Received: 24 October 2023 | Revised: 3 January 2024 | Accepted: 4 January 2024

DOI: 10.1111/gcb.17160

Global Change Biology WILEY

RESEARCH ARTICLE

Community assembly of organisms regulates soil microbial functional potential through dual mechanisms

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Funding information

Strategic Priority Research Program of Chinese Academy of Sciences, Grant/Award Number: XDA0440203; National Key Research and Development Program of China, Grant/Award Number: 2022YFD1900603 and 2022YFD1900601; National Natural Science Foundation of China, Grant/Award Number: 42177298; Double Thousand Plan of Jiangxi Province, Grant/Award Number: jxsq2023201046; Youth Innovation Promotion Association of CAS, Grant/Award Number: Y2021084

Abstract

Unraveling the influence of community assembly processes on soil ecosystem functioning presents a major challenge in the field of theoretical ecology, as it has received limited attention. Here, we used a series of long-term experiments spanning over 25 years to explore the assembly processes of bacterial, fungal, protist, and nematode communities using high-throughput sequencing. We characterized the soil microbial functional potential by the abundance of microbial genes associated with carbon, nitrogen, phosphorus, and sulfur cycling using GeoChip-based functional gene profiling, and determined how the assembly processes of organism groups regulate soil microbial functional potential through community diversity and network stability. Our results indicated that balanced fertilization (NPK) treatment improved the stochastic assembly of bacterial, fungal, and protist communities compared to phosphorus-deficient fertilization (NK) treatment. However, there was a nonsignificant increase in the normalized stochasticity ratio of the nematode community in response to fertilization across sites. Our findings emphasized that soil environmental factors influenced the assembly processes of the biotic community, which regulated soil microbial functional potential through dual mechanisms. One mechanism indicated that the high phosphorus levels and low soil nutrient stoichiometry may increase the stochasticity of bacterial, fungal, and protist communities and the determinism of the nematode community under NPK treatment, ultimately enhancing soil microbial

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functional potential by reinforcing the network stability of the biotic community. The other mechanism indicated that the low phosphorus levels and high soil nutrient stoichiometry may increase the stochastic process of the bacterial community and the determinism of the fungal, protist, and nematode communities under NK treatment, thereby enhancing soil microbial functional potential by improving the β-diversity of the biotic community. Taken together, these results provide valuable insights into the mechanisms underlying the assembly processes of the biotic community that regulate ecosystem functioning.

KEYWORDS
balanced fertilization, deterministic and stochastic processes, environmental factors, functional genes, nutrient stoichiometry, organism groups

1 | INTRODUCTION

Understanding the principles that govern the assembly of organism communities and their impact on soil biogeochemical cycling is crucial for predicting ecosystem responses to global climate change (Martiny et al., 2011). The fundamental processes of community assembly, determinism and stochasticity, work concurrently and symbiotically in species composition, and ecosystem functioning (Dini-Andreote et al., 2015). Determinism indicates that selection is the primary force that shapes soil microbiomes, including environmental filtering and species interactions, while stochasticity emphasizes dispersal events and random drift (Zhou, 2017). Currently, the extent to which stochastic and deterministic processes contribute to community assembly in agricultural ecosystems remains a subject of deliberation (Jiao et al., 2020; Wang et al., 2013). Fertilization is a common agriculture management practice that affects the patterns of organism community assembly by changing soil characteristics (Guo et al., 2010). Long-term phosphorus-deficient fertilization alters the microbial community by changing the ratios of soil nitrogen–phosphorus or carbon–phosphorus (Peñuelas et al., 2013). In contrast, balanced fertilization is considered a beneficial strategy to improve bacterial diversity, enzyme activities, soil quality, and crop yield, which is associated with the stochastic process of microbial communities (Feng et al., 2017). Studies on the impact of fertilization-induced environmental changes on the assembly processes of soil organism communities have primarily focused on individual bacterial or fungal communities (Shi et al., 2020). However, soil protists and nematodes as top-down regulators play critical roles in mediating microbial community structure and ecosystem functioning. Therefore, it is essential to consider various organism groups to understand how anthropogenic environmental changes affect the assembly processes of soil microorganisms (including bacteria, fungi, and protists) and microfauna (nematodes).

To advance the understanding of the mechanisms underlying belowground biota, it is imperative to elucidate the relationship between biotic community and assembly processes (Feng et al., 2018). The assembly processes can inevitably structure the diversity and co-occurrence network of soil biotic community (Stegen et al., 2012) and show substantial influences on ecosystem functioning (Leibold et al., 2017). Variations in community assembly processes have provided evidence for differences in the composition and species co-occurrence patterns of various biotic communities (Peay & Bruns, 2014). Deterministic processes tend to dominate in bacterial communities characterized by low diversity (Xun et al., 2019), whereas stochastic processes lead to more intricate microbial co-occurrence networks, potentially influencing microorganism interactions (Jiao et al., 2020). Microbial communities exhibit different relationships between fungal diversity and the stochasticity ratio of the community from large to small scales (Zheng et al., 2021). However, a comprehensive quantitative synthesis is yet to be undertaken to assess the impact of assembly processes on the diversity and network structures of soil organism groups at larger scales.

Biotic community diversity holds significant ecological importance in predicting ecosystem functioning responses to belowground habitat changes (Balvanera et al., 2014). Disentangling the mechanisms underpinning the diversity–function relationship is crucial for determining the contributions of abundance and composition of organisms to ecosystem functioning. While an increasing number of studies have primarily focused on the implications of microbial α-diversity for ecosystem functioning (Delgado-Baquerizo et al., 2020), the role of β-diversity, which pertains to variations in community composition, has received relatively less attention (Mori et al., 2018). Furthermore, the network complexity and stability mirrored by the functional traits of organisms play a pivotal role in determining the functional composition of ecological communities (Thébault & Fontaine, 2010). Soil microbial and nematode communities undergo significantly different assembly processes, resulting in distinct functional compositions regarding nutrient cycling (Fierer et al., 2012; Leff et al., 2015). So far, there is a paucity of studies on how the assembly processes of organism groups affect soil microbial functional potential by mediating community diversity and network stability.

In this study, we sought to explore the mechanisms of environmental factors and biotic communities affecting soil microbial functional potential associated with carbon (C), nitrogen (N), phosphorus (P), and sulfur (S) cycling. We used long-term experiments with three fertilization treatments across different agroecological sites, including no fertilization, phosphorus-deficient fertilization, and balanced fertilization. The bacterial, fungal, protist, and nematode communities were determined by high-throughput amplicon sequencing, and functional gene microarrays were examined by GeoChip-based metagenomic technology. Specifically, we attempted to answer the following questions: (1) How do the assembly processes, diversity, and co-occurrence network of organism groups respond to balanced fertilization compared with phosphorus-deficient fertilization? (2) How do the stochastic and deterministic processes of organism groups mediate diversity and network stability of biotic community? and (3) What is the biological mechanism of assembly processes of organism groups that drive soil microbial functional potential through community diversity and network stability? We hypothesized that balanced fertilization would enhance the stochastic processes of the microbial community compared to phosphorus-deficient treatment, and soil microorganisms (bacteria, fungi, and protists) and microfauna (nematodes) exhibit consistent responses to fertilization. We also supposed that stochastic processes of organism groups would lead to increased community diversity and network stability, thereby improving soil microbial functional potential.

2 | MATERIALS AND METHODS

2.1 | Experimental sites description and soil sampling

We collected soil samples in 2015 from seven agroecosystem sites of the National Ecosystem Research Network of China across a latitudinal gradient from 26° to 47° N, including Hailun (HL), Fengqiu (FQ), Qiyang (QY), Shengyang (SY), Changwu (CW), Yanting (YT), and Fukang (FK). The experimental sites are characterized by more than 25 years of different fertilization, with mean annual precipitation ranging from 160 to 1309 mm and mean annual temperatures ranging from 2.8 to 18.8°C (Table S1). Three fertilization treatments with three replicates included no fertilization (Ctrl), phosphorus-deficient fertilization (NK, nitrogen and potassium inputs), and balanced fertilization (NPK, nitrogen, phosphorus, and potassium inputs). The details of each experimental site and corresponding soil properties were given in Tables S1 and S2, respectively.

