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ABSTRACT

Body size is an important life-history trait that affects organism niche occupancy and ecological interactions. However, it is still unclear to what extent the assembly process of organisms with different body sizes affects soil biogeochemical cycling processes at the aggregate level. Here, we examined the diversity and community assembly of soil microorganisms (bacteria, fungi, and protists) and microfauna (nematodes) with varying body sizes. The microbial functional potential associated with carbon, nitrogen, phosphorus, and sulfur metabolism within three soil aggregate sizes (large macroaggregates, > 2 mm; small macroaggregates, $0.25-2$ mm; and microaggregates, < 0.25 mm) were determined by metagenomics. We found that the smallest microbes (bacteria) had higher α-diversity and lower β-diversity and were mostly structured by stochastic processes, while all larger organisms (fungi, protists, and nematodes) had lower α-diversity and were relatively more influenced by deterministic processes. Structural equation modeling indicated that the microbial functional potential associated with carbon, nitrogen, phosphorus, and sulfur metabolism was mainly influenced by the bacterial and protist diversity in microaggregates. In contrast, the microbial functional potential was primarily mediated by the assembly processes of four organism groups, especially the nematode community in macroaggregates. This study reveals the important roles of soil organisms with different body sizes in the functional potential related to nutrient cycling, and provides new insights into the ecological processes structuring the diversity and community assembly of organisms of different body sizes at the soil aggregate level, with implications for soil nutrient cycling dynamics.

1. Introduction

Soils are among the most biodiverse systems on earth, housing billions of individual organisms, including bacteria, fungi, protists, and nematodes (Bardgett et al., 2014). Body size is a fundamental eco-physiological trait that affects the life history, metabolism, physiology, community biodiversity, and ecology of organisms (Peters, 1985;

Saleem et al., 2013). Larger (micro)organisms (fungi and nematodes) may have a relatively narrow niche breadth, while smaller microorganisms (bacteria) can occupy a wider variety of spatial structures in the soil (Luan et al., 2020). Furthermore, the diversity of these organisms has been widely recognized to be associated with essential biogeochemical processes, including carbon, nitrogen, phosphorus, and sulfur cycling (Zhao et al., 2014; Guan et al., 2017; Gong et al., 2023). Despite

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several studies that have described patterns of community α-diversity (within-site diversity) and β-diversity (between-site diversity) in soils, less attention has been paid to changes in community characteristics of different body-sized organisms at the micro-scale (Xun et al., 2019). Environmental heterogeneity and dispersal dynamics play central roles in organizing naturally diverse communities, with cascading effects on β-diversity and ecosystem functioning (Wagg et al., 2019). Understanding how and to what extent the size-dependent traits of organisms drive the community α-diversity and β-diversity is one of the major challenges in linking organisms to ecosystem functioning.

Experimental evidence has demonstrated that organism body size influences the interplay of deterministic and stochastic processes that structure community assembly (Xun et al., 2019; Luan et al., 2020; Zhao et al., 2021), and consequently affects ecosystem functioning (Cadotte et al., 2006, 2011; De Bie et al., 2012). Deterministic assembly emphasizes ecological selection imposed by environmental filtering and biotic interactions, while stochastic assembly is associated with random changes in species composition through ecological drift and random dispersal (Nemergut et al., 2013). The assembly patterns of various organism groups, encompassing bacteria, fungi, protists, and nematodes across different spatial scales, are highly dependent on environmental filtering (nutrient quality/availability in different soil environments) and dispersal mode (Zinger et al., 2019). Several studies have shown that smaller organisms move faster than larger ones, resulting in a negative size-dispersal relationship (De Bie et al., 2012; Luan et al., 2020). However, recent efforts to link the microbial community assembly to ecosystem functions through environmental selection and dispersal patterns have yielded inconclusive results (Bier et al., 2015; Rocca et al., 2015; Yue et al., 2023), limiting our understanding of changes in ecosystem functions. Our goal is to improve our understanding of community ecosystem functions by uncovering the mechanisms of community assembly of soil organisms.

Soil aggregate structure depends on a combination of mineral particles (sand, silt, and clay) and organic matter, which form aggregates of varying sizes and stability (Tisdall et al., 1982). Soil physical structure, including the distribution and type of microaggregates and macroaggregates, is a critical determinant of ecosystem processes that govern soil fertility and nutrient cycling. These different soil aggregates create a wide range of microhabitats for microorganisms and microfauna, characterized by differences in soil organic matter (SOM) content, size, and composition (Davinic et al., 2012) that affect water potential, oxygen concentrations, and biological interactions (e.g., predation pressure) (Balesdent et al., 2000; Ranjard et al., 2001). Soil aggregates with different physical structures and nutrient characteristics can affect the diversity and composition of soil microbial and nematode communities (Bahram et al., 2018; Wan et al., 2022). Yet, it remains poorly understood how the community assembly of soil organisms with distinct body sizes regulates soil biogeochemical processes at the soil aggregate level.

Here, we investigated how the deterministic and stochastic processes mediated the community assembly of different-sized soil organisms at the aggregate level, including bacteria, fungi, protists, and nematodes. In this study, we used a 19-year field experiment under four fertilization regimes and separated soil samples into three aggregate sizes (large macroaggregates, small macroaggregates, and microaggregates) for determining the microbial and nematode communities. Although previous studies explored the effect of fertilization treatments on the composition of soil organismal community and ecological functions, we gave more attention to the relationships between the body size of soil organisms, community assembly processes, and microbial functional potential. This study aimed to address the following questions: (*i*) Is there a significant relationship between body size and diversity patterns of organism groups across and within soil aggregates? (*ii*) Does the relative influence of distinct ecological processes vary in mediating the assembly of soil communities with different body sizes? and (*iii*) What are the relationships of body size-driven diversity and community assembly with the microbial functional potential related to carbon,

nitrogen, phosphorus, and sulfur metabolism? We hypothesized that organism body size would be strongly associated with community diversity and assembly processes in soil aggregates. In particular, the microbial functional potential would be primarily associated with the small-sized bacterial and protist communities in microaggregates, but correlated with four organism groups (bacteria, fungi, protists, and nematodes) in macroaggregates.

2. Materials and methods

2.1. Experimental design and soil sampling

Soil samples were collected from a long-term fertilization experiment located at the Yingtan National Agroecosystem Field Experiment Station (28°15′20″N, 116°55′30″E) in Jiangxi Province, China. The experimental site is located in a typical subtropical climate with mean annual temperature and precipitation of 17.6°C and 1795 mm. The soil is classified as ferric acrisol according to the Food and Agricultural Organization of the United Nations (FAO) classification system. The long-term field experiment followed a completely randomized design with four manure treatments (three replicates), including (1) no manure (M0), (2) low manure amount with 150 kg N ha^{-1} y^{-1} (M1), (3) high manure amount with 600 kg N ha^{-1} y^{-1} (M2), and (4) high manure amount with 600 kg N ha^{-1} y^{-1} and lime application (3000 kg Ca(OH)$_2$ ha^{-1} 3 y^{-1}) (M3). The experimental system was established in 2002, consisting of 12 plots of 2 m length × 2 m width. Manure amendments consisted of pig manure containing an average of 386.5 g kg^{-1} total carbon and 32.2 g kg^{-1} total nitrogen. The field was annually planted with maize as a monoculture from April to July. The maize grains in each plot were manually harvested, i.e., manual picking and shelling. No management practices were applied to these plots except for periodic manual weed removal.

Soil samples were collected at 0−20 cm depth after maize harvest in July 2021. Ten randomized soil cores (5 cm in diameter) were collected from each plot and pooled to create one composite sample. Fresh samples were immediately chilled and transported to the laboratory in an insulated cooler with ice packs (< 12 h). Soils were sieved through a 4-mm sieve to remove large roots and gravel and then homogenized. Each soil sample was manually fractionated into three aggregate sizes, including large macroaggregates (>2 mm, LA), small macroaggregates (0.25−2 mm, SA), and microaggregates (<0.25 mm, MA) (Jiang et al., 2013). The fresh soil was placed on a 2000 μm sieve and horizontally sieved for 5 min. The soil that passed through the 2000 μm sieve was then transferred to a 250 μm sieve for further fractionation. Each of the 36 soil samples (4 manure treatments × 3 aggregate fractions × 3 replicates) was divided into three subsamples for analysis of soil chemical properties and the microbial and nematode communities.

