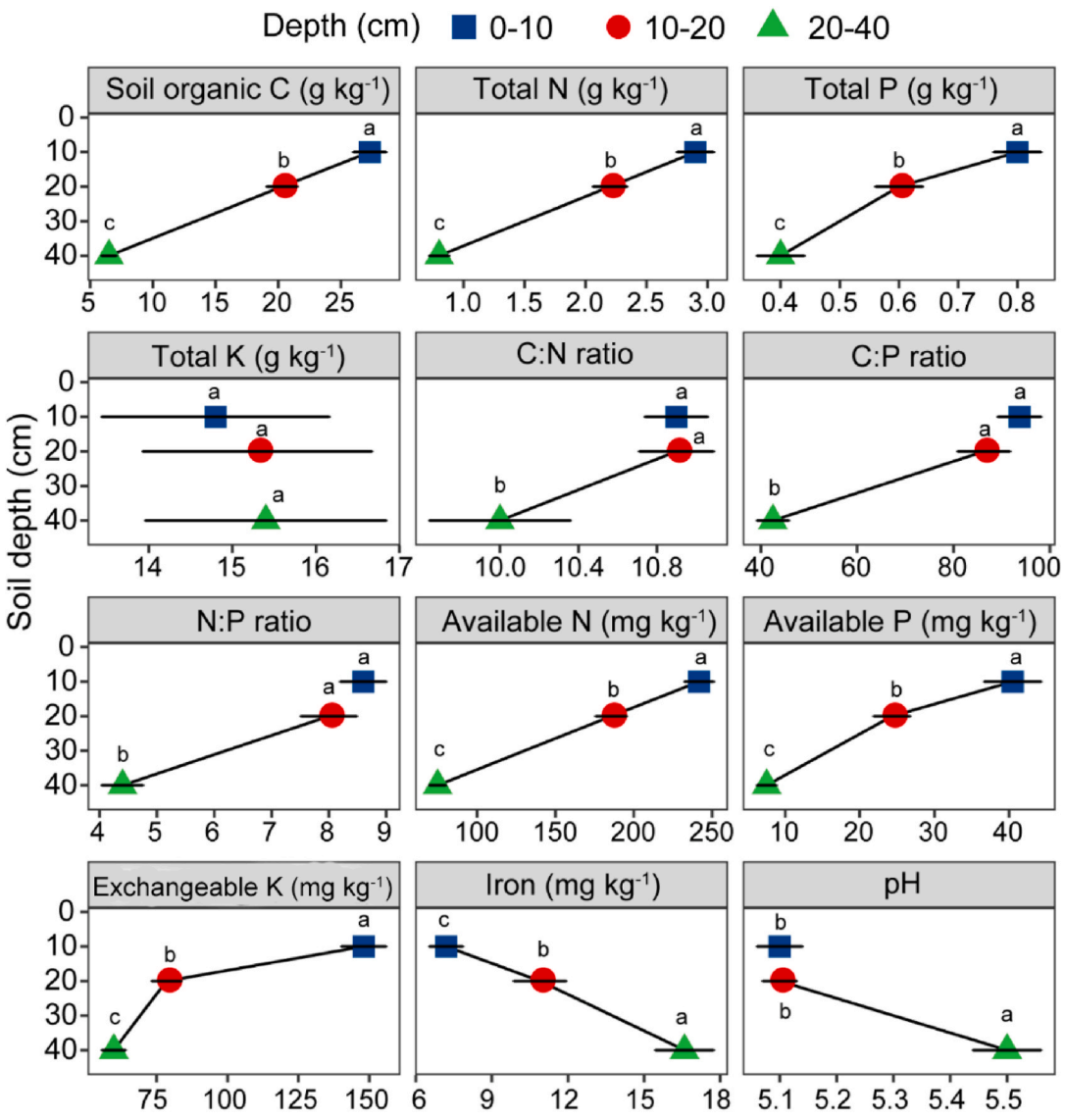
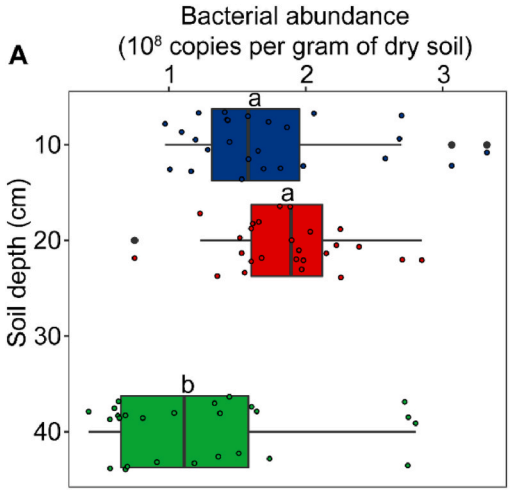


