

# Stochastic community assembly of abundant taxa maintains the relationship of soil biodiversity-multifunctionality under mercury stress

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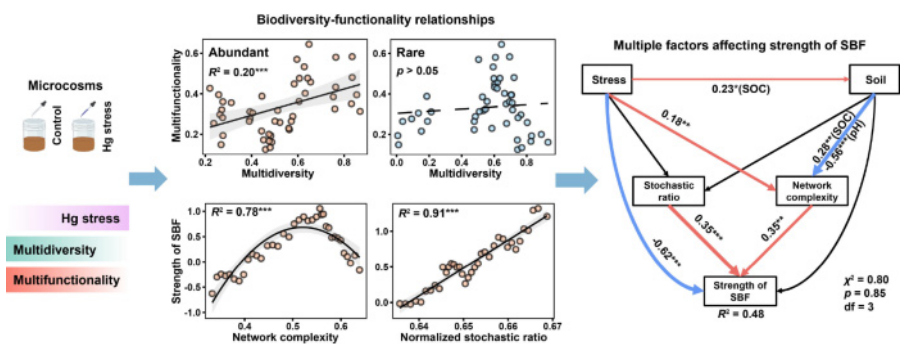
## ABSTRACT

- Soil abundant taxa diversity positively related to multifunctionality under Hg stress.
- Microbial network complexity of soil abundant taxa supported the strength of SBF.
- Stochastic assembly of soil abundant subcommunity supported the strength of SBF.
- Stochastic ratio was the most important predictor for the strength of SBF.

It is known that soil microbial communities are intricately linked to multiple ecosystem

functions and can maintain the relationship between soil biodiversity and multifunctionality (SBF) under environmental stresses. However, the relative contributions and driving forces of abundant and rare taxa within the communities in maintaining soil biodiversity-multifunctionality relationship under pollution stresses are still unclear. Here, we conducted microcosm experiments to estimate the importance of soil abundant and rare taxa in predicting these relationships under heavy metal mercury (Hg) stress in paired paddy and upland fields. The results revealed that the diversity of abundant taxa, rather than rare taxa, was positively related to multifunctionality, with the abundant subcommunity tending to maintain a larger proportion of soil functions including chitin degradation, protein degradation, and phosphorus mineralization. Soil multitrophic network complexity consisting of abundant species showed positive correlations with biodiversity and multifunctionality, and supported the strength of SBF within a network complexity range. Stochastic assembly processes of the abundant subcommunity were positively correlated with the strength of SBF, although stochastic processes decreased the biodiversity and the multifunctionality, respectively. After simultaneously accounting for multiple factors on the strength of SBF, we found that the stochastic community assembly ratio of abundant taxa was the most important predictor for SBF strength under Hg stress. Our results highlight the importance of abundant taxa in supporting soil multifunctionality, and elucidate the linkages between community assembly, network complexity and SBF relationship under environmental stresses.

**Keywords** abundant taxa, biodiversity-multifunctionality relationship, community assembly, network complexity, environmental stresses



## 1 Introduction

Soil organisms exhibit a high degree of complexity and diversity (Bardgett and Van Der Putten, 2014). Soil biodiversity is critical for fundamental ecosystem functions, such as

nutrient cycling, organic carbon turnover, plant productivity, and pathogen control (Van Elsas et al., 2012; Wagg et al., 2014; Delgado-Baquerizo et al., 2020; Lu et al., 2023). Growing evidence from large-scale investigations and controlled experiments has suggested that soil biodiversity plays a pivotal role in promoting ecosystem multifunctionality (Kardol and Wardle, 2010; Cardinale, 2011). The relationship between soil biodiversity and multifunctionality (SBF) is

generally influenced by environmental disturbance, such as climate changes, soil pollution and degradation (Rillig et al., 2019, 2021). Heavy metal pollution is a severe problem and has adverse effects on soil functions, particularly in agricultural ecosystems, and on human health (Fajardo et al., 2019; Hao et al., 2021; Liu et al., 2023). Among these heavy metals, mercury (Hg) has raised global concerns due to its potent toxicity to soil biota and transport over long distances (Mahbub et al., 2017; Liu et al., 2018). The ecological consequences of Hg pollution on soil microbiome have been increasingly evaluated (Liu et al., 2018; Du et al., 2022), but the responses of SBF relationship to Hg pollution stress are poorly understood.