2.2. Soil chemical properties

Soil chemical properties were detected according to the procedure described by Lu (2000). In brief, soil pH was determined in a 1:2.5 (*w/v*) soil:water ratio using a glass electrode. Soil organic matter (SOM) was titrated against a ferrous iron solution and digested with K$_2$Cr$_2$O$_7$-H$_2$SO$_4$. Total nitrogen (TN) and alkaline hydrolyzable nitrogen (AN) were determined by the Kjeldahl nitrogen method and alkaline hydrolysis diffusion method, respectively. Total phosphorus (TP) and available phosphorus (AP) were extracted with HF-HClO$_4$ and sodium bicarbonate, respectively, and then detected by the molybdenum-blue method.

2.3. Illumina sequencing and bioinformatics

Fresh soil (0.5 g) was used for genomic DNA extraction using the Power Soil DNA Extraction Kit (Mo Bio Laboratories, CA, USA), according to the manufacturer's protocol. The quality and quantity of DNA

were determined using a NanoDrop spectrophotometry. The V4–V5 region of the bacterial 16 S rRNA gene was amplified using primer pairs 515 F and 907 R (Biddle et al., 2008), the internal transcribed spacer (ITS) region of the fungal rRNA gene was amplified using ITS1F and ITS2 (Gardes et al., 2008), and the protist 18 S rRNA gene was amplified using TAReuk454FWD1 and TAReukREV3 (Stoeck et al., 2010). The forward and reverse primers were tagged with an adapter and linker sequence, and 8-bp barcode oligonucleotides were included to distinguish between amplicons from different soil samples. Polymerase chain reaction mixtures (20 µl) contained 4 µl of 5× FastPfu buffer, 2 µl of 2.5 mM dNTPs, 0.25 µl of each primer (10 µM), 0.4 µl FastPfu polymerase, and 10 ng template DNA. Each amplicon was purified and combined in equimolar concentration for library preparation and sequenced with Illumina MiSeq.

Raw sequence data were processed using the Quantitative Insights into Microbial Ecology (QIIME) pipeline (v1.9.1) (Caporaso et al., 2010). Sequences that fully matched the barcodes were selected and distributed into separate files for the bacterial 16 S rRNA gene, fungal ITS region, and protist 18 S rRNA gene. Additional sequence processing was performed, including quality trimming, demultiplexing, and taxonomic assignment. QIIME quality trimming was performed according to the following criteria: (1) truncated before three consecutive poor quality bases and re-evaluated for length, (2) no ambiguous bases, and (3) minimum sequence length of 469 bp (16 S rRNA), 307 bp (ITS), and 516 bp (protist) after trimming. The assembled reads were subjected to de novo chimera detection using UCHIME (Edgar et al., 2011), and the remaining sequences were further corrected for frameshifts using HMM-FRAME (Zhang et al., 2011). The sequences from each sample were clustered into operational taxonomic units (OTUs) at the 97% cut-off level (Li et al., 2006). Bacterial 16 S rRNA sequences were assigned using Silva (release 138) (Quast et al., 2013), and fungal ITS and protist 18 S rRNA sequences were assigned using UNITE (version 7) (Nilsson et al., 2019) and PR2 (Guillou et al., 2012), respectively. The α-diversity and β-diversity (based on Bray-Curtis dissimilarity) for the microbial community were determined after rarifying to equal sequencing depth per sample. Overall, a total of 11,628 bacterial, 975 fungal, and 1907 protist OTUs per sample were observed out of 2372, 805, 1570,494, and 1440,323 high-quality sequences, respectively.

2.4. Nematode assemblages

Nematodes were extracted from different soil aggregates on all samples using a modified Baermann funnel method (Barker et al., 1985). Briefly, 100 g of soil samples were placed on a cotton filter over a circular wire mesh on top of a Baermann funnel, and distilled water was poured into the funnel to about half of the conical part of the funnel. Nematodes were allowed to swim out of the soil sample and sink through the filter into the water in the funnel for 48 h, and then clean suspensions were prepared for counting and identifying nematodes. Nematode functional groups (bacterivores, fungivores, plant parasites, and omnivores-predators) were identified based on the esophagus and stomach morphology (Yeates et al., 1993). Nematode numbers were expressed as individuals per 100 g of dry soil.

2.5. Organism body size

Given the unfeasibility of obtaining individual body size information for each taxon based on taxonomic assignments and/or morphological descriptions, we partitioned the taxonomic affiliation of bacterial, fungal, and protist communities at the phylum level. We focused on the dominant phyla with relative abundances higher than 1% because functional traits of body size and trophic categories are typically conserved at the phylum level (Briones et al., 2014; Martiny et al., 2015; Zinger et al., 2019). Nematodes were grouped by functional traits because their body size is usually conserved within functional groups (Zhao et al., 2015; van den Hoogen et al., 2019).

2.6. Community assembly modeling

The relative influences of deterministic-stochastic balance on community assembly were determined using an abundance-based β-null model with the 'vegan', 'reldist', and 'bipartite' packages in R (Dini-Andreote et al., 2015; Luan et al., 2020). The null scenario involved random resampling of taxa within the complete community dataset, while maintaining sample richness and number of reads. We calculated the average Bray-Curtis dissimilarities of 999 simulated communities to obtain null expectations of community dissimilarities for each pair of samples. The null deviation value (NDV) is defined as the difference between the observed and averaged null dissimilarities (Tucker et al., 2016; Chen et al., 2020). NDV values close to 0 indicate a predominant influence of stochasticity, whereas values close to −1 or +1 indicate a substantial influence of determinism driving community assembly.

2.7. Niche breadth and dispersal rates

We estimated Levins' niche breadth index for the four organism groups according to Jiao et al. (2020). The community level *B*-value (B_{com}) was calculated as the average of the niche breadth index of all taxa present in a community using the 'spaa' package in R (Zhang and Zhang, 2013). A high B_{com} indicates a large habitat niche breadth. The Sloan neutral model was used to assess the contribution of passive dispersal to community assembly by predicting the relationship between the frequency with which each taxon occurs in a set of local communities and its abundance in the metacommunity (Sloan et al., 2007; Luan et al., 2023). The dispersal rate (*m*) refers to the probability that a random loss of an individual in a local community will be replaced by an immigrant from the metacommunity. The *m* value was calculated by observed OTU distributions and mean relative abundances. This analysis was performed using nonlinear least squares fitting with the 'minpack.lm' package. Calculation of 95% confidence intervals for the model predictions was performed using the 'Hmisc' package. The overall fit of the model was determined by comparing the residual sum of squares to the total sum of squares. The best-fitting models were determined by Akaike's information criterion based on 1000 bootstrap replicates.

