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pH-dependent divergence of microbial co-occurrence networks and taxon roles in paddy versus upland soils

Deqiang MAO[1,2,3], Chen CHEN[1,2,3], Qi ZHAO[1,2,3], Yongfu LI[4], Chengqi YAN[5], Xuebin XU[1,2,3], Jianping CHEN[1,2,3], Tida GE[1,2,3], Haoqing ZHANG (✉)[1,2,3,6]

1 State Key Laboratory for Quality and Safety of Agro-Products, Key Laboratory of Biotechnology in Plant Protection of MARA, Zhejiang Key Laboratory of Green Plant Protection, Institute of Plant Virology, Ningbo University, Ningbo 315211, China.
2 School of Marine Sciences, Ningbo University, Ningbo 315211, China.
3 International Science and Technology Cooperation Base for the Regulation of Soil Biological Functions and One Health of Zhejiang Province, Ningbo University, Ningbo 315211, China.
4 State Key Laboratory for Development and Utilization of Forest Food Resources, College of Environmental and Resource Sciences, Zhejiang A & F University, Hangzhou 311300, China.
5 Ningbo Academy of Agricultural Sciences, Ningbo 315040, China.
6 Institute of One Health Science, School of Civil & Environmental Engineering and Geography Science, Ningbo University, Ningbo 315211, China.

KEYWORDS

Abundant taxa, co-occurrence networks, land use, microbial diversity, rare taxa, soil pH

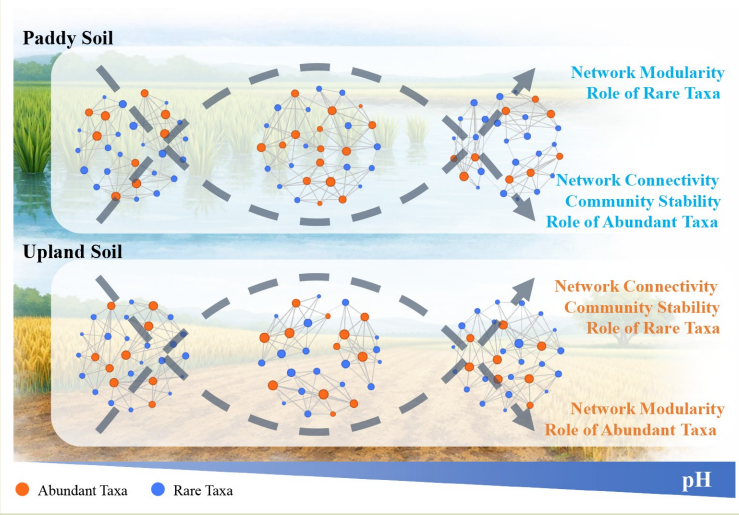
HIGHLIGHTS

- Paddy and upland soils were found to have distinct microbial communities under a shared pH filter.
- Paddy and upland networks had opposite non-linear responses to soil pH.
- Rare taxa had land-use-dependent persistence during pH-driven network reorganization.

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Correspondence: zhanghaoqing@nbu.edu.cn

GRAPHICAL ABSTRACT



ABSTRACT

Soil microbial communities and their co-occurrence networks are central to nutrient cycling and ecosystem functioning in agricultural soils, however, how environmental factors regulate their reorganization at regional scales remains poorly understood. Using a paired paddy-upland sampling design comprising

171 soil samples from Ningbo, Zhejiang, China, this study combined soil physicochemical analyses, amplicon sequencing, random forest modeling and network-based approaches to determine how soil pH shapes microbial community structure, network topology and the relative roles of abundant and rare taxa among different land-use types. The results revealed that soil pH was the primary determinant of microbial community structure in both paddy and upland soils, acting as a major environmental filter shaping microbial community differentiation (Mantel's r = 0.523–0.735 for paddy soil and 0.276–0.668 for upland soil). In addition, soil pH was closely associated with network reorganization, and microbial networks in paddy and upland soils had non-linear and contrasting response patterns to pH gradient, indicating that the effect of pH on co-occurrence patterns was strongly dependent on land use. Rare taxa were found to be potentially important structural components in maintaining network continuity during pH-driven reorganization and their relative importance in bacterial networks increased with pH in paddy soils. Collectively, these findings reveal non-linear and habitat-dependent pH responses of agricultural soil microbiomes, highlight a potential role of rare taxa in maintaining network continuity during pH-associated reorganization, and indicate that soil pH regulation should be tailored to land-use type to help sustain microbial network stability in both paddy and upland soils.

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1 Introduction

Soil microorganisms are central to numerous ecosystem functions, including nutrient cycling, organic matter decomposition, and plant health maintenance, thereby being pivotal in agricultural sustainability and global biogeochemical processes[1,2]. As the engine of soil fertility, microbial communities drive the transformation of carbon, nitrogen, and phosphorus, directly influencing crop productivity and soil C sequestration potential[3,4]. The assembly and functional performance of microbial communities are strongly modulated by a suite of abiotic and biotic factors, including climate, soil physicochemical properties and vegetation types[5,6]. While substantial progress has been made in understanding the influence of soil environmental factors on microbial taxonomic and phylogenetic turnover, the mechanisms by which these factors govern the complex ecological interactions among microbial taxa, remain insufficiently elucidated[7,8]. Of these factors, soil pH has been widely recognized as one of the strongest predictors of soil microbial communities because many microbial groups, especially bacteria, have relatively narrow pH optima[9,10]. In addition to directly affecting microbial growth and enzyme activity, soil pH can indirectly regulate microbial community differentiation by modifying nutrient availability, metal solubility and other core soil chemical properties that influence resource acquisition and competitive balance[11,12].

In agricultural ecosystems, however, the effect of soil pH may not be uniform across land-use types. In paddy soils, the effect of pH is closely coupled with flooding-induced oxygen depletion and redox fluctuations, rather than acting as an isolated chemical filter[13]. Under submerged conditions, pH interacts with Fe reduction, phosphorus mobilization and methane-cycling processes, thereby influencing both microbial assembly and functional differentiation[14]. In addition, the reductive dissolution of Fe-bound phosphorus under low-redox conditions can increase P availability, indicating that pH in paddy soils influences microbial communities partly through redox-sensitive nutrient transformations[15]. In contrast, upland soils are predominantly aerobic, and the effects of pH are more commonly expressed through acidity stress, nutrient availability and shifts in bacterial-fungal competitive balance[11,16,17]. These contrasting mechanisms indicate that pH-driven microbial turnover in agricultural soils need to be interpreted within land-use-specific hydrological and biogeochemical contexts.

Given that soil pH may shape microbial communities through distinct mechanisms in paddy and upland soils, a network-based perspective is needed to understand how these environmental filters translate into community organization. Microbial community structure is not merely an assemblage of independent populations, but rather embedded within intricate networks of cooperative and competitive interactions that collectively determine ecosystem stability and resilience[18,19]. The inference of potential interactions among microorganisms is often operationalized through the construction of ecological co-occurrence networks[20]. In these networks, nodes correspond to taxonomic units, while edges represent statistically significant associations between them[19,20]. Such an approach is particularly useful for evaluating whether similar environmental drivers, such as soil pH, generate distinct patterns of microbial association under contrasting land-use types. Recent meta-analyses have demonstrated that environmental stressors, such as extreme temperatures or pollution, often simplify network topology, reducing connectivity and making communities more vulnerable to collapse[21,22]. Thus, assessing how co-occurrence networks reorganize under shifting environmental conditions is important for predicting ecosystem responses to global change. Understanding the plasticity of network architecture provides insights into the underlying assembly rules that govern microbial community stability beyond simple taxonomic shifts[23].

Beyond the global network topological properties, the relative proportion and functional contributions of abundant versus rare taxa in microbial co-occurrence networks have garnered increasing attention. Abundant taxa, contribute disproportionately to community biomass turnover and realized metabolic activity, and are often characterized by broader niche breadth and greater environmental adaptability, allowing them to dominate resource use and many ongoing biogeochemical processes under relatively stable conditions[24]. In contrast, rare taxa, despite their low individual abundances, form a vast and phylogenetically diverse reservoir that preserves genetic and functional potential within the community; this reservoir can buffer environmental change by providing functionally redundant or even functionally distinct populations that may resuscitate, expand or replace dominant taxa when conditions shift[25,26]. Notably, rare taxa are not necessarily inactive, as some low-abundance soil bacteria have been shown to be metabolically versatile and fast-growing, indicating that rarity can also reflect ecological specialization or context dependence rather than dormancy alone[27,28]. Recent network analyses indicate that abundant and rare taxa can occupy distinct topological positions in microbial co-occurrence networks, with abundant taxa more often associated with highly connected core structures, whereas rare taxa could contribute disproportionately to network connectivity and inter-module linkage[29,30]. However, understanding the roles of abundant and rare taxa in maintaining the stability of ecological networks and the responses of their roles across environmental gradients remains a key challenge[31].

Despite growing recognition that network architecture is associated with environmental heterogeneity, evidence linking soil environmental conditions to network topology and the ecological roles of abundant and rare taxa in different land-use types remains fragmented. Therefore, an integrated assessment of microbial community composition, network topology and the roles of abundant and rare taxa across contrasting land-use types is needed to clarify how soil environmental variation structures agricultural soil microbiomes. Accordingly, soil pH was hypothesized to act as a major but land-use-dependent driver of microbial community structure and network organization, with abundant and rare taxa exhibiting distinct topological responses along pH gradients in paddy and upland soils. For this study, we investigated soil bacterial and fungal communities, as well as soil chemical and physical properties, in 171 soil samples collected from 86 paddies and 85 upland rice fields in Ningbo, Zhejiang Province, China. This study aimed to: (1) identify the key environmental drivers shaping microbial communities in paddy and upland soils; (2) elucidate how microbial network topology shifts along environmental gradients; (3) disentangle the linkages between soil environmental conditions and the ecological roles of abundant versus rare taxa. Our study offers new insights into the environmental filtering of microbial interactions in different land-use types, with implications for predicting ecosystem functioning under changing soil conditions.