Microorganisms typically present a highly unbalanced composition in a community, whereby a small number of species are extremely abundant, while a large number of other species have a low abundance (Lynch and Neufeld, 2015). The abundant species are referred to as the “abundant biosphere”, contributing most of the microbial biomass (Wu et al., 2017). In parallel, the low-abundance taxa are referred to as the “rare biosphere”, acting as a “seed bank”, with a potential to become abundant in a particular environment (Lennon and Jones, 2011; Pedrós-Alió, 2012). Abundant and rare taxa have been reported to exhibit different distribution patterns and functional traits (Pedrós-Alió, 2012; Wu et al., 2017). It has been argued that a subcommunity consisting of specific species, rather than the whole community, drives ecosystem functioning (Soliveres et al., 2016; Xun et al., 2021). For example, abundant taxa have significant influences on ecosystem processes such as productivity, nutrient cycling, and stability (Delgado-Baquerizo et al., 2020). In parallel, rare taxa may not have strong direct impacts on ecosystem processes, but they can play potential roles in maintaining ecosystem resilience and contributing to niche specialization (Jousset et al., 2017). In addition, abundant and rare taxa have different responses to environmental stresses. Previous studies have shown that abundant species can effectively adapt to various environments (Jiao et al., 2017), while rare species are more sensitive to environmental disturbances (Jiao et al., 2019). However, we still lack the comprehensive knowledge of how soil abundant and rare taxa respond to environmental stresses such as Hg pollution, and whether they influence the relationship between soil biodiversity and multifunctionality.

The relationship between biodiversity and functionality in soil ecosystems can be regulated by microbial community assembly processes, although the role of community assembly has often been overlooked in previous studies (Knelman and Nemergut, 2014; Xun et al., 2019). Stochastic assembly processes can result in positive species covariations that increase the functional performance of microbial communities (Li et al., 2019a; Yu et al., 2019). Meanwhile, deterministic processes may reduce the effect of biodiversity

on ecosystem function via selecting the species which do not affect the functioning (Knelman and Nemergut, 2014). It has been reported that stochastic community assembly processes of rare taxa could promote the biodiversity-functionality relationship (Zhang et al., 2022). Apart from community assembly, soil multitrophic interactions, which form complex ecological networks, are also pivotal in promoting ecosystem functions (Bardgett and Van Der Putten, 2014). Network topological features including connectedness and constructure have impacts on ecosystem functioning such as carbon and nitrogen cycling processes and energy flux (De Vries et al., 2013; Wagg et al., 2019). A recent study suggests that network complexity, a composite indicator of multiple topological features, shapes the biodiversity-multifunctionality relationship (Jiao et al., 2021). Nevertheless, it is still unclear whether community assembly and network complexity of abundant and rare taxa influence the relationship between biodiversity and multifunctionality under Hg pollution stress.

Here, we aimed to explore the relative contributions of abundant and rare taxa in maintaining the relationship between soil biodiversity and multifunctionality under Hg stress. We hypothesized that (i) the biodiversity of soil abundant taxa instead of rare taxa supports multifunctionality; and (ii) the relationship between biodiversity and multifunctionality is maintained by stochastic community assembly processes of abundant taxa under Hg stress. To test our hypotheses, we carried out microcosm experiments to evaluate the effects of Hg pollution stress on the relationship between soil biodiversity of abundant/rare taxa and multifunctionality, using soils collected from paddy fields and their adjacent upland fields. Then we performed moving window and random sampling analyses to evaluate the effects of network complexity and community assembly on the strength of SBF relationship. Our results provide novel insights into the different functional roles of abundant and rare taxa diversities on multifunctionality under environmental stresses, and highlight the importance of microbial community assembly processes in maintaining the relationship between soil biodiversity and multifunctionality.