We collected surface soils (0–20 cm) from each plot (5 × 5 m) under three treatments at the seven sites after harvesting the summer crop. We followed an “S” sampling pattern to collect seven soil cores from each plot and then combined them to create a composite sample. Three treatments were randomly distributed and applied to triplicate plots at each site. After collection, all samples were stored on dry ice and immediately transported to the laboratory. Subsequently, 63 soil samples (7 sites × 3 treatments × 3 replicates) were sieved to a 2 mm size, and any visible live plant material and stones were manually removed. Each soil sample was divided into two subsamples to determine soil chemical properties, microbial and nematode communities, and functional genes (Zhu et al., 2023).

2.2 | Soil chemical properties

Soil pH was measured using a pH meter (FE20 FiveEasy™, Mettler Toledo, Germany) in a 1:2.5 suspension of dry soil mass to deionized water volume (Kader et al., 2015). The dichromate oxidation method was used to determine soil organic carbon (SOC; Nelson, 1996). Soil organic matter (OM) was calculated as OM = SOC × 1.724. Total nitrogen (TN) was determined by semi-micro Kjeldahl digestion (Bremner, 1960). Total phosphorus (TP) and total potassium (TK) were digested with HF-$HClO_4$ and determined using the molybdenum-blue method (O'Halloran & Cade-Menun, 2007) and atomic absorption spectrophotometer (Kanehiro & Sherman, 1965), respectively. Available phosphorus (AP) was determined using the molybdenum blue method after extraction with sodium bicarbonate (Lu, 1999). Available potassium (AK) was extracted with ammonium acetate and detected using flame photometry (Lu, 1999). Ammonium nitrogen (NH_4^+-N) was determined by ultraviolet spectrophotometry using 0.01 mol L^{-1} calcium chloride extraction, while nitrate nitrogen (NO_3^--N) was determined by spectrophotometry through a saturated calcium sulfate extraction (Lu, 1999). The carbon–nitrogen ratio (C/N) was calculated by the ratio of SOC to TN, the carbon–phosphorus ratio (C/P) by the ratio of SOC to TP, and the nitrogen–phosphorus ratio (N/P) by the ratio of TN to TP.

2.3 | DNA extraction and high-throughput sequencing

Total genomic DNA was extracted from 0.5 g of fresh soil using the Powersoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) following the manufacturer's instructions. The extracted DNA was quantified and qualified using a Nanodrop ND-1000 spectrophotometer at 260/280 and 260/230 ratios ≥1.8 (NanoDrop Technologies, Delaware, USA). The DNA was then used for high-throughput sequencing of the biotic community (bacteria, fungi, protists, and nematodes) and functional gene analysis.

The bacterial 16S rRNA and fungal ITS genes were amplified using the primer pairs 515F/806R (Caporaso et al., 2011) and ITS7F/ITS4R (Ihrmark et al., 2012), respectively. The protist and nematode 18S rRNA genes were amplified using primer pairs S615F/S947R (Fiore-Donno et al., 2018) and 3ndf/1132rmod (Geisen et al., 2018). The taxonomic profiles of soil bacterial, fungal, protist, and nematode communities were determined via amplicon sequencing using the Illumina MiSeq platform. Bioinformatics processing was performed as described previously (Geisen et al., 2018). Briefly, primer sequences were removed using cutadapt (Martin, 2011), and chimeric reads and singletons were filtered out using VSEARCH (Rognes et al., 2016). High-quality sequences were then divided into operational taxonomic units (OTUs) with a similarity threshold of 97% using VSEARCH (Rognes et al., 2016). Taxonomic assignments of OTUs were performed using RDP Classifier (Wang et al., 2007). All samples were rarefied to ensure equal sampling depth within each organism group.

2.4 | GeoChip hybridization analysis

We examined the functional structure of the biotic community using GeoChip 5.0, including carbon (C), nitrogen (N), phosphorus (P), and sulfur (S) cycling. To obtain sufficient DNA for microarray analysis, 20 ng of template DNA from each sample was amplified using whole community genome amplification. After amplification, 2.5 μg of DNA was labeled and resuspended in a hybridization buffer. The labeled DNA was then hybridized on a GeoChip 5.0 microarray chip with 10% formamide at 67°C for 24 h in an Agilent microarray hybridization oven (Agilent Technologies, Santa Clara, CA). The chip was then washed, dried, and scanned using 100% laser power at wavelengths of 532 and 635 nm. Intensity data were collected using the Agilent Feature Extraction program. Raw intensity data were uploaded to the Functional Gene Microarray analysis pipeline for preprocessing, including normalization and log transformation. Further steps, such as hybridization, imaging, and data preprocessing, were performed as described previously (Zhou et al., 2015). Relative abundance data for soil functional gene-related C, N, P, and S cycling were determined by the ratio of the total signal intensity of each gene to the total number of probes (Zhao et al., 2016).

2.5 | Construction of co-occurrence networks

The network analysis was based on a sample combination of all sites from each fertilization treatment (7 sites ×3 replicates for each network), because the data points from different treatments within each site cannot construct a reliable network. Collecting all sampling sites of each fertilization treatment can improve sensitivity to co-occurrence events and reveal co-occurrence patterns of soil biotic communities driven by different nutrient inputs across sites. The co-occurrence networks were constructed using the “WGCNA” package based on the Spearman correlation matrix with adjusted *p* values (Langfelder & Horvath, 2012). The network properties were calculated using the “igraph” package in R, and visualization was performed using Cytoscape software (version 3.7.2). We extracted subnetworks by preserving the phylotypes of individual soil samples using the “induced_subgraph” function in the “igraph” package (Csardi & Nepusz, 2006). Furthermore, we calculated the topological properties of the networks, including average degree (the number of adjacent edges), clustering coefficient (the probability that the adjacent nodes of a node were connected), network density (the ratio of the frequency of actual edges to possible edges), and network diameter (the longest distance in the network). These metrics were used as indicators of network cohesion and stability (Jiao et al., 2022; Zhou et al., 2010). Based on the within-module degree (z-score) and participation coefficient (c-score) threshold values, nodes were categorized into network hubs (z-score > 2.5 and c-score > 0.6), module hubs (z-score > 2.5 and c-score < 0.6), connectors (z-score < 2.5 and c-score > 0.6), and peripherals (z-score < 2.5 and c-score < 0.6; Poudel et al., 2016). Network hubs, module hubs, and connectors were termed keystone taxa that play important roles in maintaining community stability (Tylianakis & Morris, 2017). Natural connectivity provided a valuable assessment of network stability, which can be achieved by removing nodes from the static network (Ming & Yan, 2015; Peng & Wu, 2016).

2.6 | Quantification of soil microbial functional potential

We employed GeoChip 5.0 to assess the soil microbial functional potential associated with C, N, P, and S cycling (Donhauser et al., 2021; Pressler et al., 2020; Sun et al., 2014; Xu et al., 2014). The gene values of 10 functional groups were initially normalized as relative abundances, including carbon degradation (from labile carbon to recalcitrant carbon), carbon fixation, nitrogen fixation, nitrification, denitrification, polyphosphate synthesis, polyphosphate degradation, phosphorus oxidation, sulfur oxidation, and sulfur assimilation. To ensure consistency with the diversity of organism groups (Z-score transformed data), we applied Z-score transformation to the functional gene data, which allowed for the calculation of associations between the two datasets in subsequent analyses. The Z-score transformation method is commonly chosen to transform already normalized data, due to its favorable statistical properties, including a normal distribution of average Z-scores, a weak correlation between means and variances of these averages, and the absence of constraints on variability observed in raw data (Delgado-Baquerizo et al., 2016; Maestre et al., 2012). The soil microbial functional potential was determined by the average values of functional genes after Z-score transformation. In addition, the microbial functional potential associated with C, N, P, and S cycling was calculated by averaging the Z-score-transformed values of their corresponding functional genes. The averaging approaches provided a clear and easily interpretable measure of different organisms to sustain soil microbial functional potential (Jing et al., 2015; Wagg et al., 2014).