2 Materials and methods

2.1 Study area

The study was conducted across the agricultural landscapes of Ningbo City, Zhejiang Province, situated in the southern Yangtze River Delta (Fig. 1(a), 120°55′ –122°16′ E, 28°51′ –

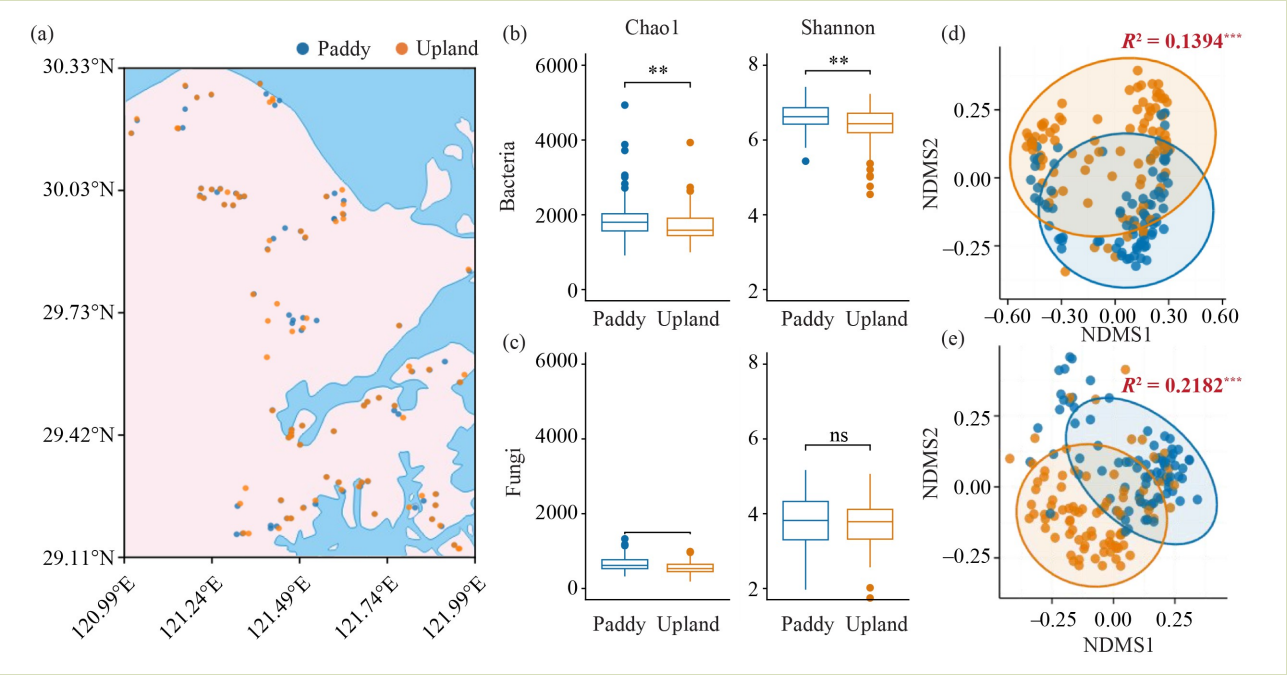


Fig. 1 Sampling information and microbial diversity of paddy and upland soils (审图号：GS 京（2026）2041 号). (a) Geographic distribution of paired paddy and upland soil sampling sites in Ningbo, China. (b,c) Comparisons of bacterial and fungal α-diversity between paddy and upland soils, based on the Chao1 and Shannon indices. $^{**}p < 0.01$ (Wilcoxon rank-sum test). (d,e) β-Diversity patterns of bacterial and fungal communities in paddy and upland soils based on NMDS ordination, with group differences further tested by analysis of similarities (ANOSIM). $^{***}p < 0.001$.

30°33′ N). This region represents a typical subtropical monsoon zone, characterized by a mean annual temperature of 16.4 °C and a mean annual precipitation of about 1480 mm. According to the Chinese Soil Taxonomy system, the dominant soil orders include stagnic Anthrosols, Ferralsols, semi-hydromorphic soils and saline-alkali soils. The agricultural landscape is dominated by two distinct cropping systems: waterlogged paddies and non-waterlogged upland fields, which serve as the primary focus of this investigation.

2.2 Soil sampling and physicochemical analysis

To ensure robust statistical power and minimize confounding environmental variables, soil sampling was executed in November 2022 during the post-harvest fallow period. A paired sampling design was implemented wherever feasible to control for spatial heterogeneity. Specifically, paddies were matched with adjacent upland fields within close geographic proximity to ensure comparable parent material, climate, and broader regional farming context. All paired sites were located within the same agricultural landscape of Ningbo and were sampled during the same post-harvest period, thereby reducing potential variation associated with seasonal crop stage and short-term management effects. At each of the 171 independent sampling locations, a composite sampling approach was adopted: six subsamples were collected within an 80-m radius using a sterile auger and thoroughly homogenized to form a single representative sample. This approach mitigates micro-scale spatial variability. Concurrently, undisturbed soil cores were retrieved using stainless steel cutting rings (100 cm^3) for the determination of bulk density (BD). The final dataset consisted of 171 topsoil samples (0–20 cm depth), partitioned into 86 paddy soil samples and 85 upland samples (Fig. 1(a)). Upon collection, visible plant debris and stones were removed, and samples were sealed in sterile bags and transported to the laboratory for immediate processing and analysis.

A comprehensive suite of 17 physicochemical indicators was quantified to characterize the edaphic properties of the

sampled soils and all variables were subsequently included in Mantel's tests to identify the main environmental drivers of microbial community structure (Dataset S1). These parameters included BD, electrical conductivity (EC), pH, soil organic C (SOC), total N (TN), total K (TK), ammonium N (NH_4^+-N), nitrate N (NO_3^--N), available K (AK), available P (AP), dissolved organic C, dissolved organic N (DON), available Fe (AFe), available manganese, available Zn (AZn), available Cu (ACu), and available Mg (AMg). Detailed measurement protocols and instrument information for all soil physicochemical properties are provided in Method S1.

2.3 DNA extraction and amplicon sequencing

Soil microbial community composition was characterized using high-throughput amplicon sequencing. Total genomic DNA was extracted from about 0.5 g of frozen soil (–80 °C) using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. The concentration and purity of extracted DNA were evaluated using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), and DNA integrity was verified by 1% agarose gel electrophoresis. All DNA extracts were stored at –80 °C before downstream molecular analyses.

Bacterial and fungal communities were profiled by amplifying the V4 region of the bacterial 16S rRNA gene and the fungal internal transcribed spacer (ITS1) region, respectively, using the primer pairs 515F/806R for bacteria and ITS5F/ITS1R for fungi[32,33]. After PCR amplification and purification, paired-end sequencing was conducted on an Illumina MiSeq platform (Illumina, San Diego, CA, USA). Raw amplicon sequence data were initially processed in QIIME 2 (version 2020.8.0) using the DADA2 plugin to perform quality filtering, denoising, chimera removal and paired-end read merging, resulting in feature tables composed of amplicon sequence variants (ASVs)[34,35]. After denoising and filtering, the dataset comprised 98,617 ASVs, whereas the fungal dataset comprised 40,050 ASVs prior to downstream normalization.

2.4 Bioinformatic analysis

To account for differences in sequencing depth among samples and to ensure comparability across datasets, rarefaction was subsequently applied using the *rrarefy* function implemented in the R (version 4.5.2) package "vegan" (version 2.7.2) package[36]. Rarefaction was conducted based on the minimum total sequencing depth observed across all samples, corresponding to 34,956 reads for bacterial communities and 33,994 reads for fungal communities, at which most samples approached saturation in the rarefaction curves (Fig. S1). Following rarefaction, the standardized bacterial ASV table retained 90,838 ASVs, whereas the fungal ASV table contained 36,024 ASVs, which were used for all downstream microbial community analyses.

Downstream microbial community analyses were conducted using the R package "microeco" (version 1.16.0)[37]. Relative abundances at multiple taxonomic levels were calculated using the *cal_abund* function. α-Diversity indices, including Shannon and Chao1 indices, were computed for each sample using the *cal_alpha* function and were subsequently used for group comparisons and model fitting. β-Diversity was assessed using the *cal_beta* function, which generated Bray-Curtis distance matrices to characterize compositional dissimilarities among samples. To evaluate the predictive capacity of microbial community composition for soil pH, random forest (RF) regression models were developed using Python (version 3.12.0) and the "scikit-learn" library (version 1.6.1)[38], and detailed information on the software environment, model training, and parameter settings is provided in Method S2. Functional Annotation of Prokaryotic Taxa (FAPROTAX, version 1.21) was used to predict the putative functions of rare and abundant bacterial taxa across the pH-defined networks in paddy and upland soils[39].

2.5 Microbial network construction and analysis

Microbial co-occurrence networks were constructed separately for bacterial and fungal communities within paddy and upland soils. To reduce matrix sparsity and limit spurious correlations caused by extremely infrequent ASVs, we retained taxa present in at least 50% of samples for network construction, thereby focusing the analysis on recurrent taxa that were more suitable for robust co-occurrence inference. Spearman correlation matrices were then calculated using the *rcorr* function in the R package "Hmisc" (version 5.2-5) based on all samples within each soil type, generating two global networks (paddy and upland). Subsequently, these global networks were partitioned into sample-level subnetworks according to the presence-absence patterns of ASVs in individual samples, such that each subnetwork retained only the nodes present in a given sample and the corresponding connections among those nodes in the global network[40].

Network quality control and refinement were performed using the analytical framework implemented in "microeco"[37]. Correlations with p values > 0.05 were removed to ensure statistical robustness. In addition, correlation thresholds were determined based on random matrix theory to identify appropriate similarity cutoffs and further eliminate weak or spurious associations[41]. The resulting adjacency matrices were used for downstream topological analyses.