2.8. Metagenome sequencing

Soil samples were subjected to shotgun metagenomics. For each sample, 1 µg of genomic DNA was used to generate sequencing libraries using the Nextera XT DNA Library Prep Kit following the manufacturer's protocols. Metagenomic sequencing was carried out on an Illumina HiSeq 4000 platform with read lengths of 150 bp to a target data size of 10 GB. Raw reads were trimmed using Sickle software to remove reads with average quality scores below 20 and less than 50 bp in length. Open reading frames (ORFs) were detected using MetaGeneMark (version 3.26) (Besemer and Borodovsky, 1999). Functional composition profiles were performed according to the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (Kanehisa et al., 2000) using DIA-MOND (version 0.9.29) (Buchfink et al., 2015). The number of each ORF was calculated as the number of base pairs mapped to the corresponding scaffold region divided by the length of the ORFs. Gene abundance was calculated as the average value of the matched ORFs. Gene abundances were summed to a total of 4150 KEGG Orthology (KO) genes that were KEGG-defined functional units. We focused the annotation on KEGG pathways associated with carbon, nitrogen, phosphorus, and sulfur metabolism according to the KEGG database and MetaCyc database. The soil microbial functional potential was determined by calculating the average values of functional genes following Z-score transformation. The Z-score transformation method is frequently applied to transform pre-normalized data, due to its advantageous statistical properties. These included the normal distribution of average Z-scores, a weak correlation between the means and variances of these averages, and the
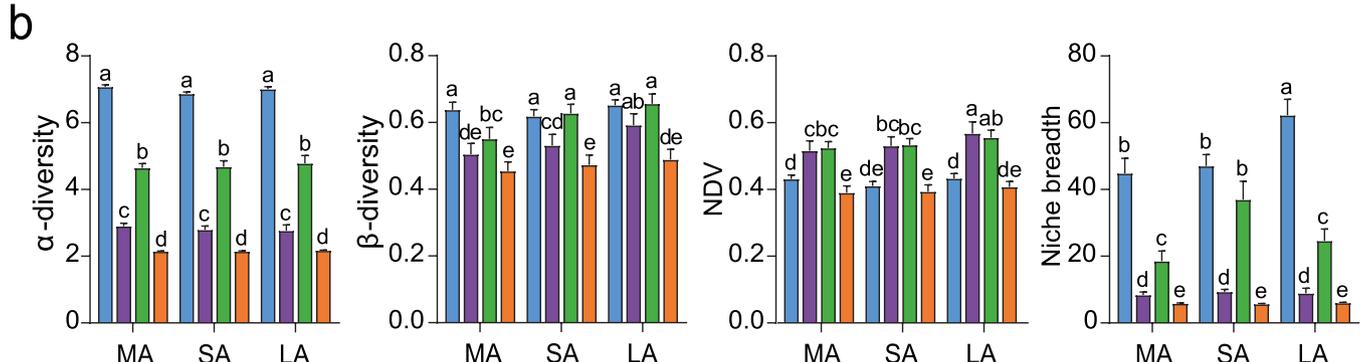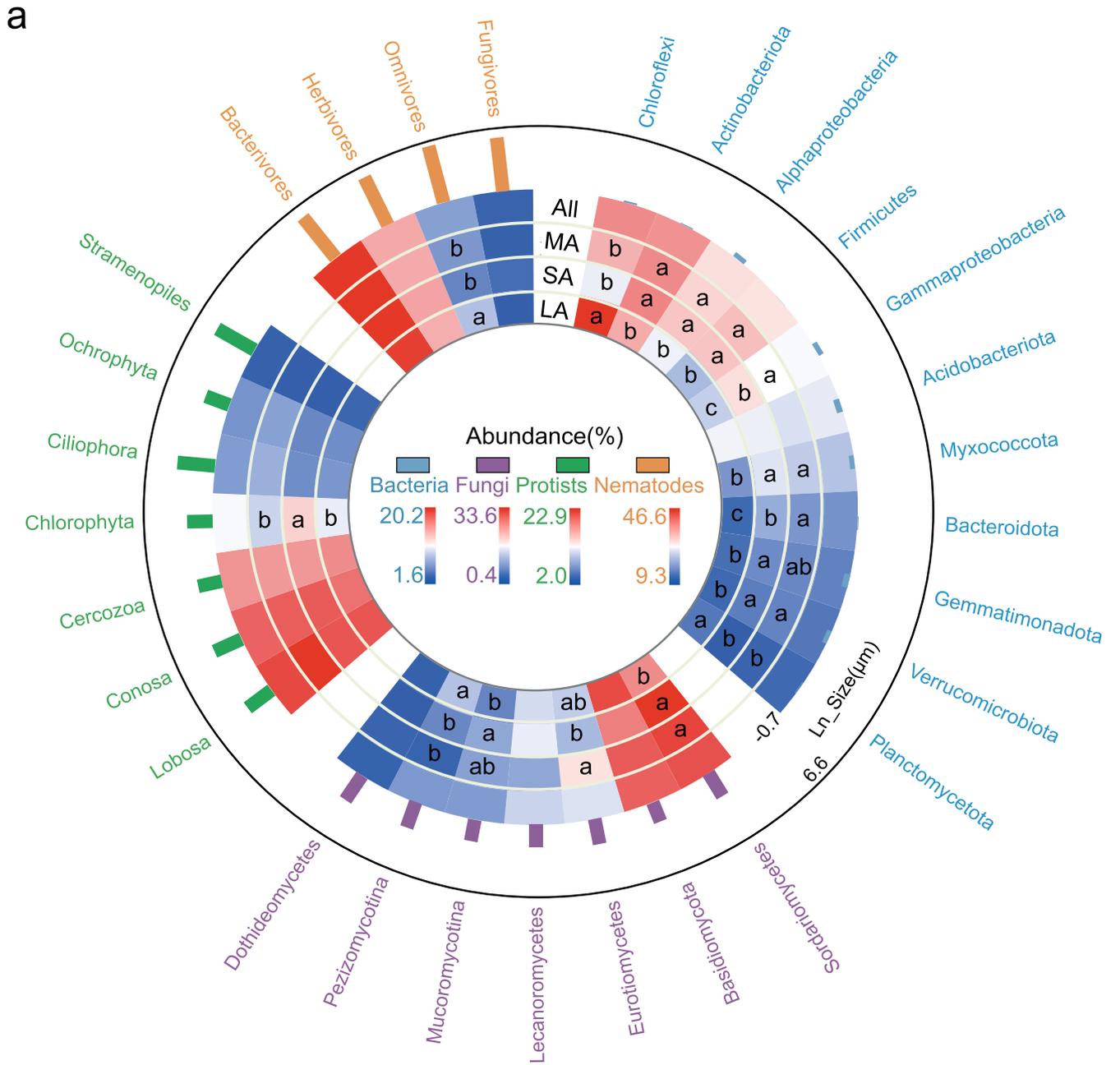
Fig. 1. The main indices of four organism groups in the three soil aggregates. (a) The abundance and body size of four organism groups; (b) The α-diversity (Shannon index), β-diversity (Bray-Curtis dissimilarity), community assembly (NDV, the null deviation value), and habitat niche breadth (*B*com) of four organism groups. Different lowercase letters inside the colored discs and on bars are significantly (*P* < 0.05) different by Tukey's post hoc tests. The bars with colors of blue, purple, green, and orange represent the bacterial, fungal, protist, and nematode communities, respectively. All, three soil aggregates; MA, microaggregates; SA, small macroaggregates; LA, large macroaggregates.