2.7 | Estimating assembly processes of biotic communities

The normalized stochasticity ratio (NST) was used to estimate the relative importance of stochastic processes in community assembly. The normalized stochasticity ratio of all treatments in each site was calculated using the “NST” package (Ning et al., 2019). NST was calculated using various similarity metrics and null model algorithms, as well as stochasticity ratios, standard effect sizes, and modified Raup-Crick metrics (Ning et al., 2019; Zhou et al., 2014). NST was modified from the stochasticity ratio by considering two alternative situations and normalizing the index to range from 0 to 1. The NST index had a boundary of 50% to distinguish between more deterministic (<50%) and more stochastic (>50%) assembly.

2.8 | Statistical analysis

The α-diversity (Shannon, Chao1, and evenness indices) of the biotic community was calculated using the Quantitative Insights into Microbial Ecology (QIIME software, version 2) pipeline. We transform the value of bacterial, fungal, protist, and nematode α-diversity, including Shannon, Chao1, and Evenness indices into Z-scores to obtain a computable index for each treatment. The standardized α-diversity values of bacterial, fungal, protist, and nematode communities were then averaged to create an overall α-diversity index for the biotic community. The average α-diversity of individual bacterial, fungal, protist, and nematode communities is calculated using the standardized Shannon, Chao1, and evenness indices (Delgado-Baquerizo et al., 2019). The β-diversity of the biotic communities (bacterial, fungal, protist, and nematode communities) as well as the β-diversity of soil microbial functional potential were estimated based on Bray–Curtis dissimilarity between samples, and the dissimilarity was calculated using the "vegdist" function in the "vegan" package. Principal coordinate analysis (PCoA) was conducted to reflect β-diversity in both biotic communities and soil microbial functional potential using the "vegan" package (Oksanen et al., 2013). The Bray–Curtis dissimilarity and PCoA analysis were performed based on a rarefied OTUs dataset for the biotic communities and a gene dataset for soil microbial functional potential. The distance decay rate (DDR) of bacterial, fungal, protist, and nematode communities and soil microbial functional potential were calculated by the slopes of ordinary least-squares regressions for their similarity (1-Bray–Curtis dissimilarity) related to geographic distance. The 1-Bray–Curtis dissimilarity represented the similarity between two samples. It ranged from 0 (when the two samples were completely dissimilar) to 1 (when the two samples were completely same in species composition). The difference in slope between two datasets was calculated using the "diffslope" function in the "simba" package.

One-way ANOVA was performed to assess the differences in both biotic community α-diversity and soil microbial functional potential across different sites and fertilization treatments using Tukey's HSD tests ($p < .05$) in SPSS 20.0 software (SPSS, Chicago, IL, USA). Two-way PERMANOVA was used to determine the interaction effect of fertilization treatments and sites on biotic communities and soil microbial functional potential in SPSS 20.0 software. Random forest modeling was conducted to quantitatively estimate the important predictors of soil microbial functional potential using the "randomForest" package (Liaw & Wiener, 2002), and the model and predictor significance levels were determined using the "A3" and "rfPermute" packages, respectively (Archer, 2016).

The partial least squares structural equation modeling (PLS-SEM) was essentially a sequence of regressions in terms of weight vectors using Smart PLS 3.0 software (Henseler et al., 2009). We used the relative abundances of functional genes for C, N, P, and S cycling, and the average degree and natural connectivity for network stability in the PLS-SEM model (Table S2). The fitting index of the model was determined by Cronbach's alpha, composite reliability, and average variance extracted, with values greater than 0.7, 0.6, and 0.5, respectively, indicating a well-fitting model. The goodness-of-fit (GoF) index was used to evaluate the overall fitness of the model (Henseler & Sarstedt, 2013). The overall fitting effect of the model was divided into weak (GoF>0.1), medium (GoF>0.25), and strong (GoF>0.36; Wetzels et al., 2009).

3 | RESULTS

3.1 | Assembly processes and distance decay pattern of organism groups

The normalized stochasticity ratio (NST) based on the null model was used to quantitatively assess the stochasticity of four organism groups under the three treatments across sites (Figure 1). The NST values of bacterial and fungal communities were significantly

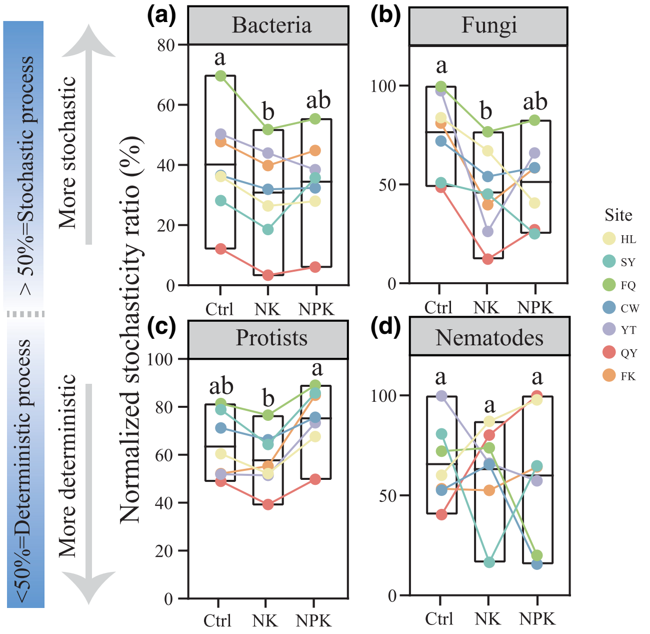

FIGURE 1 The assembly processes of biotic community under different fertilization treatments. Normalized stochasticity ratio (NST) of bacterial (a), fungal (b), protist (c), and nematode (d) communities under different fertilization treatments are presented. Black lines represent the mean value. Different lowercase letters represent significant differences in NST under different fertilization treatments by *Tukey*'s HSD post hoc tests ($p < .05$). The NST index has a boundary of 50% to distinguish between more deterministic (<50%) and more stochastic (>50%) assembly. Ctrl, no fertilization; CW, Changwu; FK, Fukang; FQ, Fengqiu; HL, Hailun; NK, nitrogen and phosphorus inputs; NPK, nitrogen, phosphorus, and potassium inputs; QY, Qiyang; SY, Shengyang; YT, Yangting.

($p < .05$) lower under NK treatment than under Ctrl treatment (6 of 7 cases for bacteria and all cases for fungi; Figure 1a,b). The NST values for the protist community were 30.34% and 18.49% higher under NPK treatment than under NK and Ctrl treatments, respectively (Figure 1c, $p < .05$). However, there was a nonsignificant ($p > .05$) difference in the NST values for the nematode community across the three treatments (Figure 1d). Furthermore, the organism groups showed significant declining DDRs characterized by negative slopes, including bacteria ($R^2 = .317$–$.333$, $p < .01$), fungi ($R^2 = .239$–$.381$, $p < .01$), protists ($R^2 = .173$–$.253$, $p < .01$), and nematodes ($R^2 = .102$–$.169$, $p < .01$), indicating lower similarities with increasing distance (Figure S1). The slopes of the bacterial, fungal, and protist communities showed significant differences between NK and NPK treatments (Figure S1, $p < .05$), in contrast to the slope of the nematode community (Figure S1, $p > .05$).

3.2 | The diversity of organism groups

The results of the two-way ANOVA analysis showed significant ($p < .001$) differences in the α-diversity of the biotic community across the different sites (Table 1, Figure S2). This was observed in Shannon, Chao1, and evenness indices of the bacterial and fungal communities ($p < .001$), as well as Shannon and evenness indices of the protist community ($p < .01$) and Shannon index of the nematode community ($p < .001$; Table S3). Overall, fertilization treatments had a nonsignificant effect on the α-diversity of the biotic community ($p = .052$; Table 1), except for Shannon and evenness indices of the bacterial community, and Chao1 index of the fungal and protist communities ($p < .05$; Table S3).

We found that sites, fertilization, and their interactions significantly ($p < .05$) influenced the β-diversity of the overall biotic community, as well as that of the bacterial, fungal, protist, and nematode communities (Table 1; Figure S3). Moreover, the bacterial and protist communities had the strongest responses to sites and fertilization treatments compared to the fungal and nematode communities (Table 1). Correlation analysis showed that TN, OM, pH, and the ratios of C/N, C/P, and N/P showed stronger correlations with β-diversity than with α-diversity in the bacterial and fungal communities. However, pH, AP, and NH_4^+-N were more strongly associated with β-diversity than with α-diversity of the protist and nematode communities (Figure S4).