Network topological properties were quantified using the workflow provided in the R package "meconetcomp" (version 0.7.0)[42]. Specifically, average degree was calculated to represent the mean number of connections per node, reflecting overall network connectivity[43]. The clustering coefficient was computed to describe the tendency of taxa to form tightly connected clusters, indicating local cohesiveness[44]. Network centralization was used to assess the extent to which the network structure is organized around highly connected hub nodes[45,46]. Modularity was calculated to evaluate the strength of network compartmentalization into modules, which reflects niche differentiation and functional grouping[47]. Network robustness was evaluated by simulating random edge removal, with 30% of edges randomly deleted from each network to mimic disturbance[48]. The resulting change in overall network connectivity was quantified to assess the resistance of the network structure to interaction loss, reflecting structural stability and resilience under perturbation.

To investigate the turnover of microbial networks across pH gradients, samples within each soil type were stratified into six pH groups, and the corresponding pH-defined networks were generated by combining the sample-level subnetworks of all samples within the same pH interval. This procedure preserved the same interaction framework used for the sample-level analyses while allowing comparison of node turnover and the representation of abundant and rare taxa across pH gradients. For paddy soils, the six pH-defined networks were: Net1 (pH 5.0–5.3, n = 16), Net2 (pH 5.3–5.6, n = 17), Net3 (pH 5.6–6.0, n = 17), Net4 (pH 6.0–7.0, n = 13), Net5 (pH 7.0–7.6, n = 10), and Net6 (pH 7.6–8.2, n = 13). For upland soils, the corresponding pH groups were: Net1 (pH 4.4–5.0, n = 13), Net2 (pH 5.0–5.3, n = 15), Net3 (pH 5.3–6.0, n = 19), Net4 (pH 6.0–7.0, n = 11), Net5 (pH 7.0–7.6, n = 10), and Net6 (pH 7.6–8.6, n = 17). Within each network, taxa were classified based on relative abundance into abundant taxa (> 0.1%) and rare taxa (< 0.01%). Their proportions within networks and their contributions to overlapping nodes among networks were quantified to evaluate their structural representation.

To compare the relative contribution of taxa to network maintenance across networks of different sizes and connectivity, a standardized index of relative degree was introduced. The relative degree of each node was calculated as its degree divided by the average degree of the corresponding network, allowing comparability among networks with different structural properties[24]. The relative importance of abundant and rare taxa in maintaining network complexity was then assessed by comparing their relative degrees. Changes in their structural roles along increasing pH gradients were examined using linear regression analyses between relative degree and soil pH. In addition, the differential contribution between abundant and rare taxa was quantified as the difference in relative degree (i.e., relative degree of abundant taxa minus that of rare taxa), revealing shifts in their relative structural dominance across environmental gradients.

2.6 Statistical analysis

Differences in α-diversity between paddy and upland soils were evaluated using the Wilcoxon rank-sum test. β-Diversity patterns based on Bray-Curtis dissimilarity were statistically assessed using analysis of similarities (ANOSIM) to determine compositional differences between paddy and upland soils. To identify environmental drivers of microbial community structure, Mantel's tests were conducted between Bray-Curtis distance matrices and soil physicochemical properties using the workflow implemented in the R package "linkET" (version 0.1.0)[49]. Mantel's R statistics were ranked to determine the relative contribution of environmental factors and to identify the primary drivers of microbial community variation.

All linear regression analyses presented in this study were performed using the *lm* function in the base R package "stats" (version 4.5.0). For relationships between network topological properties and soil pH, visual inspection indicated non-linear, threshold-like patterns; therefore, second-order polynomial regression models were fitted using the *poly* function ("stats" package) to capture potential parabolic trends along the pH gradient.

3 Results

3.1 Divergent patterns of microbial diversity and community structure between paddy and upland soils

Bacterial α-diversity, assessed by Chao1 and Shannon indices, was significantly higher in paddy soils compared to upland soils (Wilcoxon rank-sum test, $p < 0.01$; Fig. 1(b)). For fungal communities, Chao1 richness was also significantly higher in paddy soils (Wilcoxon rank-sum test, $p < 0.01$; Fig. 1(c)). However, no significant difference was observed in fungal Shannon diversity between the two land-use types (Fig. 1(c)). NMDS ordination revealed significant differentiation in bacterial community structure between paddy and upland soils (ANOSIM, $R^2 = 0.139$, $p < 0.001$; Fig. 1(d)). Fungal communities had even stronger structural divergence than bacteria (ANOSIM, $R^2 = 0.218$, $p < 0.001$; Fig. 1(e)).

3.2 Soil pH as the primary determinant of microbial community structure in both paddy and upland soils

To identify the key environmental drivers structuring microbial communities, Mantel's tests were performed (Fig. 2). In paddy soils, the results revealed that both bacterial and fungal communities were significantly influenced by a shared set of soil properties, including pH, EC, BD, AFe, AK, TN and SOC (Fig. 2(a,b)). Beyond these common drivers, the bacterial community had additional significant correlations with AMg and TK, whereas the fungal community was uniquely shaped by DON, NO_3^--N and AZn. In upland soils, pH, EC, BD, AFe, TN, SOC and ACu consistently affected both microbial groups (Fig. 2(c,d)). However, bacterial communities in upland soils were distinctly associated with AK and AP, while fungal communities were a had greater association with AZn. Notably, soil pH has the strongest association with microbial community structure in all four groups, with Mantel's r values of 0.735 for paddy bacteria, 0.523 for paddy fungi, 0.668 for upland bacteria, and 0.276 for upland fungi. Collectively,

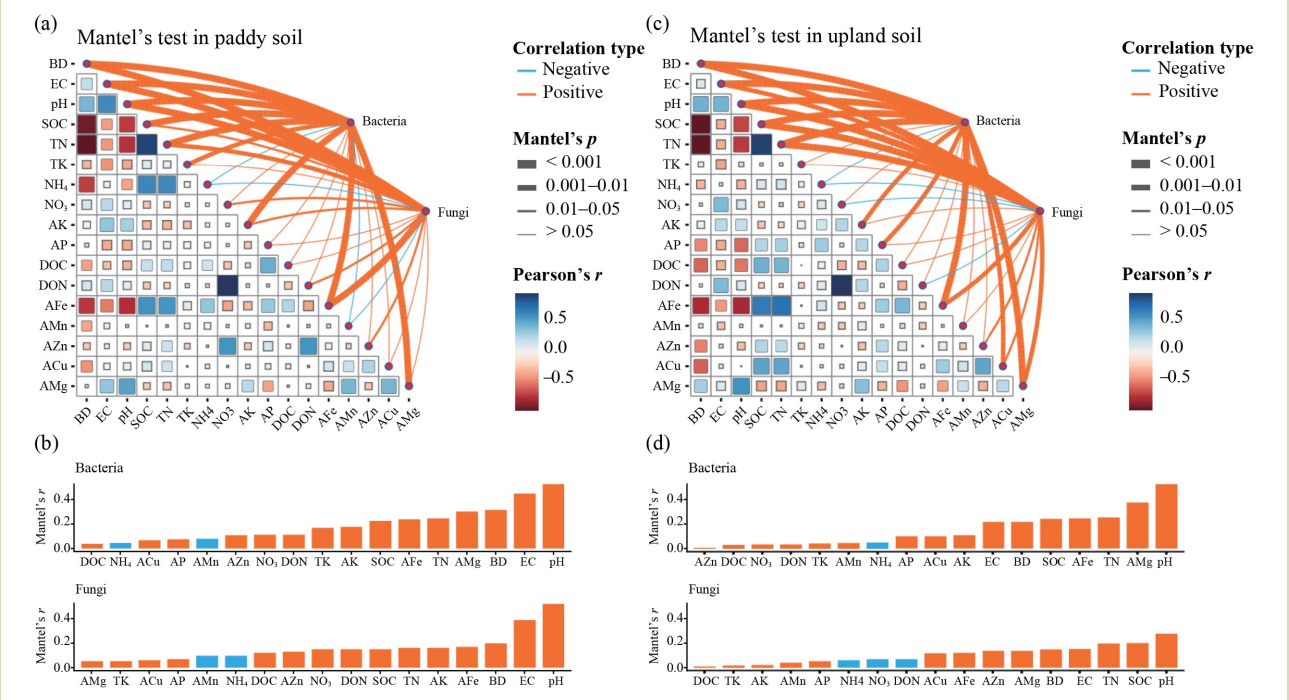


Fig. 2 Identification of the major physicochemical factors driving microbial community variation in paddy and upland soils based on Mantel's tests. (a,c) Overview of the Mantel's test results for paddy and upland soils, including the correlation heatmap showing the results of Mantel's test and associations among soil physicochemical properties for paddy and upland soils, respectively. Color indicates the direction of the correlation, and line width indicates the significance level of the Mantel's association. (b,d) Bar plots showing Mantel's r values for the relationships between individual soil physicochemical factors and bacterial/fungal communities in paddy and upland soils, respectively.

among all measured properties, soil pH was the most influential predictor across both land-use types and microbial kingdoms.

3.3 Diversity and taxonomic turnover of microbial communities along soil pH gradients

Regression analysis revealed that the Shannon index of bacterial community was positively correlated with soil pH in both paddy and upland soils (Fig. S2), and the compositions of the microbial communities fluctuated dramatically with the changing soil pH (Fig. S3). To identify microbial biomarkers most sensitive to pH fluctuations, we used RF regression models. The optimal model configurations, corresponding cross-validation errors, and test-set performance metrics for each microbial group and land-use type are summarized in Method S2 and Table S1. To improve model parsimony, we further evaluated nested feature subsets and selected the optimal subset of genera based on the minimum cross-validation error, which served as the basis for defining pH-sensitive biomarker taxa (Fig. S4 and Tables S2–S5). These pH-sensitive discriminatory taxa belonged to 11 bacterial phyla (Acidobacteriota, Bdellovibrionota, Entotheonellaeota, Proteobacteria, Actinobacteriota, Chloroflexi, Gemmatimonadota, Verrucomicrobiota, Bacteroidota, Desulfobacterota and Planctomycetota) and three fungal phyla (Ascomycota, Basidiomycota and Mortierellomycota), and were designated as pH biomarker taxa (Fig. 3; Tables S2–S5).