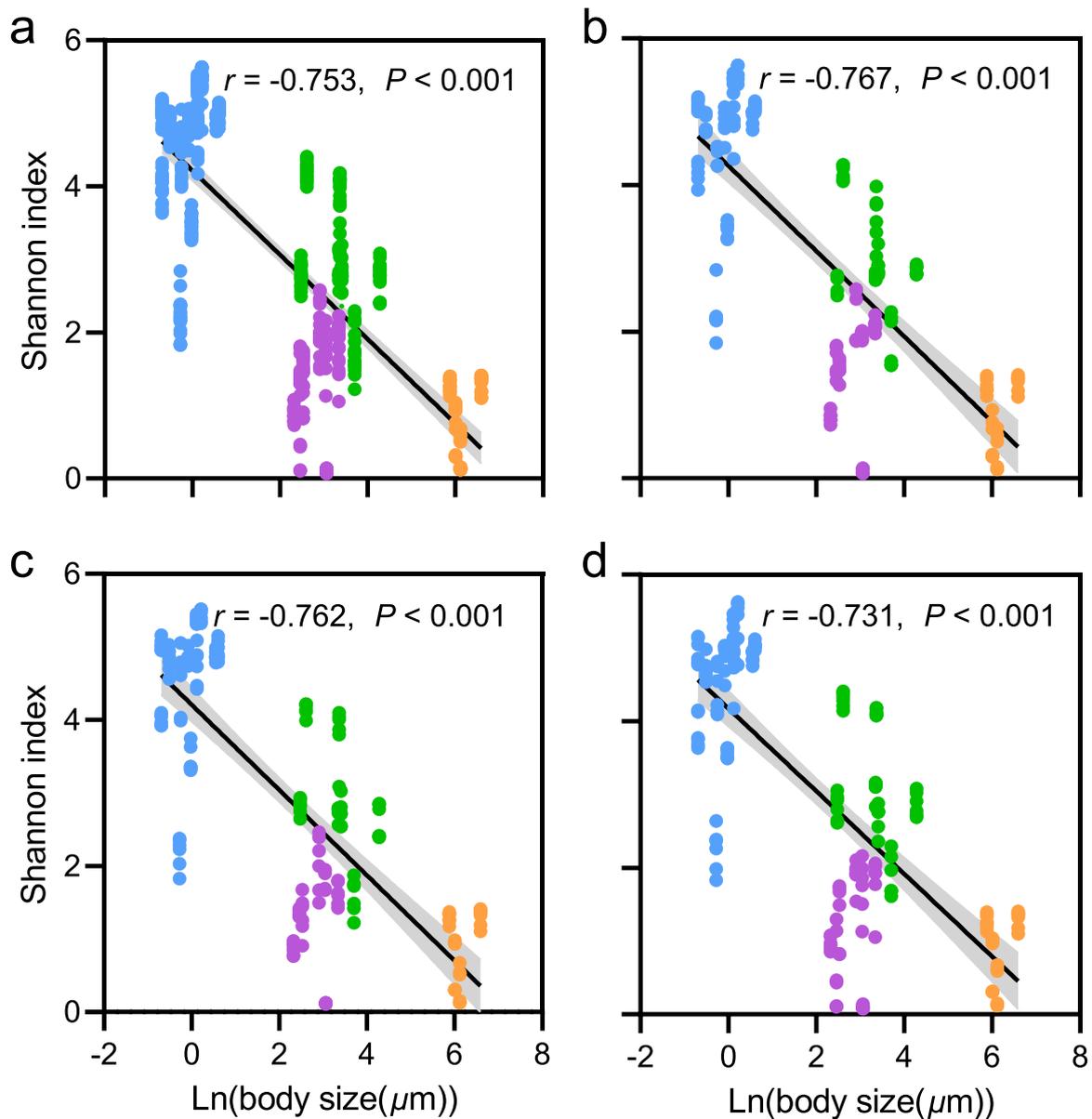**Fig. 2.** The relationships between body size and α-diversity of four organism groups across (a, All) and within (b, MA; c, SA; d, LA) soil aggregates. The points with colors of blue, purple, green, and orange represent the bacterial, fungal, protist, and nematode communities (29 taxa), respectively. The *r* and fit line are generated from unary linear regression. All, three soil aggregates; MA, microaggregates; SA, small macroaggregates; LA, large macroaggregates.

absence of constraints on variability observed in raw data (Delgado-Baquerizo et al., 2016).

2.9. Statistical analyses

One-way ANOVA was performed to assess differences in the α-diversity, β-diversity, NDV, dispersal rate, and B_{com} of the biotic community and the microbial functional potential across fertilization treatments and soil aggregates using Tukey's HSD post hoc tests ($P < 0.05$) in SPSS 22.0 software (SPSS, Chicago, IL, USA). Correlational analyses of environmental factors, diversity, body size, assembly process, and the microbial functional potential were based on Pearson's tests in SPSS 24.0 (Chicago, IL, USA). Random forest modeling was conducted to reveal important predictors of soil properties, body size, and the diversity and assembly processes for the microbial functional potential associated with carbon, nitrogen, phosphorus, and sulfur metabolism. The importance of each predictor was assessed by the increase in the mean square error between observations and predictions

when the predictor was randomly permuted (Breiman, 2001). Random forest modeling was performed using the 'randomForest' package in R (Liaw and Wiener, 2002), and model significance and predictor importance were determined using the A3R and rfPermute packages, respectively (Archer, 2013; Fortmann-Roe, 2013). The significant ($P < 0.05$) predictors identified through the random forest analyses were further chosen for structural equation modeling (SEM). The SEM was conducted to evaluate the contributions of soil properties (pH, SOM, TP, TN, AP, and AN) and body size, α-diversity, β-diversity, and assembly process of four organism groups to the microbial functional potential using AMOS version 22.0. The paths in the model represented the partial correlation coefficients, indicating the strength of the relationships between parameters. The final model retained all interactions and elements. The model fitness was assessed based on a nonsignificant chi-squared (χ^2) test, the goodness of fit index, and the root mean square error of approximation.
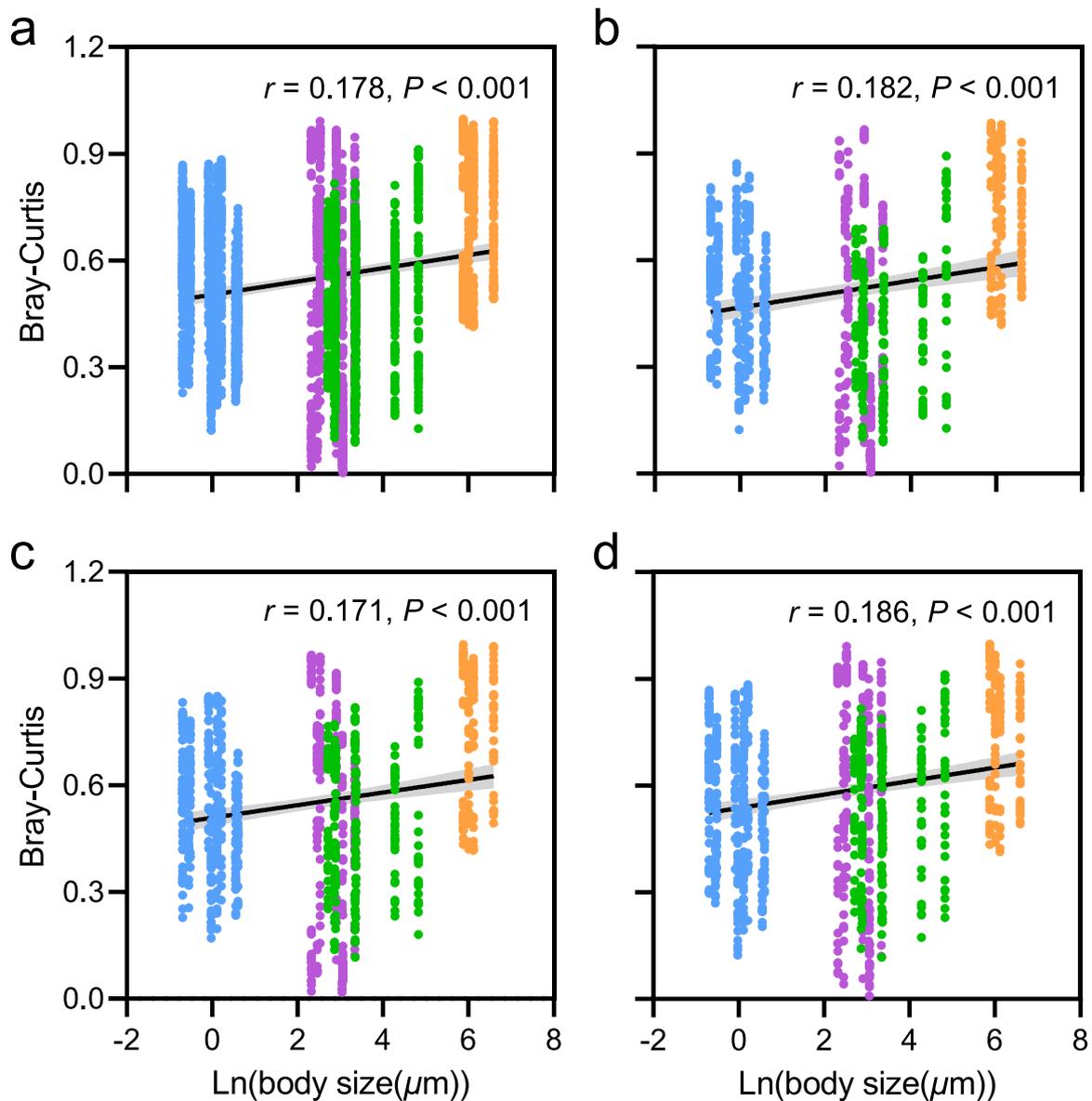
Fig. 3. The relationships between body size and β-diversity of four organism groups across (a, All) and within (b, MA; c, SA; d, LA) soil aggregates. The points with colors of blue, purple, green, and orange represent the bacterial, fungal, protist, and nematode communities (29 taxa), respectively. The *r* and fit line are generated from unary linear regression. All, three soil aggregates; MA, microaggregates; SA, small macroaggregates; LA, large macroaggregates.