TABLE 1 Two-way ANOVA of biotic community diversity and soil microbial functional potential.

		Site		Fertilization		Interaction	
		F value	*p* value	*F* value	*p* value	*F* value	*p* value
α-diversity of biotic community	Biotic community α-diversity	20.22	<.001	3.15	.052	7.92	<.001
	Bacteria	169.49	<.001	9.78	<.001	18.48	<.001
	Fungi	11.33	<.001	3.05	.058	2.44	.016
	Protists	3.69	.005	4.94	.012	1.72	.096
	Nematodes	6.85	<.001	0.24	.789	1.17	.338
β-diversity of biotic community	Biotic community β-diversity	51.06	<.001	8.66	<.001	2.37	.02
	Bacteria	1229.46	<.001	3.35	.05	10.2	<.001
	Fungi	510.19	<.001	3.99	.026	5.25	<.001
	Protists	322.93	<.001	10.83	<.001	11.34	<.001
	Nematodes	42.37	<.001	4.79	.013	10.19	<.001
Soil microbial functional potential	Soil microbial functional potential	37.84	<.001	39.02	<.001	9.75	<.001
	Carbon cycle	38.27	<.001	38.43	<.001	9.9	<.001
	Nitrogen cycle	40.95	<.001	41.06	<.001	10.14	<.001
	Phosphorus cycle	38.81	<.001	43.15	<.001	11.12	<.001
	Sulfur cycle	39.1	<.001	40.53	<.001	10.36	<.001
β-diversity of soil microbial functional potential	β-diversity of soil microbial functional potential	40.77	<.001	38.01	<.001	10.3	<.001
	Carbon cycle	41.18	<.001	38.75	<.001	10.45	<.001
	Nitrogen cycle	42.76	<.001	40.26	<.001	10.4	<.001
	Phosphorus cycle	41.45	<.001	41.82	<.001	11.42	<.001
	Sulfur cycle	41.75	<.001	40.97	<.001	10.84	<.001

Note: Interaction represents the interaction effect of site and fertilization treatment on individual parameters. The bacterial, fungal, protist, and nematode community β-diversity were represented by their composition (first principal coordinates, PCoA1). β-diversity of soil microbial functional potential and carbon, nitrogen, phosphorus, and sulfur cycling functional potential composition were represented by PCoA1.

3.3 | Co-occurrence networks of organism groups

We constructed the co-occurrence network to explore the potential niche partitioning and network stability of the biotic community. Our results indicated that the NPK network was the most complex, with the highest number of connections (edges = 16,168) among co-occurring genera (nodes = 954) compared to Ctrl and NK networks (nodes = 871 and 945, edges = 14,427 and 13,884; Figure 2a–c). Notably, the associations between nematodes and bacteria (edges = 171), nematodes and fungi (edges = 20), and protists and bacteria (edges = 66) were higher in NPK network than in Ctrl and NK networks. In addition, the networks showed variation in topological metrics, with the NPK network exhibiting the highest values of average degree, clustering coefficient, network density, and diameter (Figure S5). The ratio of negative edges to positive edges showed a general pattern of Ctrl > NPK > NK (Figure 2a–c). Compared to Ctrl and NK networks, the average degree and natural connectivity were the highest in NPK network (Figure 2d,e), indicating the highest network stability. The numbers of module hubs and connectors were higher in NPK network (6 and 7) than in Ctrl (5 and 5) and NK networks (3 and 4; Figure S6). Network stability, as indicated by average degree and natural connectivity, was positively correlated with TP and AP (R = .271–0.450, $p < .05$), but negatively correlated with NO_3^--N and the ratios of C/N, C/P, and N/P (R = .288–0.765, $p < .01$; Figure S4).

3.4 | Relationships of assembly processes of organism groups with community diversity and network stability

Linear regression analysis showed significant associations between community assembly of organism groups, diversity, and network stability (R^2 = .003–.309, $p < .05$; Figure 3). Differences in α-diversity and β-diversity were significantly positively correlated with pairwise comparisons of stochasticity for the bacterial community (R^2 = .05 and .018, $p < .001$), but negatively correlated with those for fungal, protist, and nematode communities (R^2 = .004 and .309, $p < .05$; Figure 3a–h). Moreover, differences in network stability showed significantly positive correlations with pairwise comparisons of stochasticity of bacterial, fungal, and protist communities (R^2 = .005 to .006, $p < .01$; Figure 3i–k), but a negative correlation with that of nematode community (R^2 = .003, $p < .05$; Figure 3l).

3.5 | Soil microbial functional potential

We observed that DDRs of soil microbial functional potential were significant under Ctrl and NK treatments (R^2 = .042 and .158, $p < .01$). However, there was nonsignificant DDR for soil microbial functional potential under NPK treatment ($p > .05$), indicating that the composition of functional genes was similar across different sites (Figure 4a). The slopes of DDRs were significantly lower under NPK treatment

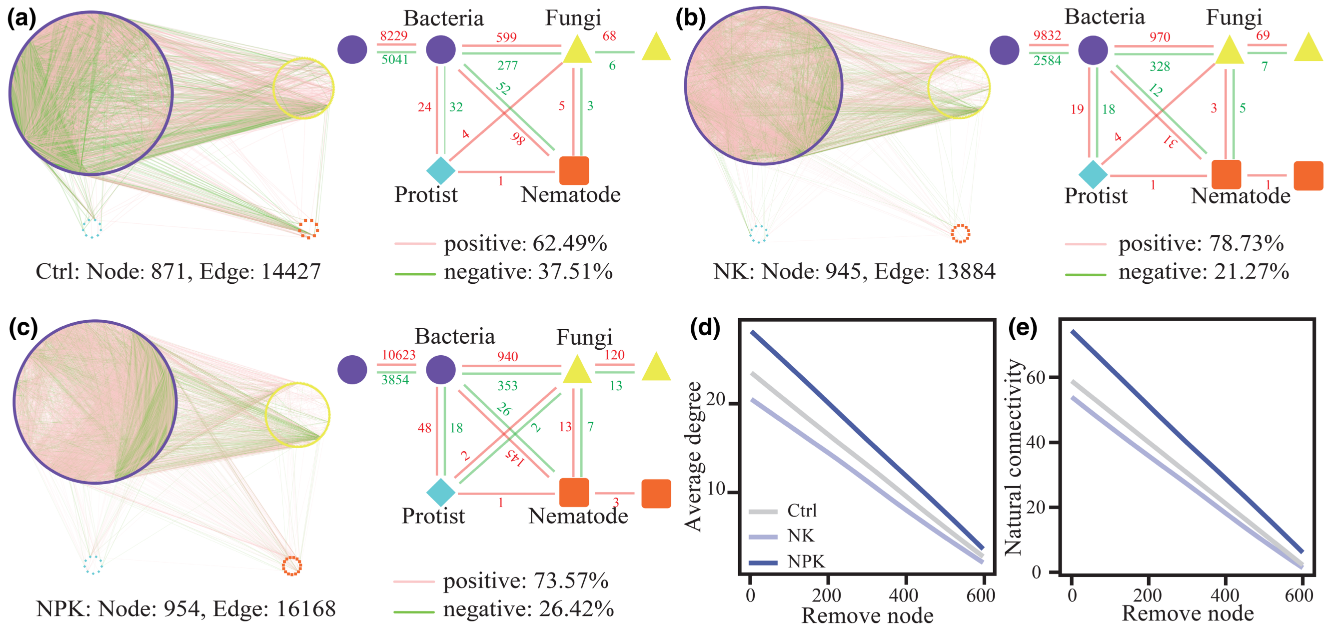

FIGURE 2 Co-occurrence patterns and network stability of biotic community under different fertilization treatments. Co-occurrence patterns of multi-trophic networks under the Ctrl (a), NK (b), and NPK (c) treatments. The node is the genera of bacteria, fungi, protists, and nematodes. A connection between two nodes (i.e., an edge) represents a strong (Spearman's $r > .8$) and significant (adjusted $p < .05$) correlation. Pink edges indicate positive correlations between two nodes, while green edges indicate negative correlations. The right of each network is the summary of the positive and negative edges among bacteria, fungi, protists, and nematodes, and the statistics of the total nodes and edges. The pink and green numbers represent the number of positive and negative edges, respectively. (d, e) Network stability is indicated by average degree and natural connectivity of biotic network. Ctrl, no fertilization; NK, nitrogen and phosphorus inputs; NPK, nitrogen, phosphorus, and potassium inputs.