We further classified these biomarkers based on their response trajectories along the pH gradient. Of the identified bacterial biomarkers in paddy soils, three genera (including *Aquisphaera*, *Conexibacter* and *Acidothermus*) were classified as low-pH associated taxa whose relative abundance decreased with increasing pH. Thirteen genera [including *Lysobacter*, *Desulfuromonadaceae*, *Desulfuromonadia*, *Geothermobacter*, MA-28-I98C (Class: Desulfuromonadia), *Hirschia*, BD2-11_terrestrial_group (Phyla: Gemmatimonadota), OM27_clade (Family: Bdellovibrionaceae), *Chryseolinea*, vadinHA49 (Phyla: Planctomycetota), A4b (Class: Anaerolineae), *Propionivibrio* and *Nordella*] were classified as high-pH associated taxa, whose relative abundance increased with increasing pH (Fig. 3(a) and Table S2). This indicates that the majority of bacterial biomarkers in paddy soils thrive under higher pH conditions, with only a minority doing so under acidic conditions. In upland soils, bacterial biomarkers comprised eight low-pH associated taxa [including HSB_OF53-F07 (Family: Ktedonobacteraceae), FCPS473 (Family: Ktedonobacteraceae), Ellin516 (Family: Pedosphaeraceae), *Candidatus Koribacter*, CWT_CU03-E12 (Order: Sphingobacteriales), subgroup_13 (Class: Acidobacteriae), *Rhodanobacter* and *Burkholderia-Caballeronia-Paraburkholderia*] and nine high-pH associated taxa [including *Azospira*, TRA3-20 (Order: Burkholderiales), *Skermanella*, SH-PL14 (Family: Rubinisphaeraceae), *Vicinamibacteraceae*, *Entotheonellaceae*, *Agromyces*, Pir4_lineage (Family: Pirellulaceae) and *Azoarcus*] (Fig. 3(b); Table S3). This indicates a relatively balanced sensitivity to pH extremes, with no strong dominance of either acidophilic or alkaliphilic taxa. For paddy fungal biomarkers, five were low-pH associated (including *Saitozyma*, *Phialocephala*, *Conlarium*, *Neurospora* and *Psilocybe*), seven were high-pH associated (including *Nigrospora*, *Chaetomella*, *Edenia*, *Acremonium*, *Hydropisphaera*, *Gaeumannomyces* and *Cirrenalia*), and seven were complex colonizer (including *Conioscypha*, *Echria*, *Mortierella*, *Tausonia*, *Pseudeurotium*, *Cyberlindnera* and *Cercophora*) (Fig. 3(c); Table S4). Also in upland soils, four were low-pH associated (including *Saitozyma*, *Solicoccozyma*, *Conioscypha* and *Conlarium*), three were high-pH associated (including *Cladorrhinum*, *Acremonium* and *Ramophialophora*) and two had complex responses (Fig. 3(d); Table S5). This indicates a more balanced distribution of pH preferences among fungal taxa in both paddy and upland soils, with a notable proportion exhibiting complex, non-linear responses to pH gradients.

3.4 Contrasting responses of microbial co-occurrence network topology to soil pH in paddy and upland soils

We then examined the relationships between soil pH and microbial co-occurrence network topology. By comparing the fits of linear and quadratic models, we found clear threshold-like responses of network topological properties to soil pH, with the quadratic model providing a better fit (linear model: R^2 = 0.000–0.543, quadratic model: R^2 = 0.052–0.702) (Fig. 4; Fig. S5). In paddy soils, all examined topological properties of bacterial networks were significantly associated with soil pH (R^2 = 0.213–0.702, all $p < 0.001$; Fig. 4(a–e)). Average degree, clustering coefficient, centralization, and robustness all had unimodal relationships with soil pH peaking at pH 5.94, 6.37, 5.62 and 6.10, respectively (Fig. 4(a–c,e)). In contrast, modularity had an inverted unimodal relationship with a minimum at pH 6.15 (Fig. 4(d)). For fungal networks in paddy soils, average degree, clustering coefficient and robustness generally declined with increasing soil pH (R^2 = 0.277–0.450, all $p < 0.01$; Fig. 4(f,g,j)), while modularity was not significantly

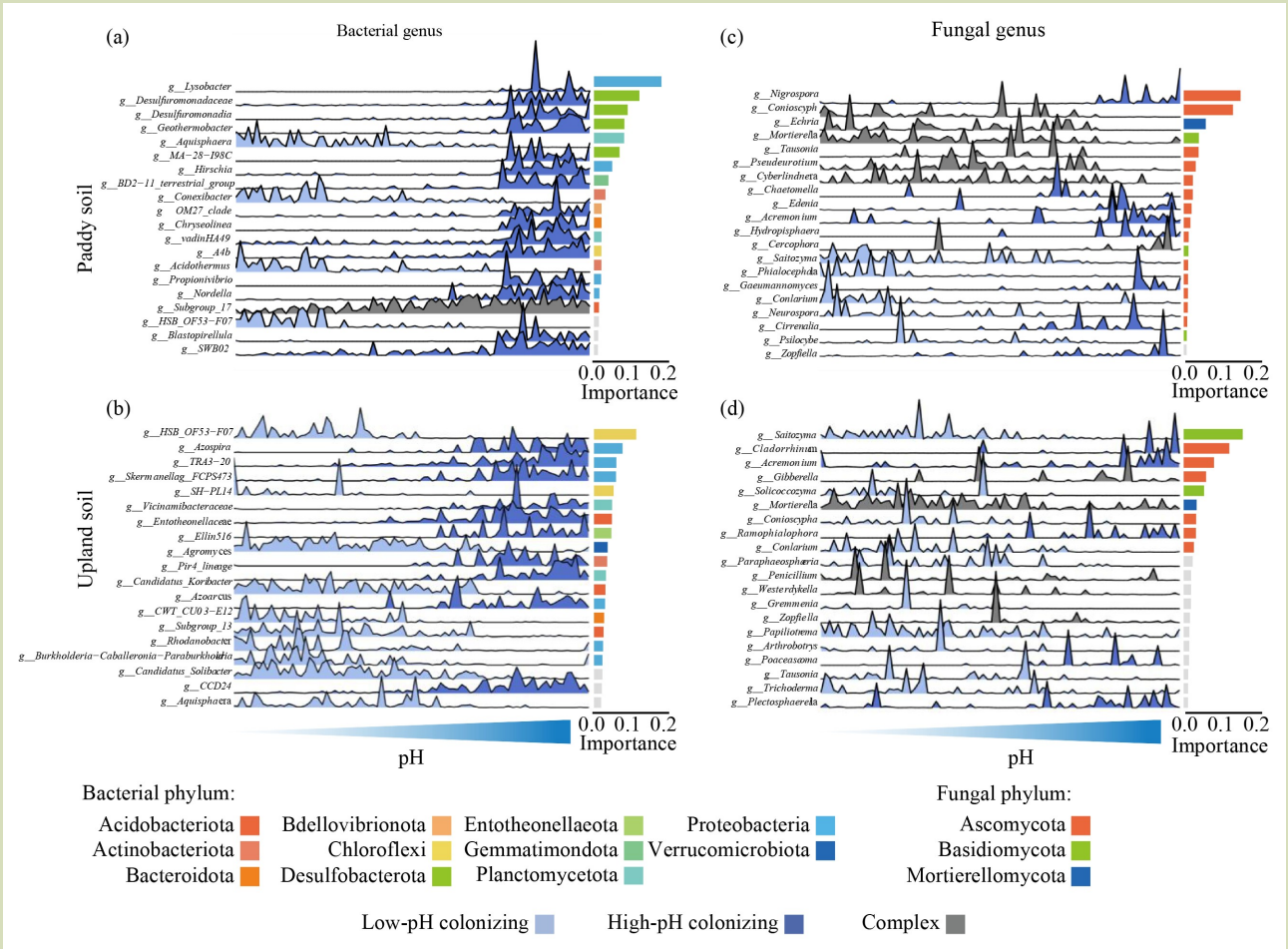


Fig. 3 Top 20 genera associated with soil pH identified by random forest analysis. (a) Paddy soil bacteria, (b) upland soil bacteria, (c) paddy soil fungi, (d) upland soil fungi. In each panel, ridge plots show genera abundance distributions along the pH gradient, with genera classified as high-pH colonizing (dark blue), low-pH colonizing (light blue) or complex-response taxa (gray). Bar plots show the importance values of the corresponding genera in the random forest models. Bar colors represent phylum-level taxonomy, but only cross-validated biomarkers are color-coded, whereas non-biomarker genera are shown in gray. The numbers of biomarkers retained were 17, 17, 19 and 9 for paddy bacteria, upland bacteria, paddy fungi and upland fungi, respectively.

related to pH (R^2 = 0.052, p > 0.05; Fig. 4(i)). These results indicate that bacterial networks in paddy soils were most connected and robust at intermediate pH (about 6), whereas fungal networks generally had higher connectivity under more acidic conditions. In contrast, bacterial modularity was lowest at intermediate pH and increased toward both acidic and alkaline conditions.