3. Results

3.1. Soil chemical properties

Soil chemical properties significantly ($P < 0.05$) differed among different soil aggregates. SOM, TN, AN, and AP in MA fraction were significantly ($P < 0.05$) higher than those in LA fraction. TP was significantly ($P < 0.05$) higher in SA fraction than in LA fraction (Fig. S1). However, no significant ($P > 0.05$) differences in pH were detected in soil aggregates.

3.2. Variation in organism body sizes

For the entire dataset, there were 11 bacterial groups representing 94.2% of all bacterial sequences, with average relative abundances ranging from 1.6% (Planctomycetota) to 20.2% (Chloroflexi), 7 fungal groups representing 77.9% of all fungal sequences, with average relative abundances ranging from 0.4% (Dothideomycetes) to 33.6% (Sordariomycetes), 7 protist groups representing 91.5% of all protist sequences,

with average relative abundances ranging from 2.0% (Stramenopiles) to 22.9% (Lobosa), and 4 nematode groups with average relative abundances ranging from 9.3% (fungivores) to 46.6% (bacterivores) (Fig. 1a). Bacterial cell sizes varied from 0.5 μm (Gemmatimonadota) to 1.1 μm (Planctomycetota), fungal spores from 10.2 μm (Basidiomycota) to 28.3 μm (Dothideomycetes), protists from 15.1 μm (Cercozoa) to 71.9 μm (Ciliophora), and nematodes from 357.1 μm (Bacterivores) to 728.0 μm (Omnivores) (Fig. 1a).

3.3. Diversity, assembly, and niche breadth of four organism groups

The bacterial community had the highest α-diversity and β-diversity compared with the fungal, protist, and nematode communities among the three aggregates (Fig. 1b). We found that the relative influences of community assembly processes varied across four organism groups, with deviations from null expectations associated with body size ($F = 3.98$, $P < 0.01$) (Fig. 1b). The β-null deviations of protist and fungal communities (NDV = 0.54 and 0.54) were significantly ($P < 0.01$) higher than those of nematode and bacterial communities (NDV = 0.40 and 0.43)
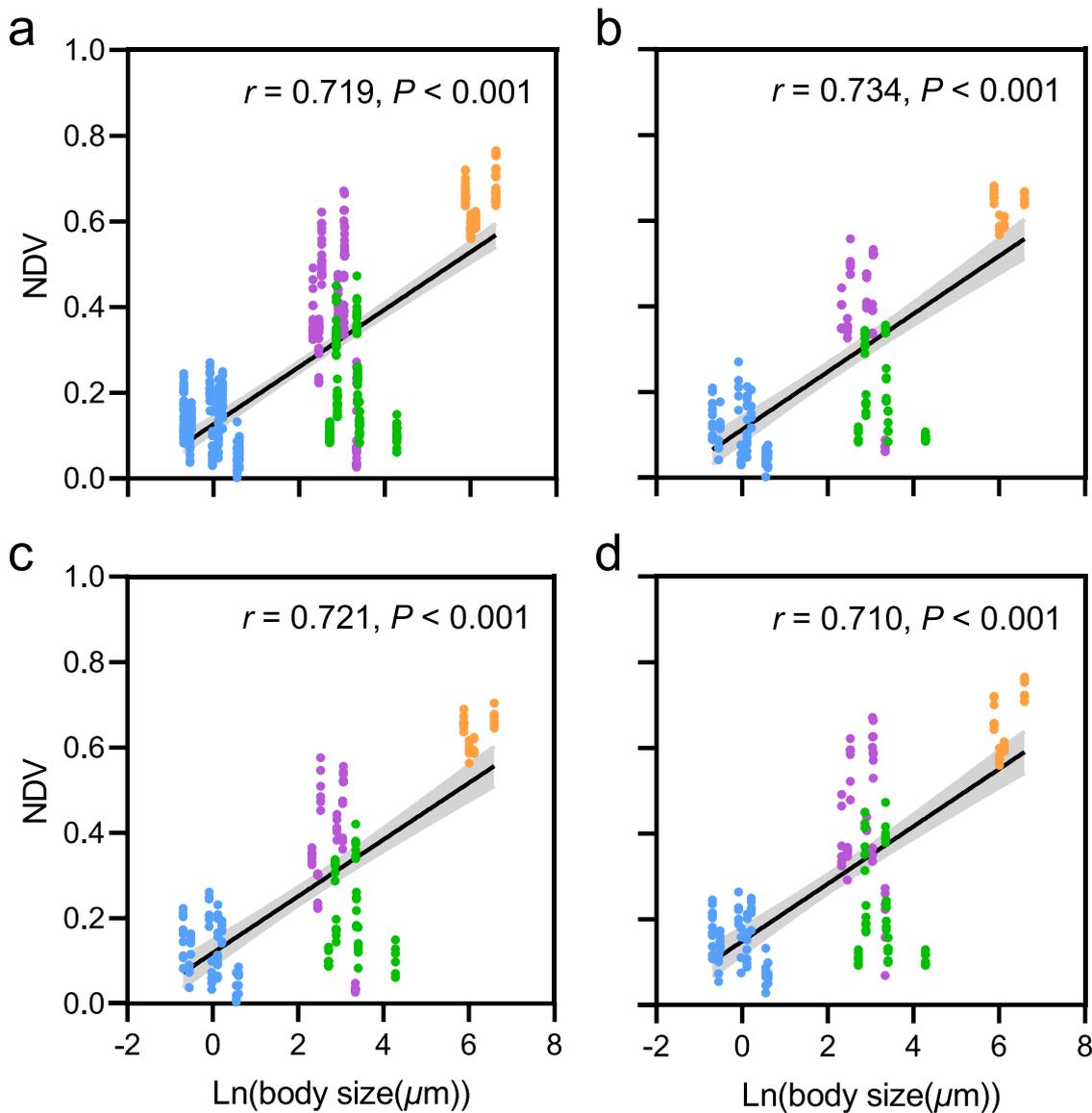
Fig. 4. The relationships between body size and community assembly process of four organism groups across (a, All) and within (b, MA; c, SA; d, LA) soil aggregates. The points with colors of blue, purple, green, and orange represent the bacterial, fungal, protist, and nematode communities (29 taxa), respectively. The r and fit line are generated from unary linear regression. NDV, null deviation value. All, three soil aggregates; MA, microaggregates; SA, small macroaggregates; LA, large macroaggregates.

(Fig. 1b). Among the four organism groups, the bacterial ($avg.R^2 = 0.71$–0.81) and nematode ($avg.R^2 = 0.52$–0.62) groups fitted the neutral model better than the fungal ($avg.R^2 = 0.06$–0.37) and protist ($avg.R^2 = 0.35$–0.59) groups, regardless of soil aggregate fractions (Table S1). The values of dispersal rate (m) were higher for the bacterial ($m = 0.47$) and protist ($m = 0.13$) communities than for the fungal ($m = 0.03$) and nematode ($m = 0.10$) communities (Table S1). Conversely, habitat niche breadth (B_{com}) was significantly ($P < 0.05$) lower for the fungal (8.86) and nematode (5.85) communities than for the bacterial (51.34) and protist (26.68) communities (Fig. 1b).

3.4. Body size associated with diversity and assembly processes of organism groups

Across the four organism groups, our results indicated that the logarithms of body size were negatively associated with α-diversity (Shannon index, $r = -0.753$, $P < 0.001$), but positively correlated with β-diversity ($r = 0.178$, $P < 0.001$) across all samples (Figs. 2 and 3). Similarly, the NDVs of soil organisms were positively correlated with

body size across all samples ($r = 0.719$, $P < 0.001$), as well as within MA ($r = 0.734$, $P < 0.001$), SA ($r = 0.721$, $P < 0.001$), and LA ($r = 0.710$, $P < 0.001$) fractions (Fig. 4). Positive correlations were also observed between dispersal rates and B_{com} values ($r = 0.416$–0.530, $P < 0.001$) (Fig. S2). However, the organism body size was negatively correlated with dispersal rates ($r = -0.702$, $P < 0.001$) and B_{com} values ($r = -0.577$, $P < 0.001$) (Figs. S3 and S4). In addition, dispersal rates were negatively correlated with NDVs ($r = -0.350$ to -0.609, $P < 0.001$) and β-diversity ($r = -0.086$ to -0.169, $P < 0.001$) (Figs. S5 and S6).