## 2 Materials and methods

### 2.1 Microcosm experiment and soil sampling

We collected paddy and upland (maize cultivation) soil samples with no documented heavy metal Hg pollution from Wuchang (127°07' E, 44°58' N), Yueyang (112°25' E, 29°32' N), and Maoming (110°50' E, 21°34' N) in China on May 2021, as previously described (Du et al., 2023). At each field, five soil cores (0–15 cm) were homogenized into a composite sample, sieved through a 2-mm mesh, and

pre-incubated in darkness at 25°C with constant moisture for one week. The details of the microcosm experiment were described in our previous paper (Du et al., 2023). Briefly, 25 g soil from each field was added to a polyvinylchloride container (6 cm × 6 cm × 8 cm), and four replicates were set for each of the Hg addition treatment (10 µg HgCl<sub>2</sub> g<sup>-1</sup> soil) and the control (equivalent volume of sterile distilled water). We selected this Hg concentration as a stress based on information regarding the Hg content range in normal agricultural soils and its toxicity on soil organisms (Du et al., 2022; Du et al., 2023). During 60 days of incubation, we supplemented sterile distilled water to maintain the flooded state for paddy soils or at 30% of water holding capacity for upland soils. We chose this sampling time based on the response and adaptability of soil microbial community to Hg stress (Zhou, et al., 2020). At the endpoint of incubation, we obtained 48 soil samples (2 treatments × 3 sampling sites × 2 land uses × 4 replicates). Each soil sample was divided into two portions, one portion was stored at 4°C for functioning measurements, and the other portion was stored at -20°C for molecular analysis. Soil pH was measured at a fresh soil-to-water ratio of 1:2.5 using a Delta pH meter, and soil organic carbon (SOC) was measured using the K<sub>2</sub>CrO<sub>7</sub> oxidation titration method (Walkley and Black, 1934). Detailed information of sampling sites, microcosm treatment, and soil properties were listed in Table S1.

## 2.2 Soil biodiversity analysis

Microbial DNA was extracted from 0.3 g soil using a DNeasy PowerSoil Kit (QIAGEN GmbH, Germany) based on the manufacturer's instructions. The purity and concentration of DNA were determined using a NanoDrop Spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA). High-throughput sequencing targeted the V4 region of bacterial 16S rRNA, the internal transcribed spacer (ITS) region of fungi, and the V4 region of protistan 18S rRNA, with corresponding primer pairs: 338F/806R (Delgado-Baquerizo et al., 2016), ITS1F/2043R (Gardes and Bruns, 1993), and TAREuk454FWD1/TAREukREV3 (Stoeck et al., 2010). Purified amplicons were mixed and carried out for paired-end sequencing on an Illumina Miseq PE 250 sequencer (Illumina Inc., San Diego, USA). Raw sequences were processed using the Quantitative Insights into Microbial Ecology (QIIME) pipeline (version 1.91) (Caporaso et al., 2010). Briefly, raw reads of each sample were trimmed, merged to paired-end reads, and filtered low quality sequences (e.g., chimera) using the UPARSE pipeline (version 7.1) (Edgar, 2013). Operational taxonomic units (OTUs) were clustered at 97% sequence similarity and a representative sequence of each OTU was selected and used for taxonomic assignments. Bacterial, fungal, and protistan OTUs were taxonomically assigned by blasting against the SILVA database

(version 123) (Quast et al., 2013), the UNITE database (Nilsson et al., 2019), and the Protist Ribosomal Reference (PR2) database (version 4.10) (Guillou et al., 2013), respectively. To obtain protistan OTU table, the non-protists taxa including Fungi, Metazoa, Rhodophyta, Streptophyta, Opisthokonta, and ambiguous taxa in Eukaryotes were excluded according to a previous study (Zhao et al., 2019).

The OTUs that contained fewer than 20 reads were discarded to avoid random effects on the identification of rare taxa (Jiao and Lu, 2020). Then the OTU tables were resampled to a minimum number of sequences from each sample (i.e., 83663 for bacteria, 40879 for fungi, and 12587 for protists). Totally, 16474 bacterial OTUs, 2370 fungal OTUs, and 1978 protistan OTUs were observed in all of the soil samples. Here, OTUs with relative abundances above 0.1% of the total sequences were defined as "abundant" taxa, those with relative abundances below 0.01% were defined as "rare" taxa, and those with relative abundances between 0.01% and 0.1% were "moderate" OTUs (Liu et al., 2015; Jiao and Lu, 2020). In this study, richness (i.e., number of phylotypes) was used as a metric of soil biodiversity, which is the most extensively used and simplest biodiversity metric (Delgado-Baquerizo et al., 2020). To obtain the multidiversity index for both the abundant and rare taxa, we combined soil biodiversity characteristics by averaging the standardized scores of richness of bacteria, fungi, and protists. The scores, standardized based on a common scale ranging from 0 to 1, were calculated according to the following formula:  $STD = (X - X_{min}) / (X_{max} - X_{min})$ ; where  $STD$  is the standardized variable and  $X$ ,  $X_{min}$ , and  $X_{max}$  are the target variable, its minimum value, and its maximum value across all samples, respectively (Delgado-Baquerizo et al., 2020). Detailed information of the multidiversity index and individual groups of soil microorganisms for abundant and rare taxa are listed in Table S2.