Fig. 1. Nutrient contents (mean ± SE, N = 26) and pH depending on soil depth. The blue square, red circle and green triangle in each subplot represent the soil layers 0–10 cm, 10–20 cm and 20–40 cm respectively. Letters indicate significant differences ($p < 0.05$). The line between the two dots is present only when they are significantly different and absent when they are not. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)



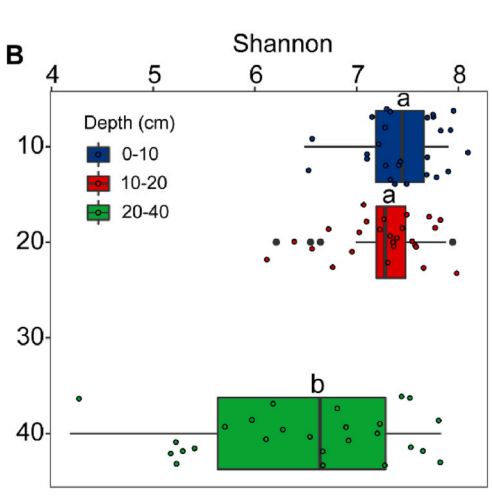


Fig. 2. Bacterial abundance (A) and alpha diversity of bacterial communities (B) depending on soil depth. The boxes in blue, red and green in each subplot represent the soil layers 0–10 cm, 10–20 cm and 20–40 cm respectively. Letters indicate significant differences ($p < 0.05$). In the box plots, the upper boundary of each box indicates the 25th percentile, the horizontal line inside each box marks the median, and the lower boundary of the box indicates the 75th percentile. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.2. Dissimilarity in alpha- and beta-diversity of bacterial communities depending on soil depth

The median bacterial quantity expressed as copies per g of dry soil was similar in the top two layers but decreased in the subsoil (Fig. 2A). The bacterial diversity (Shannon) in the topsoil was also higher than that in the subsoil (Fig. 2B). The phyla Proteobacteria, Acidobacteria, Chloroflexi, Nitrospirae and Actinobacteria were predominant in the bacterial community in the soil (Fig. 3A). The phyla Proteobacteria and Actinobacteria decreased with increasing soil depth, while the phyla Chloroflexi and Nitrospirae showed the opposite tendency (Fig. 4). There were differences in the bacterial community profile between any two soil layers, with the greatest difference between the top 10 cm and the subsoil (Fig. 3B, Table S2).

3.3. Trends in taxon-taxon and taxon-environment networks with soil depth

Linkage density, average degree, edges, and average clustering coefficient increased with soil depth, indicating that the taxon-taxon cooccurrence network became more complex and associations were more connected (Fig. 5A, C). Compared to the relatively isolated conditions of the subsoil, the topsoil was more exposed to environmental perturbations, which corresponded to a higher modularity of the bacterial community, which was important for maintaining the robustness of the bacterial community composition (Fig. 5C). Soil pH and texture were the most important factors strongly associated with bacterial taxa in the topsoil. In the subsoil (20–40 cm), however, SOC, total and available N were more strongly associated with taxa (Fig. 5B).

3.4. Bacterial community assembly depending on soil depth

Dissimilarity in the bacterial community among sampling sites within each depth increased with geographic distance and dissimilarity in environmental properties (Figs. S4A and B). This result suggested that a significant phylogenetic signal occurred within each soil depth. The proportions of absolute phylogenetic turnover (βNTI) values < 2 in the three soil layers 0–10 cm, 10–20 cm, and 20–40 cm were ~73%, ~87%, and ~70%, respectively (Fig. 6A). The proportions of absolute taxonomic turnover (RCbray) values < 0.95 in the three soil layers were 44%, 47%, and 19%, respectively (Fig. 6B). The role of stochastic processes, especially drift, was important for bacterial community assembly in the topsoil, whereas dispersal limitation was important for bacterial assembly in the subsoil (Figs. 6C and 7).