3.5. Microbial functional potential at the soil aggregate level

Metagenomic analyses focused on 19 functional categories associated with the microbial functional potential (genes) for carbon, nitrogen, phosphorus, and sulfur metabolism across different soil aggregate sizes (Fig. 5). The relative abundance of most functional modules involved in carbon, nitrogen, phosphorus, and sulfur metabolism was significantly ($P < 0.05$) higher in MA fraction than in LA fraction (Fig. 5a). Correlation analysis revealed that the diversity of four
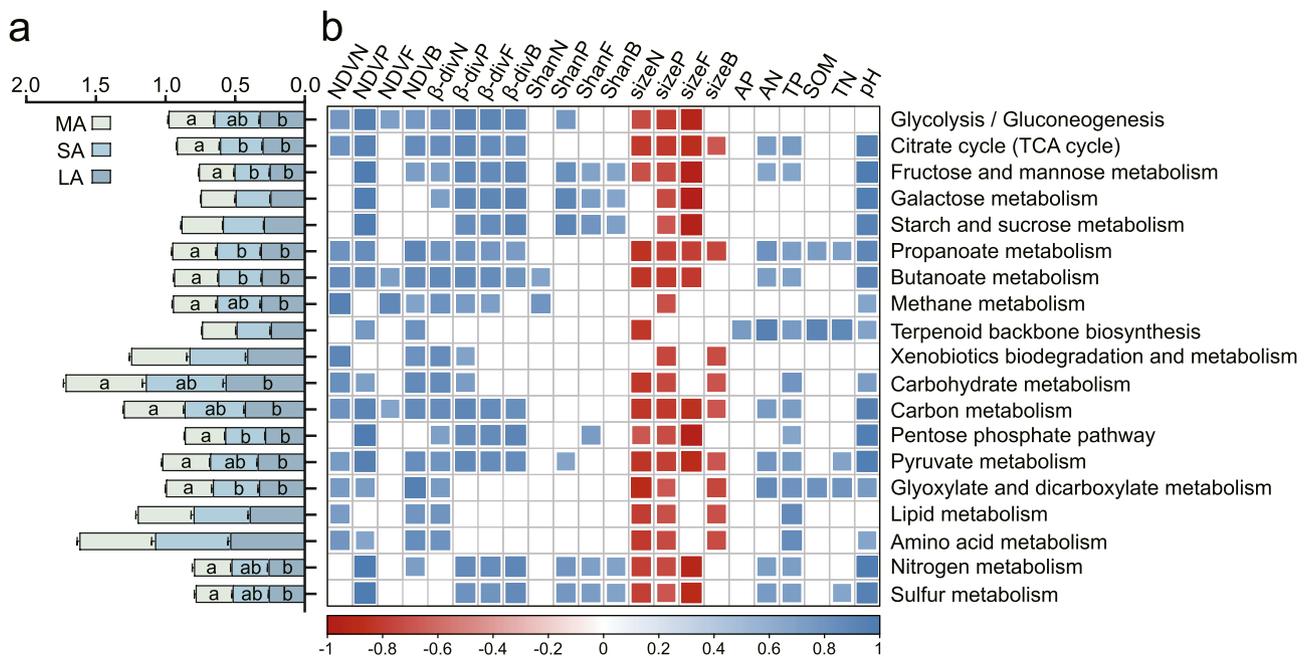
Fig. 5. The microbial functional potential associated with carbon, nitrogen, phosphorus, and sulfur metabolism in the three soil aggregates. (a) Relative abundance of carbon, nitrogen, phosphorus, and sulfur metabolism. Bars with different lowercase letters are significantly ($P < 0.05$) different by Tukey's post hoc tests. (b) The relationships between soil properties, the community of four organism groups, and the microbial functional potential using Pearson correlation analysis. The sizeB, sizeF, sizeP, and sizeN represent the body size of the bacterial, fungal, protist, and nematode communities. The NDVB, NDVF, NDVP, and NDVN represent the null deviation value of the bacterial, fungal, protist, and nematode communities. The ShanB, ShanF, ShanP, and ShanN represent the Shannon index of bacterial, fungal, protist, and nematode communities. The β-divB, β-divF, β-divP, and β-divN represent the Bray-Curtis dissimilarity of bacterial, fungal, protist, and nematode communities. SOM, soil organic matter; TN, total nitrogen; TP, total phosphorus; AN, alkaline hydrolyzable nitrogen; AP, available phosphorus. MA, micro-aggregates; SA, small macroaggregates; LA, large macroaggregates.

organism groups was positively correlated with fructose and mannose metabolism, galactose metabolism, starch and sucrose metabolism, nitrogen metabolism, and sulfur metabolism (Fig. 5b). The assembly processes of four organism groups were positively associated with glycolysis/gluconeogenesis, butanoate metabolism, and carbon metabolism. In contrast, the body sizes of the four organism groups were negatively correlated with the citrate cycle, pyruvate metabolism, propanoate metabolism, butanoate metabolism, and carbon metabolism.

Random forest modeling showed that soil properties ($P < 0.05$), including pH, SOM, TN, TP, AN, and AP, were the primary determinants of the microbial functional potential associated with carbon, nitrogen, phosphorus, and sulfur metabolism in soil aggregates (Fig. S7). Variations in the microbial functional potential were also significantly ($P < 0.05$) associated with body size, diversity, and assembly process, but the variations differed in three aggregates. The diversity of bacterial and protist communities showed significant ($P < 0.05$) influences on the microbial functional potential in MA fraction, while the community assembly of four organism groups contributed significantly to the microbial functional potential in SA and LA fractions.

Structural equation modeling revealed the potential influences of soil properties and four organism groups on the microbial functional potential across and within the three aggregate fractions. Soil properties were positively correlated with the α-diversity of four organism groups ($r = 0.58$–0.77, $P < 0.001$), except for SA fraction (Fig. 6). Body size had negative associations with bacterial α-diversity ($r = -0.04$ to -0.42, $P < 0.05$), but positive associations with the β-diversity ($r = 0.17$–0.43, $P < 0.05$) and assembly processes ($r = 0.21$–0.63, $P < 0.05$) of the bacterial, fungal, and protist communities in the three soil aggregates. Notably, the microbial functional potential showed positive correlations with the α-diversity and β-diversity ($r = 0.26$ and 0.22, $P < 0.05$) of the bacterial and protist communities in MA fraction, and positive correlations with the assembly process of four organism groups in SA and LA fractions ($r =$

0.58 and 0.67, $P < 0.01$) (Fig. 6).

4. Discussion

4.1. Variation in community assembly processes associated with organism body sizes

Our findings supported our first hypothesis that there was a robust relationship between body size and community assembly (Fig. 4). Smaller organisms (bacteria) were primarily structured by stochasticity, while larger organisms (fungi, protists, and nematodes) were strongly influenced by determinism. The relationship between body size and community assembly is also evident in the niche breadth of different organism groups, supported by the negative correlations between body size and B_{com} (Fig. S4). In this sense, small-sized bacteria can inhabit small pores within microaggregates and macroaggregates with diameters of $0.8 - 3$ μm, thus avoiding predation by larger protists and nematodes (Thakur and Geisen, 2019; Erktan et al., 2020). High nutrient resources in microaggregates may also provide an adaptive advantage for bacteria to compete with fungi for resources (Jiang et al., 2018). In the case of microbial predators, protists and nematodes essentially depend on existing pore spaces as microhabitats for survival and access to resources including bacterial prey (Jiang et al., 2023). Larger-sized nematodes are known to move through the water-filled pores in the soil matrix with a neck diameter of $30 - 90$ μm, while protists can often pass through smaller pores with a neck size > 5 μm (Wright et al., 1995; Briar et al., 2011). Therefore, environmental heterogeneity may lead to habitat preference for soil organisms of different sizes, such as nematodes are more likely to live in soils with suitable water conditions and large pores.