In upland soils, the overall pH dependence of microbial network topology differed from that observed in paddy soils. All examined topological properties of bacterial networks were significantly associated with soil pH (R^2 = 0.238–0.580, all p < 0.01; Fig. 4(k–o)). Average degree, clustering coefficient, centralization and robustness all had inverted unimodal relationships with soil pH, with minima at pH 6.41, 5.49, 5.91, and 6.37, respectively (Fig. 4(k–m,o)). In contrast, modularity had a unimodal relationship peaking at pH 5.96 (Fig. 4(n)). For fungal networks in upland soils, average degree, clustering coefficient, centralization, and robustness generally increased as pH increased (R^2 = 0.123–0.441, all p < 0.05; Fig. 4(p–r,t)). In contrast, modularity declined significantly with increasing pH (R^2 = 0.318, p < 0.001; Fig. 4(s)). These results indicate that

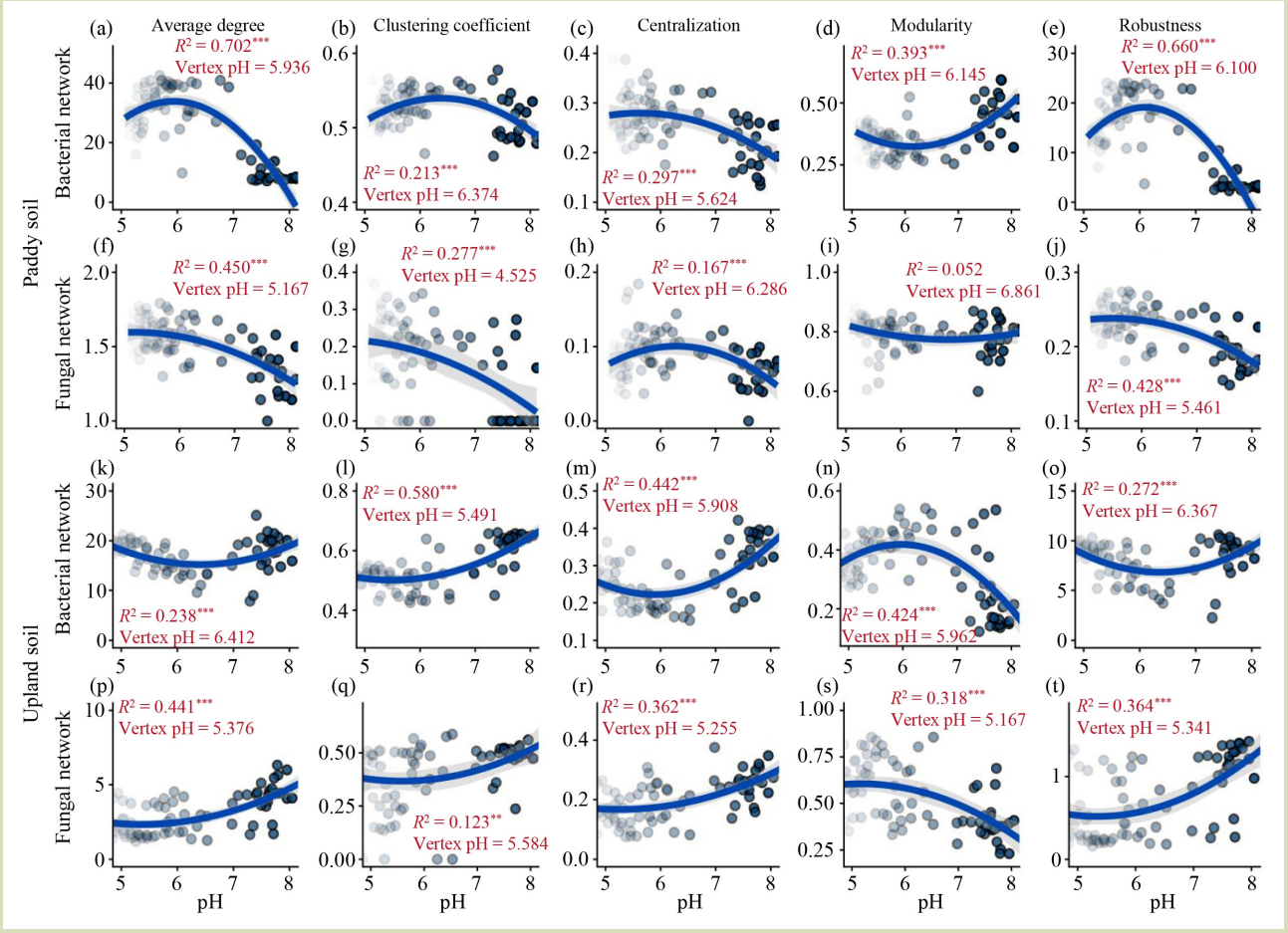


Fig. 4 Regression analysis between soil pH and topological properties of microbial co-occurrence networks in paddy and upland soils. (a–e) Paddy bacterial networks; (f–j) paddy fungal networks; (k–o) upland bacterial networks; and (p–t) upland fungal networks. The x-axis represents soil pH, and the y-axis represents network topological properties, including average degree, clustering coefficient, centralization, modularity, and robustness. Scatter points denote observed values, and curves show the fitted regression relationships. R^2 indicates the coefficient of determination. $^{**}p < 0.01$ and $^{***}p < 0.001$.

connectivity-related properties and robustness of bacterial networks in upland soils were lowest at intermediate pH (about 6), whereas those of fungal networks generally increased with pH. In contrast, bacterial modularity was highest at intermediate pH (about 6), whereas fungal modularity was higher under more acidic conditions.

3.5 Soil-pH-dependent turnover of microbial network nodes and the differential contributions of abundant and rare taxa

To further assess how microbial network composition varied along the soil pH gradient, we divided samples into six pH-defined groups and reconstructed bacterial and fungal co-occurrence networks separately for paddy and upland soils (Fig. 5(a–d)). It was found that the composition of microbial networks experienced clear pH-associated turnover, although the extent of turnover differed markedly between bacterial and fungal networks. In both soil types, bacterial networks had relatively strong continuity in node composition across adjacent pH-defined networks, with a major fraction of nodes retained from one network to the next (Fig. 5(a,b)). In contrast, fungal networks had more apparent node turnover, especially in paddy soils, where node replacement among successive pH-defined networks was more pronounced (Fig. 5(c,d)).

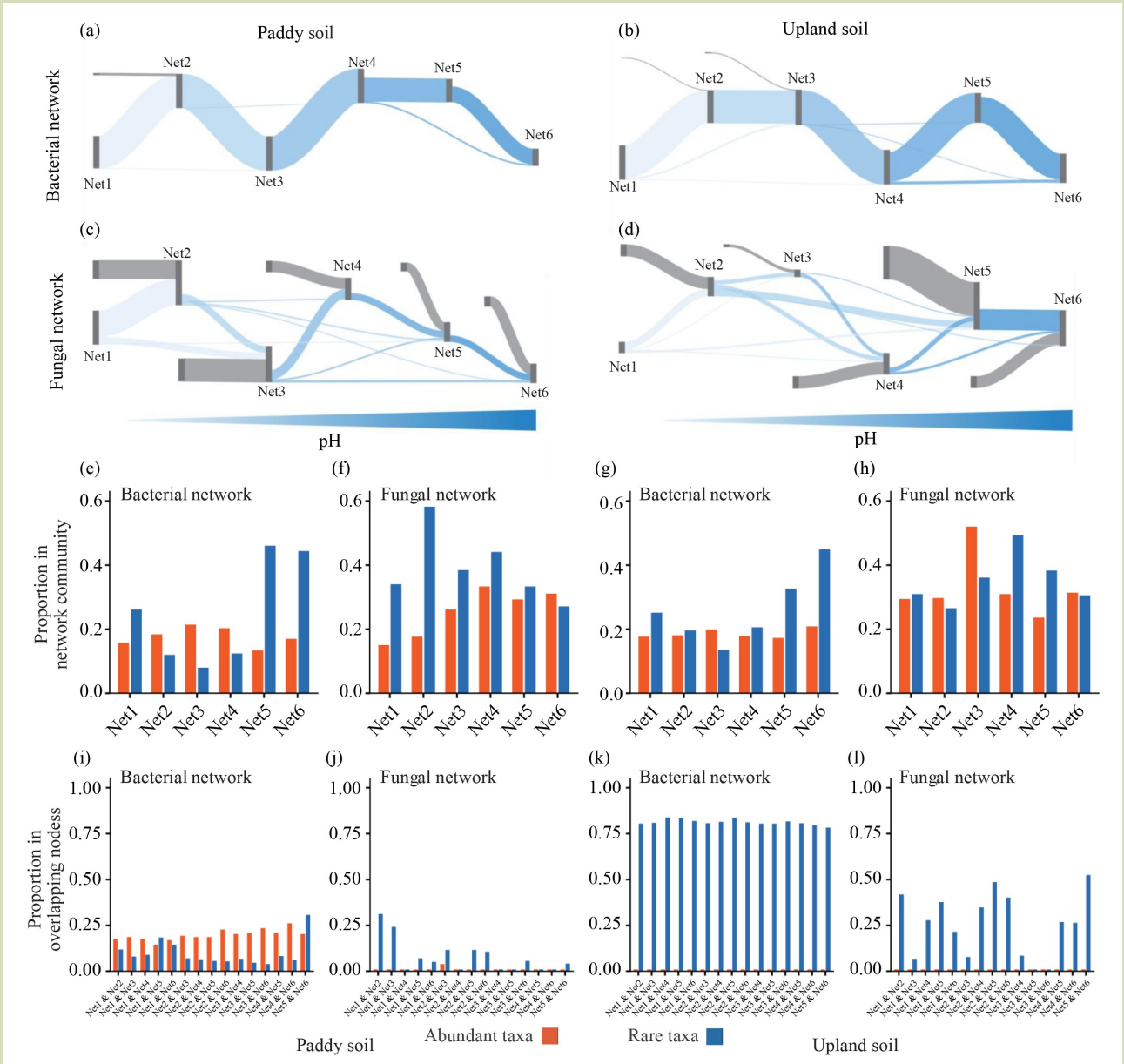


Fig. 5 Contributions of abundant and rare taxa to microbial networks across pH gradient. (a–d) The turnover of nodes in microbial networks in paddy and upland soils. For paddy soils, the six pH-defined networks were: Net1 (pH 5.0–5.3, *n* = 16), Net2 (pH 5.3–5.6, *n* = 17), Net3 (pH 5.6–6.0, *n* = 17), Net4 (pH 6.0–7.0, *n* = 13), Net5 (pH 7.0–7.6, *n* = 10), and Net6 (pH 7.6–8.2, *n* = 13). For upland soils, the corresponding pH groups were: Net1 (pH 4.4–5.0, *n* = 13), Net2 (pH 5.0–5.3, *n* = 15), Net3 (pH 5.3–6.0, *n* = 19), Net4 (pH 6.0–7.0, *n* = 11), Net5 (pH 7.0–7.6, *n* = 10), and Net6 (pH 7.6–8.6, *n* = 17). (e–h) The proportion of abundant and rare taxa in each network. (i–l) The proportion of abundant and rare taxa overlapped between each two networks.