In the top 10 cm, the soil properties (N:P ratio, pH, mineral N, and sand content) and spatial distance between sampling sites were significant factors affecting phylogenetic turnover (βNTI). The C:P and N:P ratios and iron and sand contents were crucial factors directly defining taxonomic associations in the topsoil (Fig. 8A). For the 10-20-cm layer, the soil properties (SOC, available N, iron, and clay content) and taxonomic associations directly influenced βNTI. SOC, available N, available P, C:N, and pH influenced taxonomic associations and indirectly influenced βNTI (Fig. 8B). For the 20-40-cm layer, iron and spatial distance were more important for βNTI; available P was important for taxonomic associations (Fig. 8C).

4. Discussion

4.1. Dissimilarity in bacterial communities within soil profiles

The relative abundance of Nitrospirae increased with depth by 95%, whereas that of Proteobacteria decreased by 24% (Fig. 4). Proteobacteria are better adapted to the high C input from root exudates (Hernandez et al., 2015), while Nitrospirae survive better under the low air permeability conditions common in paddies below 20 cm (Elul et al., 2021). The sharp decrease in soil nutrients with increasing depth, especially the 81% decrease in the phosphorus availability (Fig. 1) shapes the bacterial community. A low ratio of Proteobacteria or α-Proteobacteria to Acidobacteria is common in oligotrophic soils. The autotrophic nitrifying bacteria Nitrospira occupy the ecological habitat of the subsoil (Hayatsu et al., 2008). Further analysis revealed that the relative abundance of Geobacter was higher in the subsoil than in the topsoil (Fig. S3), where it belongs to the class Deltaproteobacteria and is crucial for anaerobically oxidize aromatic hydrocarbons (Lovley et al., 2011). All these results clearly show that nutrient (especially N and P) availability in the soil is an important explanatory factor affecting the distribution of bacterial communities with depth (Li et al., 2017).

Consistent with the 1st hypothesis, dissimilarity in the bacterial community composition between soil layers and the beta diversity within each layer increased with depth. We also found the largest number of genera with significant differences between the upper 10 cm and the subsoil (Fig. S3). The same pattern was common in other agricultural systems (Li et al., 2019; Hao et al., 2021). Although parent material was crucial for the establishment of the soil bacterial community (Sheng et al., 2015), long-term rice cultivation buffered the effects of parent material in the plough horizon (Ap, 0–20 cm) (Shahbaz et al., 2017). Below the Ap horizon, exposure to external environmental disturbances decreased sharply, and the dissimilarity of the microbial community due to dispersal limitations was closely related to the parent material.

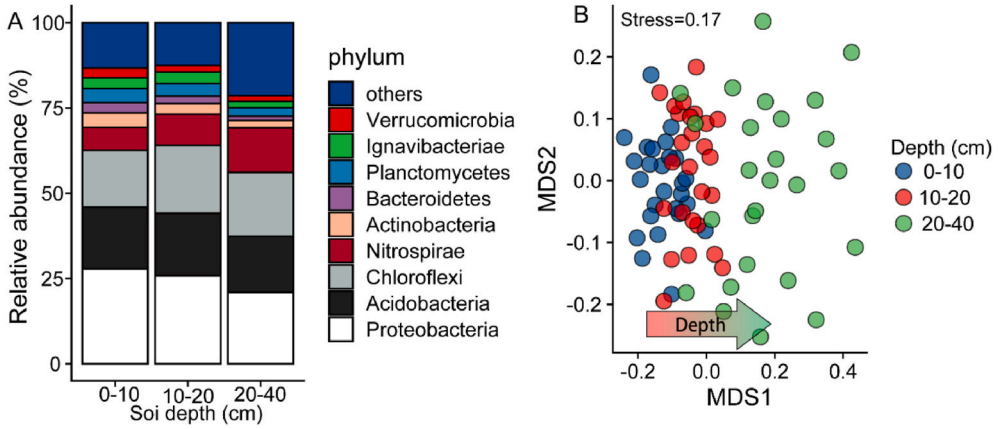


Fig. 3. Relative abundance of phyla present greater than 1% (A) and sample dissimilarity in the bacterial community (B) based on NMDS analysis with soil depth.