## 2.3 Soil functioning measurements

In this study, soil functions regulated by microorganisms, including extracellular enzyme activities involved in carbon cycling (sugar degradation [ $\beta$ -glucosidase, GLU;  $\beta$ -xylosidase, XYL; and  $\beta$ -cellobiohydrolase, CBH]), nitrogen cycling (chitin degradation [N-acetylglucosaminidase, NAG] and protein degradation [leucine aminopeptidase, LAP]), and phosphorus cycling (phosphorus mineralization [acid phosphatase, ACP]), were measured using fluorometry as described previously (Bell et al., 2013). All individual soil functions were standardized to a common scale ranging from 0 to 1, as described above. To obtain the multifunctionality index, the standardized scores of all functions were averaged. Detailed information of the multifunctionality index and individual soil functions are listed in Table S3.

## 2.4 Linking soil biodiversity to multifunctionality

We conducted ordinary least-squares linear regressions between soil multidiversity and multifunctionality for abundant and rare taxa in all, control, and Hg stress samples, respectively. Functional effects of multidiversity on multifunctionality were calculated using the standardized slopes i.e., mean  $\pm$  s.e.m. from linear regressions (Jiao et al., 2021). We performed Spearman correlation analyses between the diversity of individual kingdoms and single soil functions. To further explore the effects of abundant and rare taxa on soil functions, we estimated the Spearman correlations between individual phylotypes and single soil functions. The phylotypes which were significantly ( $p < 0.01$ ) and positively correlated with soil functions were defined as “supporting phylotypes” (Zhang et al., 2022). The relationships between the proportion of supporting phylotypes and the number of functions they support via ordinary least-squares linear regressions were also calculated as previously described (Zhang et al., 2022).

## 2.5 Structural equation model and random forest analyses

We conducted structural equation modeling (SEM) to evaluate the direct link between the biodiversity of soil abundant and rare taxa and (averaging) multifunctionality, and between multitrophic network complexity, community assembly processes, and the strength of SBF (explained below), after accounting for multiple drivers such as Hg stress and soil properties (SOC and pH) simultaneously. The SEM analyses were conducted in IBM SPSS Amos 22 (Chicago, IL: Amos Development Corporation). To ensure adequate fit of the model, the parameters including root mean square errors of approximation (RMSEA  $< 0.08$ ),  $\chi^2$  value ( $p > 0.05$ ), and goodness-of-fit index (GFI  $> 0.90$ ) were used (Schermelleh-Engel et al., 2003). With a good model fit, we were able to interpret the path coefficients of the model and their associated  $p$  values. A path coefficient was analogous to the partial correlation coefficient, and described the strength and sign of the relationship between two variables. The standardized total effects (STEs) were calculated to visualize the impact of each factor on the dependent variable (i.e., multifunctionality or the strength of SBF).

In addition, random forest (RF) analyses were performed to identify the main predictors for multifunctionality or the strength of SBF, via the “rfPermute” package in R (Archer, 2016; Trivedi et al., 2016). The accuracy importance measure was computed for each tree and averaged over the forest (1 000 trees). Percentage increases in the MSE (mean squared error) of variables was used to estimate the importance of these predictors; higher MSE% values implied more important predictors (Breiman, 2001). Significance of the

models and cross validated  $R^2$  values were assessed with 1000 permutations of the response variable, using the “A3” package in R (Fortmann-Roe, 2015).