Dispersal has been generally considered as both deterministic and stochastic (De Bie et al., 2012; Lowe and McPeek, 2014), and body size is expected to interact with dispersal mode in determining dispersal
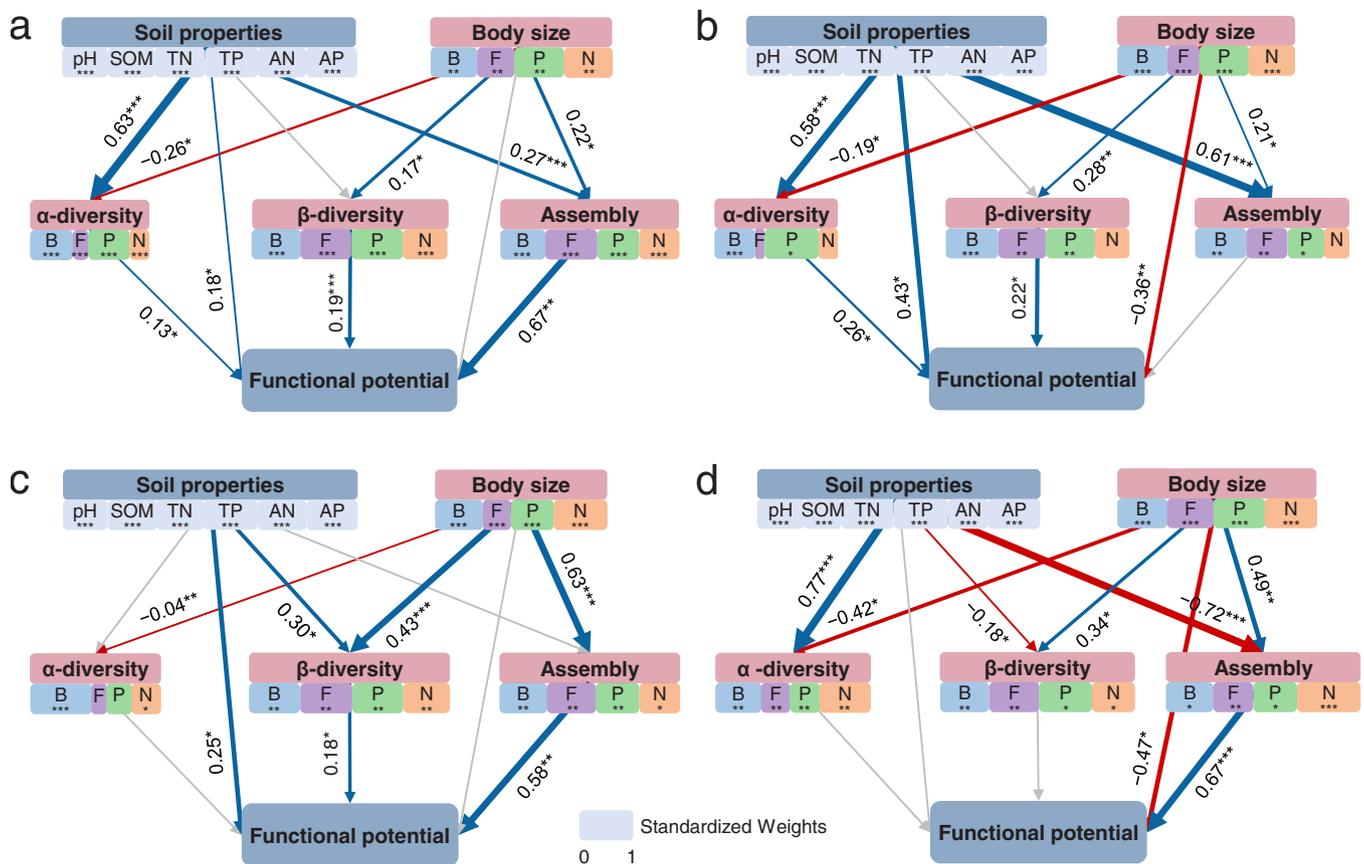
Fig. 6. Influence of soil properties and the community of four organism groups on the microbial functional potential associated with carbon, nitrogen, phosphorus, and sulfur metabolism across (a, All) and within (b, MA; c, SA; d, LA) soil aggregates using structural equation modeling. B, bacterial community; F, fungal community; P, protist community; N, nematode community. Blue lines indicate positive correlations, and red lines indicate negative correlations. Widths of arrows indicate strength of significant standardized path coefficients ($P < 0.05$). Paths with non-significant coefficients are presented as grey lines. SOM, soil organic matter; TN, total nitrogen; TP, total phosphorus; AN, alkaline hydrolyzable nitrogen; AP, available phosphorus. All, three soil aggregates; MA, microaggregates; SA, small macroaggregates; LA, large macroaggregates. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

ability, which has a strong potential to mediate the relative influence of dispersal on community composition (De Bie et al., 2012). Our results showed that the dispersal rate decreased with increasing organism body size (Fig. S3). Smaller bacteria tend to occupy broader niches than larger protists and nematodes because they disperse more easily to suitable habitats and are more metabolically diverse at the community level. High dispersal ability and rapid life-history strategies are important to balance the variation in community composition. However, some discrepancies in dispersal ability have been observed for distinct organism groups, such as large-sized nematodes having higher dispersal rates than smaller fungi. Methodological biases between study systems may have contributed to these counterintuitive results, including the potential underestimation of fungal communities with large hyphae and limited dispersal potential (Woo et al., 2019). The tendency for some fungi to differ in size and community assembly may also be due to a lack of an explicit comparison between spore and hyphal structures, which vary across taxa and environmental conditions (Luan et al., 2020). Although the complex soil micro-food web is influenced by both deterministic and stochastic processes, the relative importance of these processes can considerably vary between four organism groups in soil aggregates.

4.2. Diversity changes with organism body size

Our results showed that α-diversity decreased with increasing body size in the four organism groups (Fig. 2). According to the metabolic theory of ecology, smaller organisms (bacteria) often have higher metabolic rates, faster growth rates, shorter generation times, and

higher energy requirements than larger organisms (fungi, protists, and nematodes) (Peters, 1985; Brown et al., 2004). The population ecology of different microbial groups is primarily determined by intrinsic density-dependent factors that control population size such as growth, reproduction, and mortality (Trosvik et al., 2010). In addition, the short generation time and large population sizes of microbial communities lead to rapid evolutionary adaptation and promote microbial diversity (Blazewicz et al., 2020). As such, species at higher trophic levels are often more vulnerable to extinction than species at lower trophic levels due to reduced energy transfer and smaller population sizes.