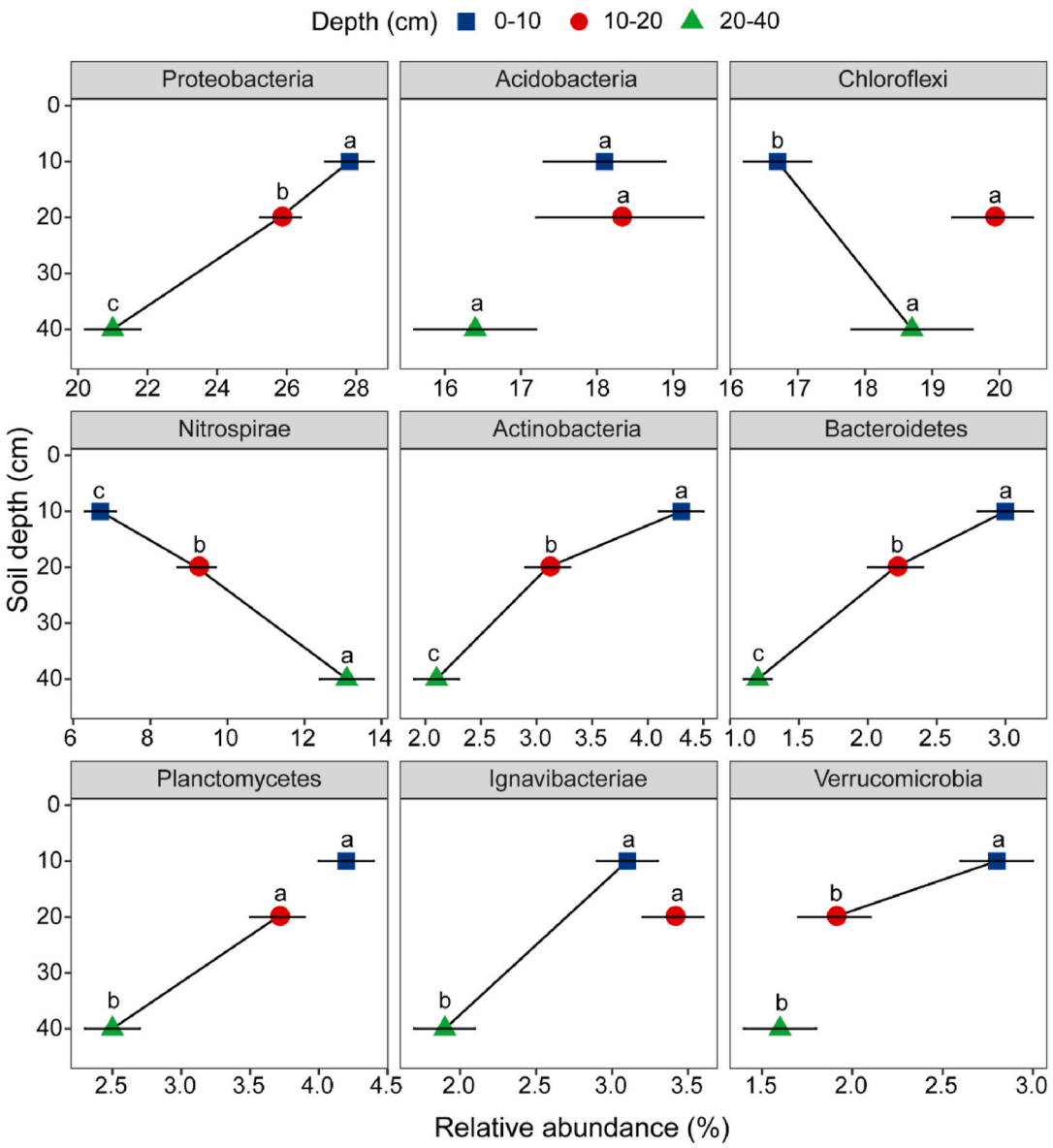


Fig. 4. Dissimilarity in the relative abundance of phyla (means ± SE, N = 26) greater than 1% with soil depth. The blue square, red circle and green triangle in each subplot represent the soil layers 0–10 cm, 10–20 cm and 20–40 cm respectively. Letters indicate significant differences ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4.2. Shifts in taxon-taxon and taxon-environment associations with soil depth

Consistent with the 2nd hypothesis, network analysis showed that the complexity of the bacterial community networks and the intensity of taxa-taxa interactions increased with soil depth. The drastic decrease in nutrients with depth and greater anoxia allow taxa with similar physiological ecotypes to cooccur, resulting in higher taxa-taxa associations in the subsoil to reduce functional redundancy (Kuzyakov et al., 2009). Land use intensification decreased the network density and reduced the average number of neighbours (Creamer et al., 2016). This result supports that paddy cultivation in our study resulted in lower beta diversity and fewer biotic associations in the topsoil than in the subsoil. Moreover, the modularity of the bacterial network was higher in the topsoil (Fig. 5C), which indirectly indicated the resistance of bacteria to environmental filters (flooding, fertilization, tillage, etc.). In microbial ecology, module means a demarcation of microbial groups, with species within the module closely related to each other and less to species outside the module, typically due to differentiation in ecological niches and/or divergent selection (Olesen et al., 2007). Therefore, one module has little or no influence on another module, and the effects of environmental perturbations within one module are unlikely to be transmitted to the other, reducing the impact of environmental perturbations on the microbial community as a whole (Kitano, 2004). Therefore, high modularity of bacterial network in the topsoil is associated with greater environmental perturbations to maintain the robustness of the microbial community composition.