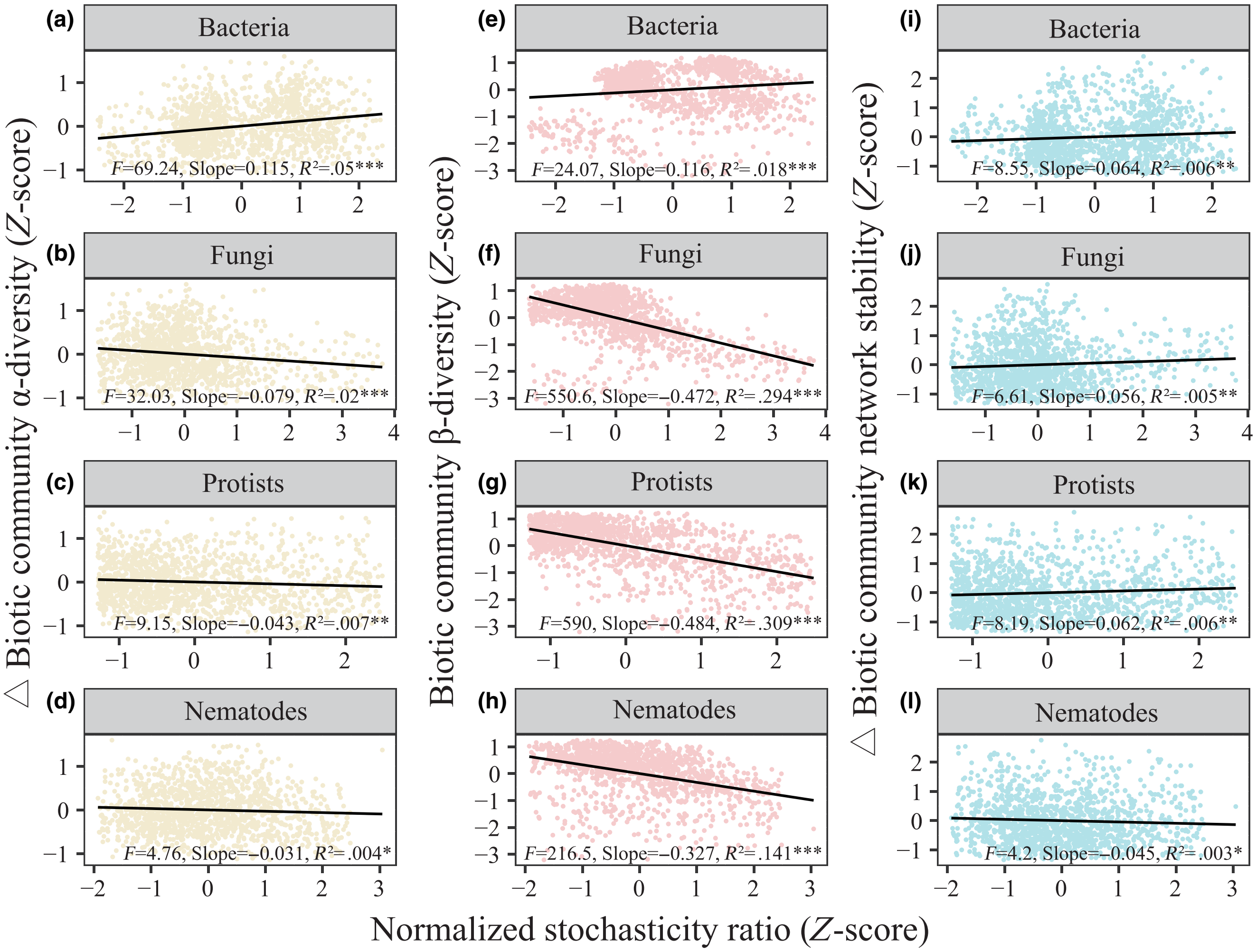

FIGURE 3 Relationships between the assembly processes of organism groups and biotic community diversity and network stability. Relationships of the stochasticity of bacterial (a), fungal (b), protist (c), and nematode (d) communities with the biotic α-diversity. Relationships of the stochasticity of bacterial (e), fungal (f), protist (g), and nematode (h) communities with the biotic community β-diversity. Relationships of the stochasticity of bacterial (i), fungal (j), protist (k), and nematode (l) communities with the network stability of biotic community. Linear regression models (shown as black lines) and correlation coefficients are provided on each panel. $^*p < .05$; $^{**}p < .01$; $^{***}p < .001$.

than under Ctrl and NK treatments (Figure 4a). The DDRs for functional potential related to carbon, nitrogen, phosphorus, and sulfur cycling exhibited a similar pattern to the overall soil microbial functional potential (Table S4). We found that balanced fertilization significantly ($p < .05$) increased the relative abundance of functional genes associated with carbon, nitrogen, phosphorus, and sulfur cycling (Figure 4b; Figure S7). Soil microbial functional potential, as well as functional potential related to carbon, nitrogen, phosphorus, and sulfur cycling, was significantly ($p < .001$) influenced by site, fertilization, and their interactions (Table 1; Figures S8–S10).

3.6 | The impacts of soil properties and biotic communities on soil microbial functional potential

Fertilization treatments significantly influenced the α-diversity of the biotic community (4 of 7 cases) and soil microbial functional potential (all cases), but the observed effects of fertilization on these factors were not consistent at each site (Figure S11, $p < .05$). There was a nonsignificant ($p > .05$) correlation between the α-diversity of the biotic community and soil microbial functional potential across all samples (Figure 5a). Significant positive relationships were observed between the β-diversity of the biotic community and the β-diversity of soil microbial functional potential ($R^2 = .018$, $p < .001$), and between network stability and soil microbial functional potential ($R^2 = .594$, $p < .001$; Figure 5b,c). Random forest modeling determined the highly significant ($p < .01$) predictors of soil microbial functional potential, including soil properties (AP, pH, TP, OM, TK and the ratios of C/N, C/P, and N/P), bacterial Shannon and evenness indices, fungal Chao1 index, protist and nematode evenness index, β-diversity of all organism groups, average degree, and natural connectivity (Figure 5d).

We combined with the analysis of correlation, linear regression, and random forest modeling; PLS-SEM was applied to evaluate the

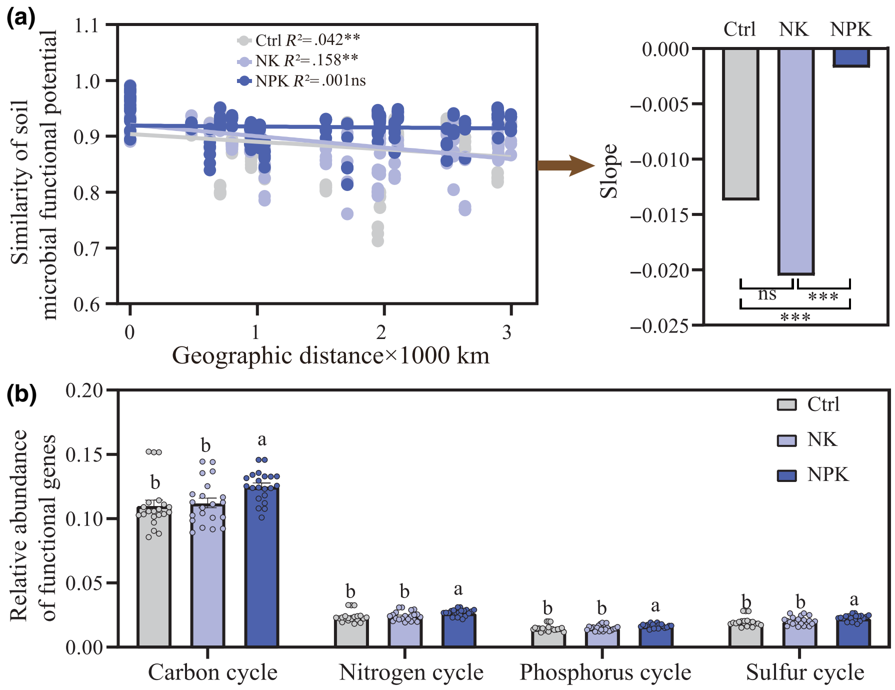

FIGURE 4 Distance decay curves of soil microbial functional potential and different functional genes under different fertilization treatments. (a) Similarity of soil microbial functional potential against geographic distances among sampling sites. The bar graph represents the value of the slope. The slopes were pairwise compared among three fertilization treatments by permutation tests. Asterisks denote significant correlations. (b) The relative abundance of functional genes associated with carbon, nitrogen, phosphorus, and sulfur cycling under different fertilization treatments. Different lowercase letters represent significant differences of individual parameters under fertilization treatments by *Tukey*'s HSD post hoc tests ($p<.05$). Ctrl, no fertilization; NK, nitrogen and phosphorus inputs; NPK, nitrogen, phosphorus and potassium inputs. $^*p<.05$; $^{**}p<.01$; $^{***}p<.001$.