We identified significant relationships between β-diversity with body size and dispersal rate across the four organism groups (Fig. 3 and S6), suggesting that high dispersal ability reduces compositional differences (β-diversity). Smaller-sized bacteria, which are passive dispersers, are less sensitive to local extinctions and drift effects and produce more propagules, thereby increasing the potential for dispersal events (Blackburn and Gaston, 1999; Fenchel and Finlay, 2004; Martiny et al., 2006). In contrast, larger-sized nematodes, which are active dispersers, are typically constrained by the connectivity of soil pores and the water film within soil aggregates, resulting in strong dispersal limitation at small spatial scales (Wright et al., 1995; Briar et al., 2011). An increase in dispersal rate leads to a trend toward homogeneity and a decrease in community heterogeneity (i.e., lower β-diversity), which can be explained by two non-exclusive reasons. First, higher dispersal rates may increase α-diversity through increased immigration and/or decreased local extinction rates of species. As a result, an increase in species-level occurrence probability, equivalent to an increase in
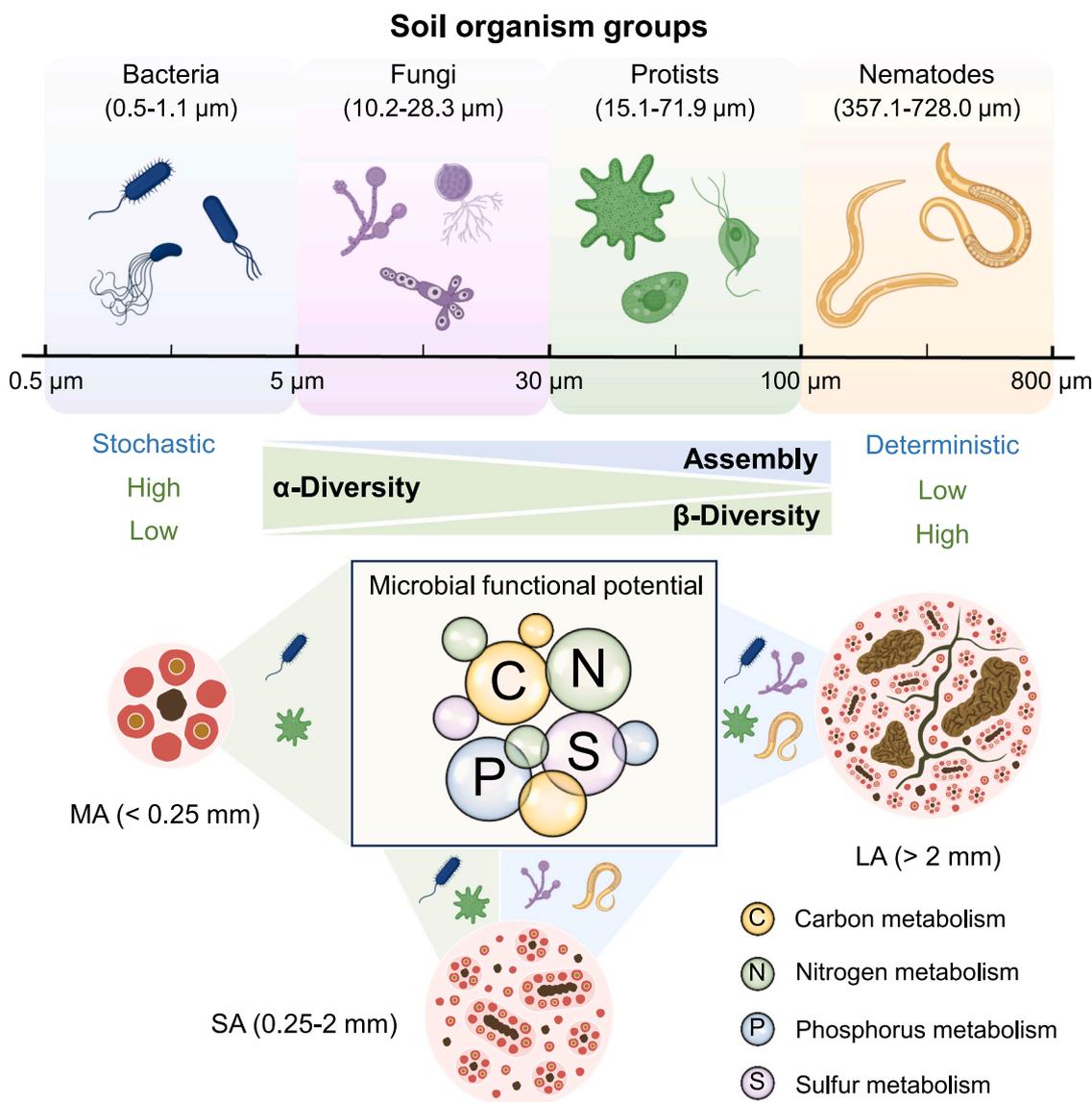
Fig. 7. The overview diagram of the diversity and community assembly of soil organism groups with distinct body size mediating soil microbial functional potential in three aggregate sizes. The smaller body size of bacterial community with higher α-diversity and lower β-diversity was relatively more influenced by stochastic processes, while the larger body size of fungal, protist and nematode communities were more structured by deterministic processes. Soil microbial functional potential was mainly influenced by the diversity of organism groups in microaggregates, but affected by the community assembly in macroaggregates. MA, microaggregates; SA, small macroaggregates; LA, large macroaggregates.

α-diversity, will lead to community homogeneity due to the prevalence of widespread species. Second, high dispersal rates may lead to a stronger deterministic signal that promotes competitive species interactions within the local community, resulting in a higher degree of homoplasy in the community's species composition. Thus, an organism's body size affects species niche and community composition, ultimately influencing species diversity through the relative interplay of community assembly processes that determine patterns of species distribution and coexistence.

4.3. Body size affects the microbial functional potential in soil aggregates

We found a significant relationship between body size and the microbial functional potential through the influence of bacterial and protist α- and β-diversity in MA fraction (Fig. 6b). Pore size and soil nutrient properties in MA fraction may provide a heterogeneous microenvironment for diverse soil microorganisms. Bacteria and protists dominated the microhabitats in MA fraction, as the pore structure characteristics of

soil microaggregates provide refuge for the bacterial community and limit the occupancy by larger predators (nematodes). High nutrient levels support the relatively higher α-diversity and lower β-diversity of bacterial and protist communities to maintain the microbial functional potential. A perspective based on β-diversity is particularly important in the context of microbial community functions, as the maintenance of multiple functions requires different sets of species to coexist in a heterogeneous environment (Hillebrand and Matthiessen, 2009). Microbial communities with low species richness exhibit low functional potential associated with carbon, nitrogen, phosphorus, and sulfur metabolism due to reduced redundancy to support essential functions in a system. In particular, the 'insurance effect of β-diversity' on ecosystem functioning highlights the importance of assembly processes that organize diversity and community composition to determine the provision of multiple ecosystem functions (Pasari et al., 2013; Mori et al., 2018).

We observed significant relationships between community assembly processes of four organism groups in SA and LA fractions (Fig. 6c, d). The results suggested that the increasing determinism of protist and

nematode communities could lead to an increase in the microbial functional potential. The ecological function of protist and nematode communities depends largely on the soil physical structure in terms of available pore space, which defines their physical niche and limits foraging for favorable resources (Jiang et al., 2017; Erktan et al., 2020). The macroaggregates support the coexistence of multiple protist and nematode species and maintain the complex trophic interaction with higher abundances of protists and nematodes. Selective predation of bacterivorous and fungivorous nematodes, as well as phagotrophic protists, can induce compositional and functional variation in soil microbial communities (Neher, 2010; Gebremikael et al., 2014). Predatory nematodes capture and consume protists and nematodes at lower trophic levels and influence the structure of protist and nematode communities (Thakur et al., 2014; Erktan et al., 2020). The higher number of nematode-protist-microbe associations may represent stronger predation of protists and nematodes on microorganisms, with consequent effects on the microbial functional potential. We speculate that selective predation by large-sized protists and nematodes may lead to the replacement of some maladapted microbial taxa by highly functional specialist taxa, thereby increasing the microbial functional potential. Constraints imposed by soil physical structure on trophic interactions are important drivers of community composition and functioning, particularly by favoring a variety of adaptations and optimizing trophic interactions through the dynamic occupancy and movement of species in the soil.

5. Conclusion

In summary, the body size of soil organisms showed consistent correlations with ecological processes in three soil aggregates. The smaller body size of the bacterial community with higher α-diversity and lower β-diversity were relatively more influenced by stochastic processes, while the larger body sizes of fungal, protist, and nematode communities were more structured by deterministic processes. In addition, the size-mediated diversity and assembly process of four organism groups affected the microbial functional potential at the aggregate level. Soil microbial functional potential was mainly influenced by the α-diversity and β-diversity of bacterial and protist communities in microaggregates, but affected by the community assembly of four organism groups in macroaggregates (Fig. 6 and Fig.7). This study emphasizes the importance of body size in regulating the diversity and assembly processes of the microbial and nematode communities, which deepens our understanding of soil nutrient cycling at the soil aggregate level.

CRediT authorship contribution statement

Francisco Dini-Andreote: Writing – review & editing. **Shungui Zhou:** Writing – review & editing. **Lu Luan:** Writing – review & editing, Funding acquisition, Conceptualization. **Guofan Zhu:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Mohammad Bahram:** Writing – review & editing. **Stefan Geisen:** Writing – review & editing. **Yunfeng Yang:** Writing – review & editing. **Yuji Jiang:** Writing – review & editing, Visualization, Funding acquisition, Data curation. **Shaopeng Wang:** Writing – review & editing. **Jie Zheng:** Methodology, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

I have shared the link to my data at the Attach File step

Acknowledgments

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.micres.2024.127669.

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