The contributions of abundant and rare taxa to network composition also varied across the pH-defined networks (Fig. 5(e–h)). In paddy soils, bacterial networks had a clear redistribution of abundant- and rare-associated nodes along the pH gradient. Abundant taxa accounted for a larger proportion of nodes in Net2-4 (pH 5.3–7.0), whereas rare taxa

became more prominent in Net1 (pH 5.0–5.3) and in the high-pH networks, particularly Net5 and Net6 (pH 7.0–8.2; Fig. 5(e)). In paddy fungal networks, rare taxa represented a larger proportion of nodes than abundant taxa across most pH-defined networks, especially in Net1-4 (pH 5.0–7.0), whereas the difference narrowed in Net5 and Net6 (pH 7.0–8.2; Fig. 5(f)). In upland bacterial networks, abundant taxa had slightly more nodes in Net3 (pH 5.3–6.0), while rare taxa accounted for a greater proportion in Net1 (pH 4.4–5.0) and Net4-6 (pH 6.0–8.6; Fig. 5(g)). In upland fungal networks, the relative contributions of abundant and rare taxa were more variable. Abundant taxa contributed markedly more in Net3 (pH 5.3–6.0), rare taxa dominated in Net4 and Net5 (pH 6.0–7.6), and the two groups were nearly equal in Net1 (pH 4.4–5.0) and Net6 (pH 7.6–8.6; Fig. 5(h)).

Pairwise overlap analysis further revealed contrasting patterns in the persistence of abundant and rare taxa across pH-defined networks (Fig. 5(i–l)). In paddy bacterial networks, overlap proportions were generally modest, with abundant taxa usually showing slightly higher overlap than rare taxa across most pairwise comparisons (Fig. 5(i)). In paddy fungal networks, overall overlap was low, especially for abundant taxa, whereas rare taxa retained measurable overlap across several network comparisons, indicating strong turnover of microbial networks (rare taxa in bacterial and abundant taxa in fungal networks) along the pH gradient (Fig. 5(j)). In contrast, upland bacterial networks had consistently high overlap proportions for rare taxa across all pairwise comparisons (Fig. 5(k)), indicating strong persistence of rare-associated bacterial nodes across the pH gradient. A similar but less uniform pattern was observed in upland fungal networks, where rare taxa generally had higher overlap than abundant taxa across most network comparisons (Fig. 5(l)).

In combination, these results show that bacterial networks tended to maintain greater continuity across pH-defined networks, whereas fungal networks underwent stronger node turnover. Rare taxa contributed disproportionately to network composition in many networks, particularly in upland soils, where they had stronger persistence across pH-defined networks.

3.6 Soil-pH-dependent shifts in the relative importance of abundant and rare taxa in microbial networks

To further evaluate the relative importance of abundant and rare taxa in microbial co-occurrence networks, we calculated the relative degree of each node by standardizing its degree against the average degree of the corresponding network (Fig. 6(a–d)). In paddy bacterial networks, the relative degree of abundant taxa decreased significantly with increasing soil pH, whereas that of rare taxa increased significantly (abundant, $R^2 = 0.068$, $p < 0.01$; rare, $R^2 = 0.048$, $p < 0.01$; Fig. 6(a)). In paddy fungal networks, the relative degree of abundant taxa increased significantly with pH ($R^2 = 0.102$, $p < 0.01$), whereas that of rare taxa had no significant relationship with pH ($R^2 = 0.000$, $p > 0.05$; Fig. 6(b)). In contrast, in upland bacterial networks, neither abundant nor rare taxa had significant changes in relative degree along the pH gradient (abundant, $R^2 = 0.008$; rare, $R^2 = 0.002$; Fig. 6(c)). In upland fungal networks, the relative degree of abundant taxa increased significantly with pH ($R^2 = 0.033$, $p < 0.05$), whereas that of rare taxa again had no significant change ($R^2 = 0.000$, $p > 0.05$; Fig. 6(d)). These results indicate that pH altered the relative network positions of abundant and rare taxa mainly in paddy bacterial networks, where abundant taxa became less central and rare taxa became more central with increasing pH, whereas in fungal networks the pH effect was expressed primarily through abundant taxa.

We further quantified the difference in relative degree between abundant and rare taxa (i.e., the relative degree of abundant taxa minus that of rare taxa) to compare their relative contributions to network organization (Fig. 6(e–h)). In paddy bacterial networks, the relative degree difference declined significantly with increasing pH ($R^2 = 0.078$, $p < 0.001$; Fig. 6(e)), indicating that the relative advantage of abundant taxa over rare taxa weakened under higher-pH conditions. A similar but weaker decreasing trend was observed in upland fungal networks ($R^2 = 0.018$, $p < 0.01$; Fig. 6(h)). In contrast, no significant relationship was detected for paddy fungal networks ($R^2 = 0.003$, $p > 0.05$; Fig. 6(f)) or upland bacterial networks ($R^2 = 0.001$, $p > 0.05$; Fig. 6(g)). In combination, these results indicate that the relative importance of abundant and rare taxa in supporting microbial networks is pH dependent, but the response differs among habitats and microbial groups. In particular, the contribution of rare taxa became increasingly comparable to that of abundant taxa with increasing pH in paddy bacterial networks whereas such convergence was weak or absent in the other networks.

3.7 Soil-pH-associated functional differentiation of rare and abundant bacterial taxa across paddy and upland networks

FAPROTAX-based prediction revealed clear land-use-

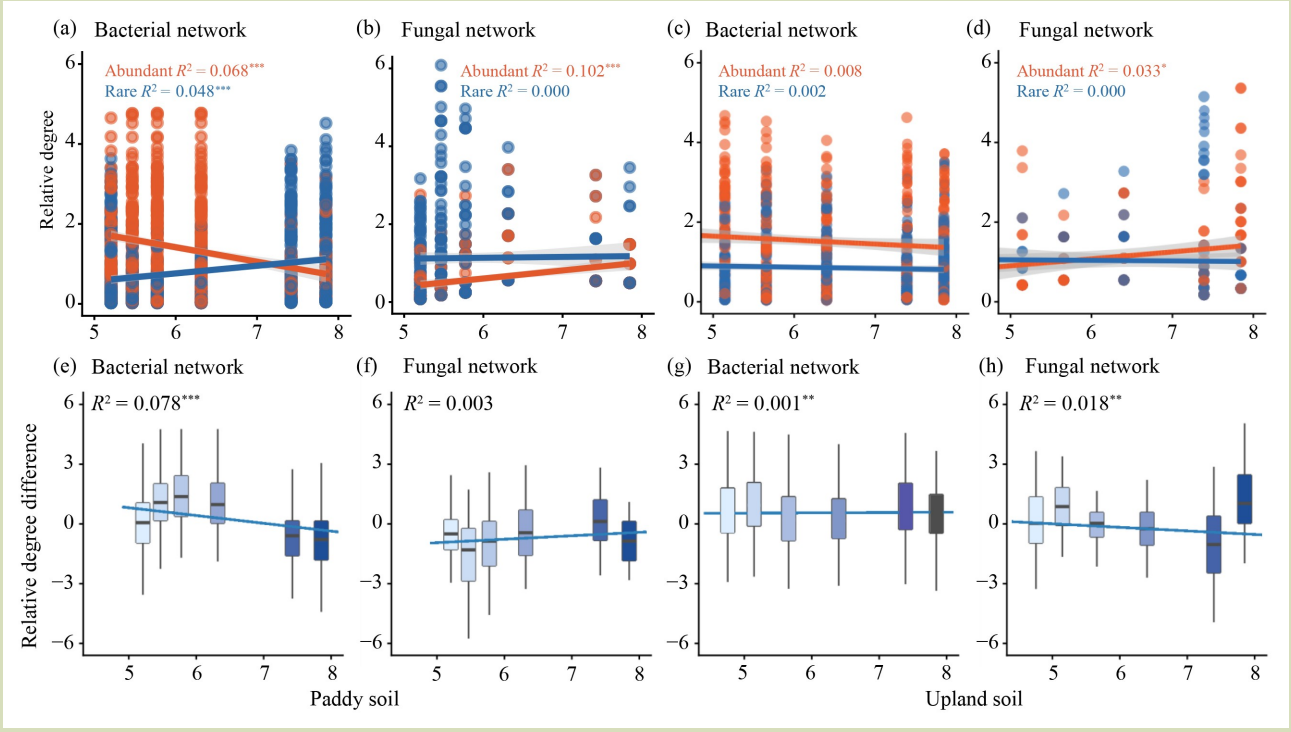


Fig. 6 Relative degree of abundant and rare taxa across six pH-defined networks in paddy and upland soils. (a,b) Relative degree of abundant and rare taxa in paddy bacterial and fungal networks, respectively; (c,d) relative degree of abundant and rare taxa in upland bacterial and fungal networks, respectively. (e,f) Differences in relative degree between abundant and rare taxa (abundant minus rare) in paddy bacterial and fungal networks, respectively; (g,h) Differences in relative degree between abundant and rare taxa (abundant minus rare) in upland bacterial and fungal networks, respectively. Panels (a–d) are scatter plots with fitted linear regressions, and panels (e–h) are box plots with fitted linear regressions. R^2 denotes the coefficient of determination. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$.

dependent functional differentiation for both rare and abundant bacterial taxa across the pH-defined networks, but pH-associated functional shifts were generally more pronounced for rare taxa than for abundant taxa (Fig. S6). In rare bacterial taxa, the quantum of several putative functions related to anaerobic or redox-sensitive carbon and sulfur transformations were consistently greater in paddy networks than in upland networks, including methanotrophy, methanogenesis, methylotrophy, fermentation and sulfate respiration (Fig. S6(a)). Additionally, these rare-taxon-associated functions were clearly differentiated along the pH gradient in paddy soils. Methanotrophy, methylotrophy, fermentation and sulfate respiration generally increased toward higher-pH paddy networks whereas methanogenesis had the opposite tendency, being greater in lower-pH paddy networks. In contrast, rare bacterial taxa in upland soils were more strongly associated with functions linked to aerobic decomposition and nitrogen turnover, such as aerobic chemoheterotrophy, cellulolysis, nitrate reduction and denitrification, although the pH-related trends of these functions were generally weaker and less directional than those observed for several paddy-enriched anaerobic categories. Nitrification-related functions had a different pattern, increasing with pH in both paddy and upland networks, indicating that this process may represent a shared pH-responsive function across land-use types.