direct and indirect effects of soil factors (AP, TP, and the ratios of C/P and N/P) and biotic communities (β-diversity and network stability) on soil microbial functional potential (Figures 6 and 7). The overall model fit was strong with a GoF value of 0.641 (Table S5). Our results indicated that AP and TP were positively associated with the network stability of the biotic community (path coefficient = 0.088, $p<.001$; Figure 6). Furthermore, AP and TP were positively correlated with the stochastic processes of bacterial, fungal, and protist communities (path coefficient = 0.111 to 0.138, $p<.001$), but negatively correlated with that of nematode community (path coefficient = −0.193, $p<.01$). Soil nutrient stoichiometry (the ratios of C/P and N/P) exhibited negative relationships with the network stability (path coefficient = −0.095, $p<.001$) and stochastic processes of the biotic community (path coefficient = −0.215 to −0.430, $p<.001$), but a positive relationship with the biotic β-diversity (path coefficient = 0.103, $p<.001$). Notably, we revealed that the network stability was positively correlated with the stochastic processes of bacterial, fungal, and protist communities (path coefficient = 0.065 to 0.177, $p<.05$), but negatively correlated with that of nematode community (path coefficient = −0.077, $p<.01$; Figure 6). However, the β-diversity showed positive relationships with the stochastic process of the bacterial community (path coefficient = 0.353, $p<.001$), but displayed negative relationships with those of fungal, protist, and nematode communities (path coefficient = −0.112 to −0.262, $p<.05$). Importantly, soil microbial functional potential was positively correlated with network stability (path coefficient = 0.469, $p<.001$) and β-diversity (path coefficient = 0.068, $p<.05$) of the biotic community.

4 | DISCUSSION

4.1 | Community assembly, diversity, and network of biotic community in response to nutrient input

Long-term nutrient input has been demonstrated to decrease the relative importance of stochasticity within bacterial and fungal communities (Liu et al., 2021). Insufficient P availability limits microbial metabolic activity and exerts significant stress on microbial communities through environmental filtering (Feng et al., 2017). Conversely, a balanced nutrient input mitigates the impact of environmental stress on community assembly (Liu et al., 2015). As P availability increases, the community may exhibit greater stochasticity to counteract the influence of niche partitioning (Zhou et al., 2014). However, the NST and DDR pattern of the nematode community did not significantly respond to nutrient input, which appears to contradict the first hypothesis. Nevertheless, the nematode community exhibited a significant DDR pattern under all three fertilization treatments, indicating that microfaunal community composition may be strongly affected by geographic factors, such as local climate

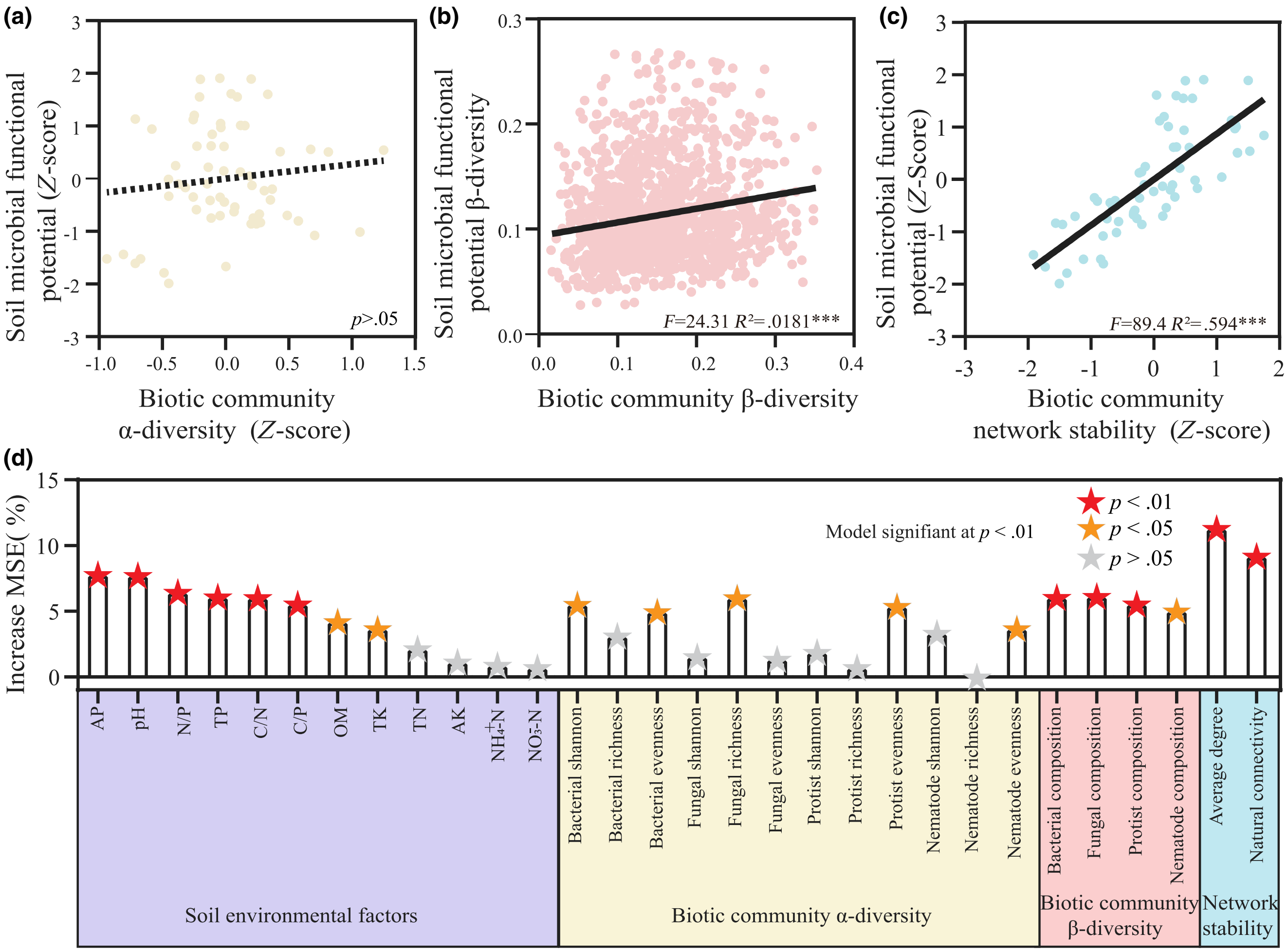

FIGURE 5 Driving factors of soil microbial functional potential based on linear and random forest modeling. (a) Correlation between the biotic community α-diversity (*Z*-score) and soil microbial functional potential (*Z*-score) across all samples. (b) Correlation between the biotic community β-diversity and the soil microbial functional potential β-diversity across all samples. (c) Relationships between network stability (*Z*-score) and soil microbial functional potential (*Z*-score) across all samples. (d) Mean predictor importance (% of increased mean square error, MSE) of abiotic and biotic factors on soil microbial functional potential. AK, available potassium; AP, available phosphorus; C/N, the ratio of soil organic carbon to total nitrogen; C/P, the ratio of soil organic carbon to total phosphorus; N/P, the ratio of total nitrogen to total phosphorus; NH_4^+-N, ammonia nitrogen; NO_3^--N, nitrate nitrogen; OM, organic matter; TK, total potassium; TN, total nitrogen; TP, total phosphorus. *$p < .05$; **$p < .01$; ***$p < .001$.

and soil type (Figure 1d; Figure S1). However, the influence of fertilization on nematode communities may not be consistent across contrasting soil types and locations (Sarathchandra et al., 2001; Zhao et al., 2014). This result underscored the importance of local conditions and the pitfalls of extrapolating results from a single site to other environments has its limitations when studying the nematode community.