In this study, the taxon-environment network analysis showed that soil texture and pH were more strongly correlated with bacterial taxa in the topsoil (0–20 cm), while soil nutrients were more important for the bacterial community in the subsoil. Based on extensive research, soil texture and pH are important factors determining the geographic distribution patterns of microorganisms in the topsoil (Karimi et al., 2018; Xia et al., 2020). The texture in the nutrient-rich Ap horizon indirectly influences bacterial abundance by affecting the flow of nutrients, water, and air, which are the main factors needed for microbial growth. Consistent with our study, the enriched OTUs in the topsoil were the genus Ohtaekwangia involved in the transformation of plant C, while the

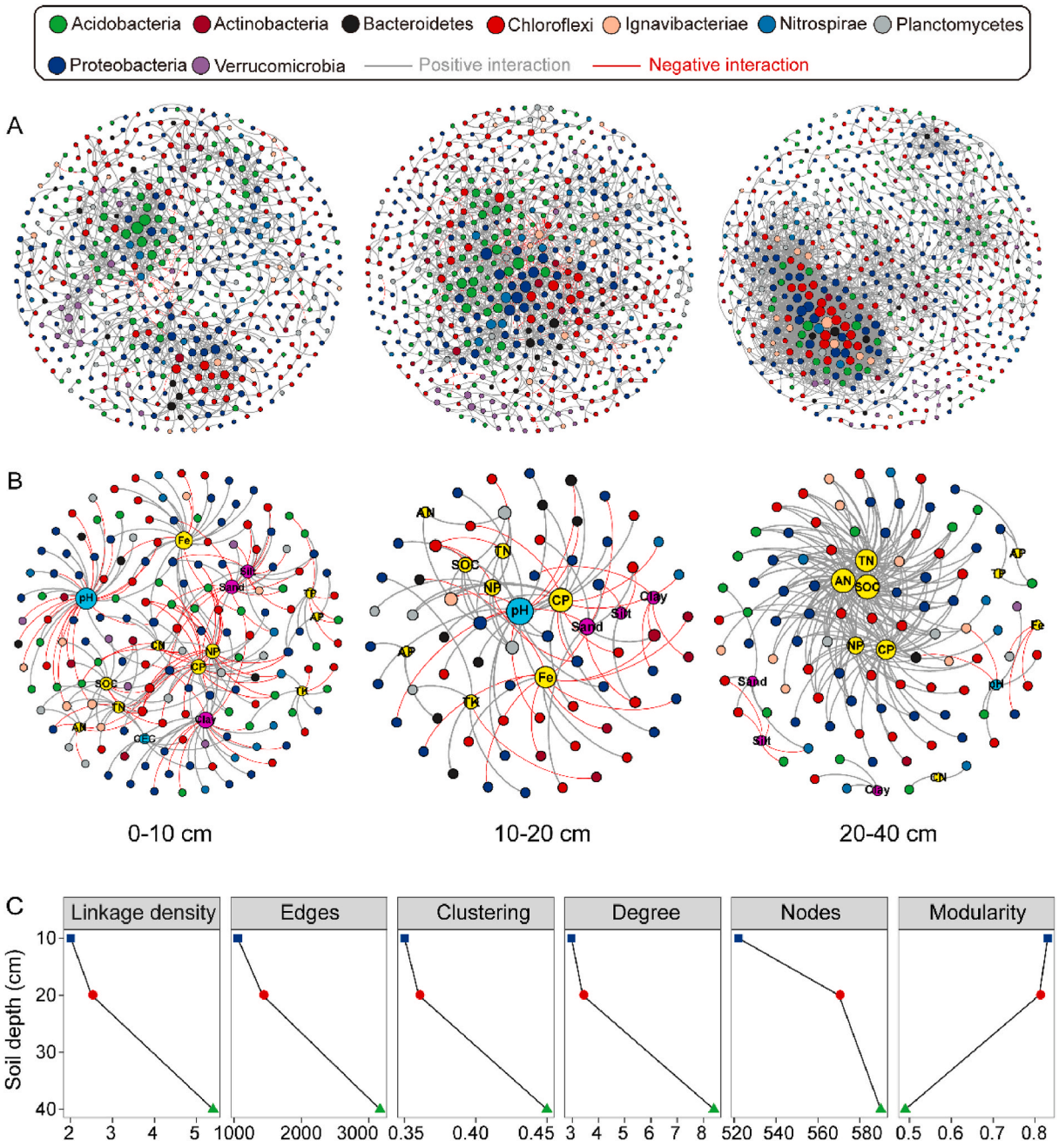


Fig. 5. Taxon-taxon networks (A), taxon-environment networks (B) and statistics for taxon-taxon networks (C) in the three soil layers. In panels A and B, the connection indicates a strong and significant ($p < 0.001$) correlation; the nodes represent unique sequences in the data sets; the size of each node is proportional to the relative abundance. In panel C, the blue square, red circle and green triangle in each subplot represent the soil layers 0–10 cm, 10–20 cm and 20–40 cm respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

enriched OTUs in the subsoil were the Subdivision 3 genera incertae sedis involved in the transformation of N and P due to nutrient limitation (Gu et al., 2017).

4.3. Effects of soil depth on bacterial community assembly processes