Compared with rare taxa, abundant bacterial taxa also displayed clear habitat-level functional partitioning, but their patterns were expressed mainly as broad differences between paddy and upland soils rather than strong functional reorganization along the pH gradient (Fig. S6(b)). Specifically, abundant taxa in paddy soils were more strongly associated with methanotrophy, methanogenesis, methylotrophy and sulfate respiration-related functions, whereas abundant taxa in

upland soils were more strongly associated with aerobic chemoheterotrophy and cellulolysis. In addition, nitrification increased with pH in both paddy and upland networks for abundant taxa, similar to the pattern observed for rare taxa. Overall, these results indicate that abundant bacterial taxa mainly reflected the baseline functional partitioning between flooded and aerated soils, whereas rare bacterial taxa had relatively stronger functional differentiation along the pH gradient, particularly in paddy soils.

4 Discussion

4.1 Importance of pH in structuring microbial communities across paddy and upland soils

This study revealed marked ecological differences between paddies and upland rice fields, particularly in hydrology, aeration and organic matter turnover rates. These differences were accompanied by significant divergence in microbial community structure[50,51]. Nevertheless, among the measured soil properties, pH consistently had the strongest association with variation in both bacterial and fungal communities (Fig. 2(a–d)), although other environmental factors, including SOC, TN, EC and available mineral elements, also contributed significantly. This finding corroborates the widely held view that pH is the primary driver of soil microbial communities and extends its applicability from single land-use types to significantly divergent paddy and upland cropping systems[17,52].

The high explanatory power of pH likely stems from its direct constraints on microbial physiology and metabolism[53]. By influencing cell membrane permeability[53], enzymatic activity[54] and energy production efficiency[55], pH directly determines the survival and proliferation capabilities of many microbial species[16]. Also, pH indirectly modulates microbial competitive interactions by governing the chemical speciation and bioavailability of nutrients (e.g., phosphorus and trace metals)[56,57]. In our study, while certain available mineral elements had some driving effects, the Mantel's tests confirmed their importance was substantially lower than that of pH. This further reinforces the importance of pH, indicating it may serve as an integrative proxy for multiple nutrient availability factors, acting as a comprehensive indicator of environmental filtering.

It is noteworthy that the fungal community were more strongly determined by land-use type compared to bacteria in the present study. This may be partly attributable to a greater sensitivity of fungi to variations in soil moisture and organic matter inputs[58]. However, even against this backdrop, pH remained the primary explanatory factor for shifts in the fungal community. This indicates that the intensity of environmental filtering by pH on eukaryotic microorganisms surpasses even the influence of habitat-specific ecological processes. This further solidifies the core conclusion of this study: in agricultural landscapes featuring coexisting paddy and upland management, pH serves as a universal core metric that transcends differences in management practices and biological kingdoms, enabling robust comparison and prediction of microbial community dynamics. Future research should aim to validate this pattern across broader pH gradients or soils derived from different parent materials, and integrate functional gene or metatranscriptomic analyses to unravel the underlying physiological mechanisms.

4.2 Soil pH drives directional taxonomic replacement rather than a simple diversity response

Our results showed that pH influenced microbial communities not simply by altering diversity, but by driving directional taxonomic replacement along the gradient (Fig. 3(a–d)). In both paddy and upland soils, bacterial Shannon diversity increased significantly with increasing pH, while community composition shifted markedly and the bacterial RF models retained high predictive power for soil pH. In combination, these patterns indicate that pH acted less as a background correlate of diversity and more as an ecological filter that sorted taxa with contrasting pH preferences. This interpretation is consistent with previous studies showing that bacterial richness generally increases from acidic toward near-neutral soils and that pH is often the strongest predictor of bacterial community composition across regional and global scales[9,10,59].

The pH-associated bacterial taxa further indicate that this filtering operated differently in paddy and upland soils. In paddy soils, high-pH-associated taxa clearly outnumbered low-pH-associated taxa, indicating that increasing pH mainly released bacterial communities from strong acidic constraints and allowed a broader set of lineages to establish. This asymmetric pattern is ecologically reasonable because paddy soils are simultaneously shaped by acidity and flooding-related redox constraints; under such conditions, low pH may

compress the viable niche space for many bacterial groups, whereas increasing pH can promote a relatively one-sided expansion of taxa better adapted to less acidic conditions[60,61]. In contrast, upland soils had a more balanced distribution of low-pH- and high-pH-associated bacterial taxa, indicating that pH filtering in aerated soils was expressed more as reciprocal replacement between lineages occupying different pH windows than as simple release from acidity stress[62]. Thus, the role of pH was not identical across land-use types, but depended on the broader ecological context in which pH operated.

Several representative taxa provide additional support for this interpretation. For example, in paddy soils, the BD2-11 terrestrial group, which belongs to Gemmatimonadota, a phylum frequently reported to be favored by neutral rather than acidic soils, was more abundant at higher pH in the present study[63]. In contrast, *Aquisphaera*, identified here as a low-pH-associated taxon, has recently been reported to show a negative correlation with soil pH in agricultural soils[64]. In upland soils, Acidobacteriae Subgroup 13 and *Rhodanobacter*, which were identified as low-pH-associated taxa, are consistent representatives of acid-associated lineages. Acidobacteriota are widely recognized as characteristic of acidic soils and many members possess traits that improve tolerance to acidic environments[65]. The members of *Rhodanobacter* have been reported to dominate acidic nitrate-rich environments and to have physiological adaptation to acidic and metal-stressed conditions[66,67]. In contrast, some high-pH-associated taxa in upland soils in our study, especially *Azoarcus* and *Azospira*, belong to groups whose cultured representatives generally are favored by neutral to slightly alkaline pH, supporting their positive response to increasing soil pH[68,69].

Compared to bacteria, fungal parameters were notably less directional. In both paddy and upland soils, fungal biomarkers were distributed among low-pH-associated, high-pH-associated and complex-response groups, indicating that pH influenced fungal communities, but not in a uniformly monotonic way. This agrees with previous studies showing that fungal composition can respond to pH, and this is often more strongly co-regulated by vegetation, substrate quality, land-use legacy and moisture regime than bacterial composition[70–72]. The mixed low-, high-, and complex-response trajectories observed here therefore indicate that fungal pH responses were shaped by the interaction of pH with habitat-specific ecological filters, rather than by pH alone. Overall, these patterns indicate that bacterial communities had clearer pH-dependent lineage sorting whereas fungal communities had more context-dependent response in which pH operated together with other environmental controls.

4.3 Soil pH reshapes microbial co-occurrence networks, but the direction of its effects is jointly modulated by land-use type and microbial kingdom

Beyond shaping community composition, soil pH profoundly influenced the assembly of microbial co-occurrence networks, with markedly different patterns emerging between paddy and upland soils. These divergent pH-network relationships indicate that the ecological mechanisms by which pH governs microbial interactions are contingent upon the broader environmental context imposed by land-use type. This also indicates that changes in network topology may reflect shifts not only in taxonomic composition, but also in the physiological conditions under which microorganisms maintain growth, tolerate stress and establish associations with neighboring taxa[73].

For bacterial networks, paddy and upland soils had contrasting pH-dependent topological responses. In paddy soils, bacterial network topological properties, including average degree, clustering coefficient, centralization and robustness, had unimodal relationships with pH, peaking near circumneutral conditions (about pH 5.6–6.4), while modularity had a inverted unimodal relationship with a minimum at intermediate pH. This unimodal response of network connectivity and stability to pH aligns with intermediate-disturbance or optimal-niche hypotheses[74,75], where moderate pH conditions alleviate physiological constraints and promote diverse metabolic strategies, facilitating cooperative and competitive interactions that yield complex and robust networks[76]. The minimized modularity at intermediate pH further supports this interpretation, as lower modularity typically indicates greater network integration and potential for cross-module resource exchange[47,77]. In flooded paddy soils, pH likely operates together with redox-sensitive processes rather than as an isolated chemical factor: oxygen depletion after submergence promotes Fe(III) and Mn(IV) reduction, alters proton consumption and Fe mineral transformation, and thereby affects phosphorus mobilization as well as the release of soluble carbon and nutrients under anaerobic conditions[60,78,79]. Against this background, the stronger bacterial connectivity observed at intermediate pH may reflect a condition in which acidity constraints are alleviated while reductive Fe-Mn cycling

and associated nutrient release remain sufficiently active to support metabolically complementary interactions and denser microbial associations. Conversely, the decline in network connectivity and robustness toward both acidic and alkaline extremes indicates that pH-induced environmental filtering becomes increasingly stringent, eliminating species with narrow pH tolerance and simplifying potential interaction niches[10,17]. Thus, although the taxa retained at low and high pH are not necessarily the same, both ends of the gradient may impose stronger physiological constraints that reduce the number of populations able to engage in dense and integrated co-occurrence networks[80]. In upland soils, in contrast, bacterial network connectivity and robustness had inverted unimodal relationships, with minima near pH 6, while modularity peaked at intermediate pH. Unlike paddy soils, upland soils remain predominantly aerobic and lack strong redox-driven Fe-Mn buffering, so pH is more likely to regulate microbial interactions through its effects on organic matter decomposition rates, extracellular enzyme activity and nutrient acquisition under oxic conditions[81]. In this context, intermediate pH was associated with stronger modular partitioning of bacterial co-occurrence networks in upland soils[73,77,82] whereas more acidic or alkaline conditions may have selected for a smaller set of pH-tolerant taxa with more similar ecological strategies[80,83,84]. However, this interpretation remains inferential and should be further tested using experimental or temporal datasets. Together, these results indicate that the pH dependence of bacterial network organization differs fundamentally between paddy and upland soils: intermediate pH favors greater integration and robustness in paddy soils, but greater modular partitioning in upland soils, likely reflecting differences in hydrological regime, redox buffering capacity and resource competition[73,77,82].