It was not surprising that the diversity of the biotic community significantly varied across the seven sites (Figures S2 and S3; Table 1; Table S3), as soil acidity and type have been reported to cause changes in the belowground biota (Bardgett & Van Der Putten, 2014; Oliverio et al., 2020). Nutrient addition caused little or only marginal effect on microbial α-diversity, but it did significantly alter β-diversity. Balanced nutrient input results in the homogeneous selection of copiotrophic species, favoring their survival (Feng et al., 2017). Phosphorus-deficient conditions can promote the growth of oligotrophic species, leading to different community compositions (Leff et al., 2015). Concomitantly, fertilization-induced changes in the soil environment have a more pronounced effect on β-diversity than α-diversity due to soil pH and nutrient stoichiometry (in 't Zandt et al., 2019; Liu et al., 2018).

Network analysis can be applied to identify co-occurrence patterns and potential interactions in a complex biotic community (Faust & Raes, 2012). Our study focused on a complex multi-trophic network that included bacteria, fungi, protists, and nematodes, rather than a single taxonomic network. Our findings revealed that the NPK network displayed the highest topological metrics and module hubs, indicating highly complex and robust network stability (Deng et al., 2012; Peng & Wu, 2016). Additionally, we found that the associations between different organism groups were the strongest in the balanced fertilization treatment (Figure 2). In the belowground ecosystem, nutrient availability can influence

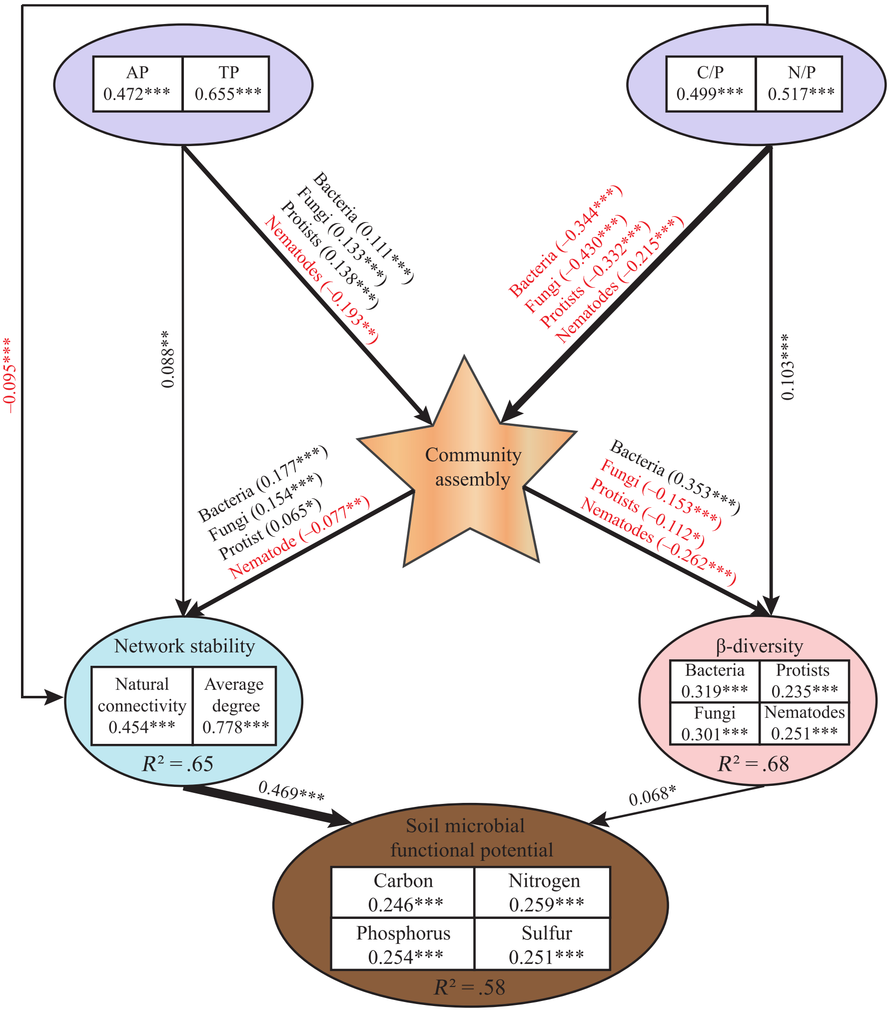

FIGURE 6 Soil environmental factors mediate the soil microbial functional potential through the bacterial, fungal, protist, and nematode communities using structural equation modeling. The ellipses represent the latent variables, and the rectangles represent the observed variables. The symbol of the pentagram represents the community assembly of four organism groups. The data below the observation variable in the white box represent the weight. Black lines indicate significant relationships. The text on the black line represents the path coefficient, the black text represents the positive effect, while the red text represents the negative effect. Paths with nonsignificant coefficients are not presented. Community assembly is represented by normalized stochasticity ratio. The β-diversity is represented by the Bray–Curtis dissimilarity of bacterial, fungal, protist, and nematode communities. Network stability is represented by average degree and natural connectivity of biotic network. AP, available phosphorus; C/P, the ratio of soil organic carbon to total phosphorus; N/P, the ratio of total nitrogen to total phosphorus; TP, total phosphorus. *$p < .05$; **$p < .01$; ***$p < .001$.

biodiversity across multi-trophic levels through bottom-up forces (Schulz-Bohm et al., 2017). An ample supply of nutrients likely leads to rapid growth of organisms in the basal trophic level, thereby increasing the populations of their predators and enhancing potential multi-trophic interactions (Geisen et al., 2021). Positive and negative edges are often considered indicative of potential cooperative and competitive relationships in the network (Chen et al., 2019). The balance between cooperation and competition in multiplex networks is the fundamental mechanism for species coexistence (Godoy et al., 2018). The presence of moderately positive and negative edges in the NPK network resulted in a greater number of coexisting species and a more stable network structure.

4.2 | The influence of assembly processes on diversity and network stability

Partially supporting our second hypothesis, the stochastic process of the bacterial community and the deterministic processes of the fungal, protist, and nematode communities enhanced β-diversity. In contrast, the stochastic processes of the bacterial, fungal, and protist communities and the deterministic process of the nematode community promoted network stability (Figures 3 and 6). The stochastic process indicates that when bacteria occupy similar abilities to compete for common resources, they can coexist in highly overlapping ecological niches without eliminating species due to competition, which supports higher bacterial diversity (Chase & Myers, 2011; Jiao et al., 2020). The heightened bacterial diversity, in turn, amplifies metabolic networks and nutrient availability, thereby promoting the stable coexistence of bacteria with specialized functions (Xun et al., 2019). In addition, bacteria are more affected by stochastic processes due to their smaller size, and the random colonization or extinction events can result in ecological drift and dispersal limitation, ultimately contributing to high levels of β-diversity (Chase, 2010; Martiny et al., 2011). Conversely, the rise in deterministic processes within the eukaryotic community, characterized by larger body sizes, is linked to habitat heterogeneity that creates diverse ecological niches across regions, promoting β-diversity (Chase, 2010). A microbial community dominated by stochastic processes tends to exhibit greater stability within the complex ecological network in the face of environmental perturbations (Li et al., 2020; Pandit et al., 2009). Nematodes are essential components of soil food webs, participating in complex trophic interactions with organisms, such as plants and microorganisms (Klass