In subtropical paddy soil, stochastic processes were predominant in bacterial community assembly up to 40 cm. Inconsistent with our results, higher contributions of deterministic over stochastic processes were common up to 60 cm across three sodicity/salinity gradients (Xu et al., 2021). Under extreme environmental conditions, the process of bacterial community assembly is deterministic, and this process is magnified in proportion (Stegen et al., 2012). In contrast, rice cultivation at the regional level, where climatic conditions and cropping background are relatively uniform (Table S1) (Liu et al., 2021), is not dominated by the influence of human disturbance on bacterial community assembly. Bacterial community assembly at a regional scale was driven by dispersal from regional species pools and local selection depending on pH and other soil properties affected by land use (Barnett et al., 2020).

Inconsistent with the 2nd hypothesis, the bacterial community assembly was more stochastic at 10–20 cm because paddy cultivation mixed this layer with the top 10 cm. The soil in the 10–20 cm depth, however, was isolated from the direct influence of environmental filtering factors such as alternate flooding and drying, and were less affected by diurnal temperature dynamics. Bacteria from the top 10 cm

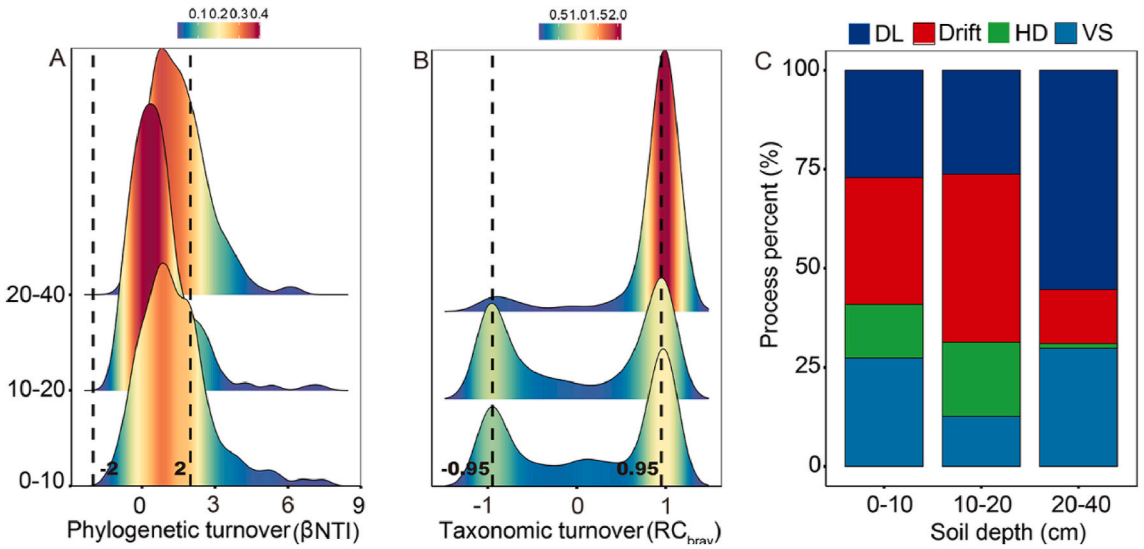


Fig. 6. Distribution of standardized phylogenetic turnover (βNTI; panel A) and taxonomic turnover (RC_{bray}; panel B) and the percentages of the four assembly processes (panel C). The vertical dashed lines mark the positions of −2 and 2 in panel A and −0.95 and 0.95 in panel B. The four assembly processes are VS: variable selection; HD: homogenizing dispersal; DL: dispersal limitation; and Drift.