For fungal networks, the pH responses also differed clearly between paddy and upland soils, but in a pattern distinct from that of bacteria. In paddy soils, connectivity-related properties generally decreased with increasing pH, indicating that fungal associations were stronger under more acidic conditions. This pattern indicates fundamental differences in how bacterial and fungal communities respond to pH gradients. Fungi, as eukaryotic organisms with broader pH tolerance and greater capacity for hyphal exploration, may experience reduced competitive pressure for spatial niches under more acidic conditions, allowing for more extensive co-occurrence networks[53,85]. This could indicate that, under acidic paddy conditions, fungal associations are maintained less by chemical relief from stress and more by hyphal foraging capacity and the ability to bridge spatially separated microsites[16,86]. In addition, under flooded paddy conditions, pronounced redox heterogeneity between surface oxidized layers, reduced bulk soil and rhizosphere microsites may further favor fungal persistence and co-occurrence under relatively low pH by increasing microscale niche diversity and allowing hyphal networks to connect chemically contrasting resource patches[87,88]. In upland soils, however, fungal network connectivity increased and modularity decreased with rising pH, a trend opposite to that observed in paddy soils. This indicates that in well-aerated upland environments, near-neutral pH may improve organic matter decomposition and resource availability, enhancing fungal hyphal connectivity and broader ecological interactions[16,89]. Under these aerobic conditions, pH is more likely to influence fungal associations through changes in decomposition rate, extracellular enzyme functioning and substrate accessibility, rather than through the strong redox-coupled nutrient mobilization characteristic of flooded paddy soils[90]. Overall, the opposite pH responses of fungal networks between paddy and upland soils further evidences the interactive effects of pH and land-use-specific environmental conditions; in flooded paddy soils, acidic conditions may better support fungal spatial linkage through hyphal exploration, whereas in aerated upland soils, higher pH may favor fungal network expansion by improving decomposition-related resource accessibility[86,89].

In combination, these results show that both bacterial and fungal network responses to pH were strongly land-use dependent, but the direction and ecological implications of these responses differed between the two microbial groups. In paddy soils, bacterial networks were most connected and robust at intermediate pH, whereas fungal networks tended to maintain stronger associations under more acidic conditions. In upland soils, bacterial networks were more modular near intermediate pH, while fungal networks had greater connectivity as pH increased. These contrasting patterns indicate that the effects of pH on microbial network organization are jointly shaped by microbial life-history traits and land-use-specific environmental contexts, including hydrological regime, aeration status and resource accessibility[73,82,89]. Nevertheless, because these topological patterns were inferred from correlation-based co-occurrence networks, they should be interpreted as changes in association structure rather than direct evidence of pairwise ecological

interactions. Further validation using experimental manipulation or process-based approaches will be needed to determine how pH reshapes the underlying biotic interactions.

4.4 Rare taxa contribute to network continuity during pH-driven microbial network reorganization

To further examine how microbial communities changed along the pH gradient, we integrated sample-level networks within different pH intervals. The resulting pH-gradient-defined networks showed that bacterial networks had greater overall node continuity among different pH intervals, whereas fungal networks, particularly those in paddy soils, had stronger node turnover. This pattern indicates that, compared with bacterial communities, fungal networks are structurally more sensitive to pH variation. Previous studies and our results consistently show that, compared with bacterial taxa, fungal taxa have a more balanced distribution of pH preferences[52,91]. This result indicates that compositional sensitivity and network structural sensitivity are not necessarily fully synchronized. Therefore, the stronger node turnover observed in fungal networks in this study may reflect not merely taxonomic replacement per se, but also a greater propensity for interaction structures to be reorganized under pH variation. Such habitat-dependent differences may reflect the contrasting environmental contexts of flooded and aerated soils: paddy soils are characterized by stronger redox constraints and chemically heterogeneous microsites, whereas upland soils are more consistently aerobic and may favor different modes of network reorganization under pH change[60,92].

Concurrently, the proportions of abundant and rare taxa within the networks were not fixed, but were systematically redistributed along the pH gradient. In bacterial networks, abundant taxa tended to dominate at intermediate pH ranges whereas rare taxa contributed more under low- or relatively high-pH conditions. This pattern was broadly consistent in both paddy and upland bacterial networks, and is consistent with previous studies on the ecological differentiation between abundant and rare taxa, which indicate that abundant taxa generally have broader niche adaptations and greater environmental tolerance, whereas rare taxa are more likely to exhibit substitutive or compensatory responses when environmental fluctuations intensify, stress increases or conditions deviate from the optimal range[29,93]. Node overlap analysis further showed that rare-associated nodes did not appear randomly across pH-specific networks, but instead had relatively strong persistence across multiple networks. This pattern was particularly evident in upland bacterial networks, where rare taxa displayed the strongest continuity across the pH gradient. Together, these patterns indicate that rare taxa are not merely incidental background members, but may act as a reservoir of low-abundance yet recurrent taxa that contributes to network continuity during reorganization[94,95]. Notably, this role was not confined to bacteria. In fungal networks, rare taxa also retained measurable overlap across multiple pH-defined networks, especially in upland soils, even though fungal networks as a whole had stronger turnover. This indicates that the contribution of rare taxa in fungi may be expressed more through persistence across reorganized networks than through stable dominance within any single network[96]. FAPROTAX-based prediction further showed that rare bacterial taxa in paddy soils were more strongly associated with putative anaerobic or redox-sensitive functions, including methanotrophy, methanogenesis, methylotrophy, fermentation and sulfate respiration, whereas rare bacterial taxa in upland soils had relatively stronger associations with aerobic chemoheterotrophy, cellulolysis and selected N-cycling functions[97]. Such habitat-dependent functional differentiation may provide a potential basis for functional complementarity of rare taxa under contrasting flooded and aerated soil conditions[24,48,96].

Relative degree analysis further supports this interpretation. In paddy bacterial networks, the relative degree of abundant taxa decreased significantly with increasing pH whereas that of rare taxa increased significantly, indicating a detectable but relatively limited pH-associated shift in the relative network position of rare taxa. This trend is consistent with recent studies indicating that rare taxa may assume critical network roles under environmental disturbance. Previous work has shown that rare taxa often account for a higher proportion of keystone nodes or are important in maintaining community stability; notably, under conditions of environmental perturbation or shifting resource availability, their topological importance may increase substantially[31,98]. At the same time, some studies have shown that abundant taxa are still key to maintaining the fundamental framework of the overall community, whereas rare taxa are more likely to manifest their importance in local interaction adjustment and disturbance responses[29]. In contrast, no comparable pH-related shift in relative degree was detected for rare fungal taxa, indicating that their contribution was expressed mainly through cross-network persistence rather than through changes in within-

network topological position. Accordingly, our results support a more conservative interpretation: under higher-pH conditions in paddy bacterial networks, the structural difference between abundant and rare taxa became smaller, indicating that rare taxa may partially complement, rather than replace, abundant taxa during network reorganization.

These patterns should, however, be interpreted with caution, because cross-network persistence and relative degree in correlation-based networks do not by themselves establish that rare taxa directly stabilize the community. Rather, our results indicate a potential structural contribution of rare taxa at the co-occurrence network level, which should be further tested using temporal datasets and targeted experimental validation.

5 Conclusions

In conclusion, this study shows that the response of soil microbial communities and their co-occurrence networks to pH is distinctly non-linear rather than simply linear across paired paddy and upland agricultural soils. Along the pH gradient, microbial communities were reorganized not only through marked taxonomic turnover but also through pronounced shifts in network topology. Importantly, these non-linear responses followed opposite trends between land-use types: in paddy soils, microbial networks generally tended to have higher connectivity and stability under intermediate pH conditions, whereas in upland soils, network topology responded to pH in the opposite direction. This contrast indicates that the effect of pH on microbial network reorganization is strongly habitat dependent, likely reflecting fundamental differences between flooded and aerated soils in water regime, redox conditions and resource-use patterns. In addition, abundant and rare taxa make distinct structural contributions during this process, with rare microbes showing relatively high continuity and topological importance in several networks, indicating that they may contribute to network continuity during pH-driven network reorganization. Overall, our findings reveal a general pattern in which soil pH non-linearly regulates microbial community assembly and network organization, generates opposite response trajectories in paddy versus upland soils, and highlights a potential supporting role of rare taxa in maintaining network continuity. Future studies integrating temporal observations, experimental manipulation and process-based validation will help further resolve how rare taxa contribute to pH-driven network reorganization and whether their structural persistence is linked to specific ecological functions under contrasting land-use conditions.

Supplementary materials

The online version of this article at https://doi.org/10.15302/J-FASE-2027726 contains supplementary materials (Methods S1–S2; Figs. S1–S6; Tables S1–S5; Dataset S1).

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Compliance with ethics guidelines

Deqiang Mao, Chen Chen, Qi Zhao, Yongfu Li, Chengqi Yan, Xuebin Xu, Jianping Chen, Tida Ge, and Haoqing Zhang declare that they have no conflicts of interest or financial conflicts to disclose. This article does not contain any studies with human or animal subjects performed by any of the authors. The authors declare no conflicts of interest. No artificial intelligence tools were used in the preparation of this manuscript.

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