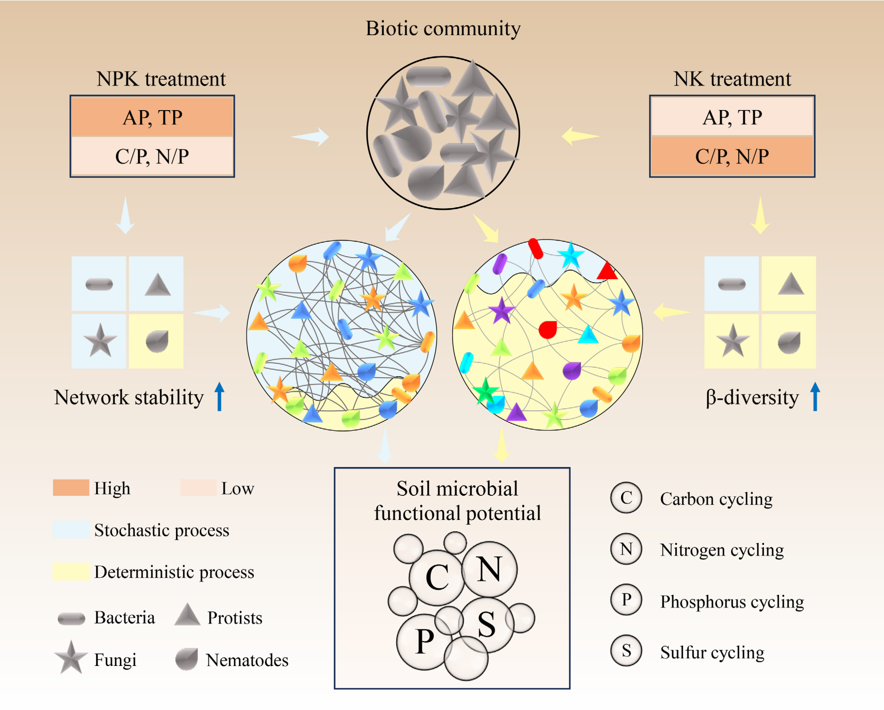

FIGURE 7 A conceptual figure displays the proposed dual mechanisms that regulate soil microbial functional potential. One mechanism indicates that the high phosphorus levels (AP and TP) and low nutrient stoichiometry (C/P and N/P ratios) may increase the stochastic processes of bacterial, fungal, and protist communities and deterministic process of the nematode community under balanced fertilization (NPK) treatment, ultimately enhancing soil microbial functional potential by reinforcing network stability of the biotic community. The other mechanism indicates that the low phosphorus levels (AP and TP) and high nutrient stoichiometry (C/P and N/P ratios) may increase the stochastic process of the bacterial community and deterministic processes of the fungal, protist, and nematode communities under phosphorus-deficient fertilization (NK) treatment, thereby enhancing soil microbial functional potential by improving the biotic β-diversity. AP, available phosphorus; C/P, the ratio of soil organic carbon to total phosphorus; N/P, the ratio of total nitrogen to total phosphorus; TP, total phosphorus.

et al., 2012). Stochastic fluctuations in nematode communities may cause disruptions in these trophic interactions, thereby affecting the overall stability of the biotic network (de Vries et al., 2013).

4.3 | Dual mechanisms of biotic community affecting soil microbial functional potential

Taking multi-trophic species into account in the network can improve our ability to predict soil microbial functional potential in agricultural ecosystems. Our study suggested that soil environmental factors altered the assembly processes of organism groups, thereby influencing the soil microbial functional potential through network stability and β-diversity of the biotic community (Figure 6). We have created a conceptual figure to illustrate the dual mechanisms that regulate soil microbial functional potential with varying soil environmental factors (Figure 7). One mechanism indicated that high phosphorus levels and low nutrient stoichiometry (C/P and N/P ratios) increased the stochastic processes of bacterial, fungal, and protist communities and the deterministic process of the nematode community under balanced fertilization (NPK) treatment, which enhanced soil microbial functional potential by reinforcing the network stability of the biotic community (Figure 7). Increasing soil P resources boosts nutrient availability for soil microorganisms and accelerates their metabolic activities, ultimately improving the stochastic processes of the microbial community and enhancing network stability (Yang & van Elsas, 2018). However, phosphorus addition can also cause environmental stress (e.g., salt toxicity) and increase the deterministic process of the nematode community (Sarathchandra et al., 2001; Zhao et al., 2014), which may improve network stability through top-down regulation (Karakoç et al., 2020). Additionally, low nutrient stoichiometry (C/P and N/P ratios) can foster complex and stable ecological networks between nematodes and microorganisms, with higher average degree and natural connectivity (de Vries et al., 2018; Xun et al., 2021). Stable multi-trophic networks can improve soil microbial functional potential by enhancing nutrient cycling and carbon utilization efficiency in agroecosystems (Chen et al., 2022; Cotrufo et al., 2013), as guild interactions are considered important drivers of ecosystem functioning (Allen et al., 2022).

As an alternative mechanism, low phosphorus levels and high nutrient stoichiometry (C/P and N/P ratios) enhanced the stochastic process of the bacterial community and the deterministic processes of the fungal, protist, and nematode communities under phosphorus-deficient fertilization (NK) treatment, which improved the soil microbial functional potential by increasing the β-diversity of the biotic community (Figure 7). In the absence of external phosphorus resources, the enhanced deterministic processes in fungal, protist, and nematode communities may be attributed to the intensified competition for phosphorus (Wang et al., 2023). Through the evolution of specific mechanisms, such as symbiotic associations, nutrient uptake efficiency, or phosphorus storage strategies, these eukaryotic organisms have developed diverse survival strategies, contributing to an increase in β-diversity (Eldridge et al., 2018). Furthermore, high soil nutrient stoichiometry has the potential for creating environmental heterogeneity to influence the P-driven deterministic processes, thereby contributing to the formation of distinct niches for various organisms and fostering the biotic β-diversity (Nguyen et al., 2020; Zhang et al., 2020). Phosphorus-deficient environments usually have higher β-diversity, fostering the survival of specialized species and offering unique functional potential (Souza et al., 2008). High β-diversity is crucial for maintaining multiple functions in the local environment since there is no ubiquitous combination of species capable of supporting all functions simultaneously (Mori et al., 2018). As such, the contribution of multi-trophic networks and β-diversity to soil microbial functional potential under different management regimes may hold considerable ecological importance and warrant further investigation.

AUTHOR CONTRIBUTIONS

Lingyue Zhu: Conceptualization; data curation; formal analysis; visualization; writing – original draft; writing – review and editing. **Lu Luan:** Writing – review and editing. **Yan Chen:** Data curation; formal analysis; funding acquisition; investigation; project administration; resources; supervision. **Xiaoyue Wang:** Visualization. **Shungui Zhou:** Methodology; writing – review and editing. **Wenxiu Zou:** Investigation. **Yinghua Duan:** Investigation. **Bo Zhu:** Investigation. **Yan Li:** Investigation. **Wenzhao Liu:** Investigation. **Jizhong Zhou:** Methodology; resources. **Jiabao Zhang:** Funding acquisition; investigation; project administration; resources; supervision; writing – review and editing. **Yuji Jiang:** Conceptualization; funding acquisition; writing – original draft; writing – review and editing. **Bo Sun:** Conceptualization; formal analysis; funding acquisition; investigation; resources; supervision.

ACKNOWLEDGMENTS

This research was financially supported by the Strategic Priority Research Program of Chinese Academy of Sciences (XDA0440203), National Key Research and Development Program of China (2022YFD1900603 and 2022YFD1900601), National Natural Science Foundation of China (42177298), Double Thousand Plan of Jiangxi Province (jxsq2023201046), and Youth Innovation Promotion Association of CAS (Y2021084). In memoriam of the distinguished Professor Bo Sun, we acknowledge his pivotal role in the development of this work. His insights and expertise will always be a cherished part of this research.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The raw sequence data of bacteria, fungi, protists, and nematodes were deposited to the National Center for Biotechnology Information (NCBI) under PRJNA747872, PRJNA747891, PRJNA747906, and PRJNA747905, respectively. Data on the relative abundance of genes related to the carbon, nitrogen, phosphorus, and sulfur cycle can be accessed at https://doi.org/10.6084/m9.figshare.21502401.v1.

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SUPPORTING INFORMATION

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

How to cite this article: Zhu, L., Luan, L., Chen, Y., Wang, X., Zhou, S., Zou, W., Han, X., Duan, Y., Zhu, B., Li, Y., Liu, W., Zhou, J., Zhang, J., Jiang, Y., & Sun, B. (2024). Community assembly of organisms regulates soil microbial functional potential through dual mechanisms. *Global Change Biology*, *30*, e17160. https://doi.org/10.1111/gcb.17160