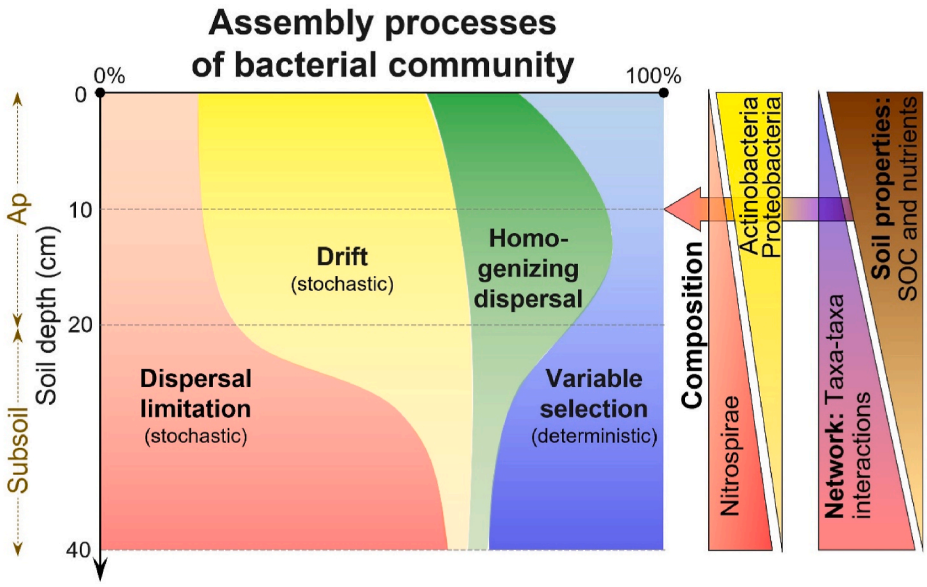


Fig. 7. Concept of the effects of soil depth on bacterial community assembly processes: Dispersal limitation, Drift, Homogenizing dispersal and Variable selection (see Fig. 6C for details). The corresponding dominance of the three main bacterial groups, the interactions and the effects of soil properties with depth are presented at the right.

layer could migrate into the 10–20 cm via soil porosity (Xu et al., 2021), which explained why homogeneous dispersal accounted for a greater proportion of the bacterial community assembly processes in this layer. All of the above factors may contribute to a rather stochastic structure of the bacterial community at the 10–20 cm depth.

Only 5–17% of the variance in phylogenetic turnover of the bacterial community was explained by environmental factors, spatial distance, and interspecies interactions (Fig. 8). This result thus confirmed that the bacterial community assembly is dominated by stochastic processes. Consistent with our study, spatial distance and soil properties together explained 16% of the variance in phylogenetic turnover of the bacterial community within islands (Wang et al., 2020). Numerous studies have shown that the large portion of variation in microbial communities is not truly explained by environmental and distance effects (Zhou et al., 2008; Caruso et al., 2011; Stegen et al., 2012). The main reason is that there are many stochastic processes (ecological drift, dispersal limitation) that influence community assembly.

A set of soil properties has been determined, but the gradient of abiotic factors along soil depth, such as temperature, are important for bacterial community composition. The network approach has been widely used in microbial ecology to calculate the intensity of taxa-taxa associations. Further validation, such as co-culture methods, is necessary to confirm true bacterial interactions in environmental samples (Wang et al., 2017). Finally, as in numerous previous studies (Zhang et al., 2018; Chalmandrier et al., 2019), piecewise structural equation modelling was used to disentangle the contributions of spatial distance and environmental factors to bacterial community assembly (Fig. 8). However, because spatial distance is associated with environmental changes and dispersal limitations, it is difficult to thoroughly disentangle them.

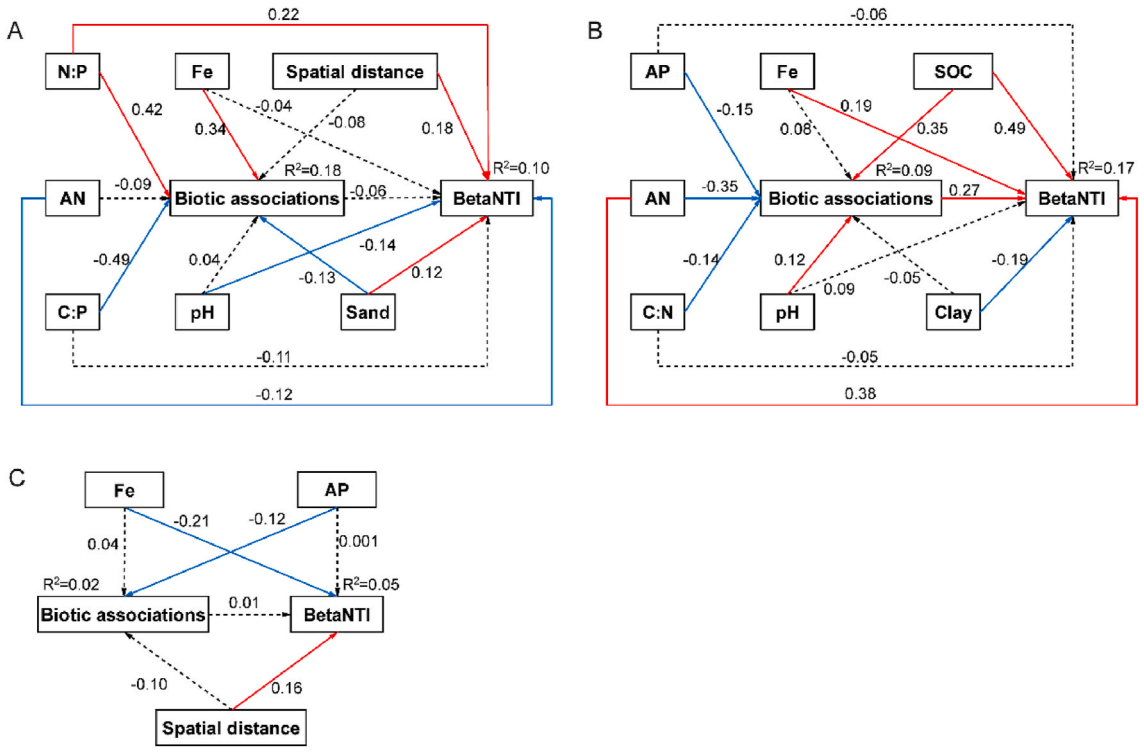


Fig. 8. Piecewise structural equation model (piecewise SEM) shows the direct effects of environmental variables, spatial distance, biotic associations and their indirect effects on phylogenetic turnover within each soil layer. Numbers above the arrows indicate path coefficients. R^2 values represent the proportion of variance explained by each variable. Solid red and solid blue arrows indicate positive and negative relationships, respectively, while dashed black arrows indicate no significant relationship. SOC refers to soil organic C; AN refers to available nitrogen; AP refers to available phosphorus; C:P refers to the ratio between soil organic C and total phosphorus; C:N refers to the ratio between soil organic C and total nitrogen; N:P refers to the ratio between total nitrogen and total phosphorus; sand and clay refer to the content of the sand and clay fractions, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

5. Conclusions

Bacterial community composition and mechanisms of its assembly were evaluated in soils of 26 rice fields in subtropics up to 40 cm depth. Nutrient contents strongly decline from topsoil to subsoil, in particular available phosphorus content (81% decline). The topsoil harboured more of the dominant phyla Proteobacteria and Actinobacteria, but less phylum Nitrospirae. Network complexity and taxa-taxa interactions of the bacterial communities increased with soil depth. Stratified bacterial community characteristics were strongly coupled with soil nutrients (available N and P) and pH. More severe environmental disturbances (flooding, diurnal temperature dynamics etc.) and anthropogenic effects (fertilization, tillage) in the topsoil lead to high network modularity, that is responsible for the robustness of the bacterial community composition. The processes of bacterial community assembly were more random in the topsoil than in the subsoil because of more dispersion. In addition to altered microbial community composition and biotic interactions, paddy cultivation increased the differences of physico-chemical properties between topsoil and subsoil, all of which influenced bacterial community assembly. This is the first study evaluating the networks and assemblages of bacterial communities depending on soil depths at a large geographical scale. The understanding of the mechanisms of bacterial community diversity formation is a prerequisite to recognize microbial functions of C and nutrient cycles in agricultural ecosystems. This study challenges future proves of the basic macro-ecology principles of organism's distribution at the level of micro-ecology in soil.

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.soilbio.2021.108517.

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