## 2.6 Soil multitrophic co-occurrence network

Co-occurrence network consisting of phylotypes from soil bacteria, fungi, and protists was constructed for abundant taxa. Robust correlations with Spearman correlation coefficients ( $\rho > 0.8$  or  $< -0.8$  and false discovery rate (FDR)-corrected  $p$ -values  $< 0.01$  were used to construct the network with the R package “WGCNA” (Langfelder and Horvath, 2012). A soil multitrophic network was constructed using the “igraph” package in R (Csardi and Nepusz, 2006), and imported into the Gephi platform (version 0.9.2) and visualized by the Frucherman-Reingold algorithms (Bastian et al., 2009). Pearson correlation coefficients ( $r$ ) were also calculated between each individual phylotype and multifunctionality to estimate the contribution of each phylotype to multifunctionality. Only nodes with  $r > 0$  were displayed in the network to highlight their support of multifunctionality (Zhang et al., 2022).

In addition, we extracted subnetworks by preserving the phylotypes of individual soil samples using the induced\_subgraph function in “igraph” package. The topological features were calculated to estimate the network complexity of individual soil samples. Higher numbers of nodes and edges, average degree, clustering coefficient, graph density, modularity, and lower average path length and diameter suggest a more connected network, reflecting more potential complexity of soil networks (Barberán et al., 2012; Ma et al., 2016). We then calculated one index to reflect the complexity of soil multitrophic networks via averaging the standardized scores (ranging from 0 to 1) of each topological feature (as explained above). Note that average path length and diameter were calculated as the inverse of the variables ( $\times -1$ ) before the calculation of the index. We then conducted ordinary least-squares linear regressions between soil multitrophic network complexity and multidiversity as well as multifunctionality for abundant taxa in all, control, and stress samples. Functional effects of network complexity on multidiversity and multifunctionality were calculated using the standardized slopes, i.e., mean  $\pm$  s.e.m., from linear regressions. Detailed information of the network complexity index and individual topological features are listed in Table S4.

## 2.7 Community assembly processes of soil organisms

Null model analysis was carried out to evaluate community assembly processes for both abundant and rare taxa, using the previously described framework (Ning et al., 2019). The

relative importance of stochastic processes was assessed using the normalized stochasticity ratio (NST), which is the ratio of the mean expected similarity in the null model to the observed similarity. The null communities were generated by randomizing the observed community structure 1000 times based on a null model algorithm (Stegen et al., 2013). We revealed the NST values along different gradients of multidiversity and multifunctionality, and then conducted ordinary least-squares linear regressions between community assembly processes (represented as NST values) and multidiversity as well as multifunctionality for both abundant and rare taxa.

### 2.8 Linking community assembly and network complexity to strength of SBF

We performed the moving window approach to analyze the effects of network complexity and community assembly on SBF relationship. The technique facilitates the analysis of multivariate data along an ecological gradient (Carlson et al., 2010; Jassey et al., 2014). To ensure adequate data amounts, we selected two window sizes of 12 and 16 consecutive samples, accounting for 1/4 and 1/3 of total samples, respectively. After reordering the samples along the values of network complexity and stochasticity ratios, 37 (e.g., 1–12, 2–13, ..., 37–48) and 33 (e.g., 1–16, 2–17, ..., 33–48) subsets were generated, respectively. Based on each subset, we first calculated the slopes of the effects of multidiversity on multifunctionality to represent the strength of SBFs, and the mean values of network complexity (or stochasticity ratio). Then we estimated the relationships of the strength of SBFs with network complexity and with stochasticity ratio (Meyer et al., 2018; Jiao et al., 2021; Zhang et al., 2022).

We further applied the random sampling approach to decipher the effects of multiple factors (e.g., Hg stress, soil properties, network complexity, and community assembly) on SBF relationships. As in the case of the moving window approach, this technique can also disentangle the relationships of multivariate data via randomly sampling from the original data set. To ensure adequate data amounts, we selected two numbers (i.e., 12 and 16) of random samples. We conducted random sampling 100 times (50 times for the control samples of 50 times for Hg-treated samples) and generated 100 subsets for both sampling sizes. Based on each subset, the mean values of multiple factors were calculated, respectively. The relationships between multiple factors and the strength of SBFs were estimated as described above. Subsequently, SEM and RF analyses were performed to evaluate the effects of network complexity and stochastic ratio on SBF strength (as explained above).

## 3 Results

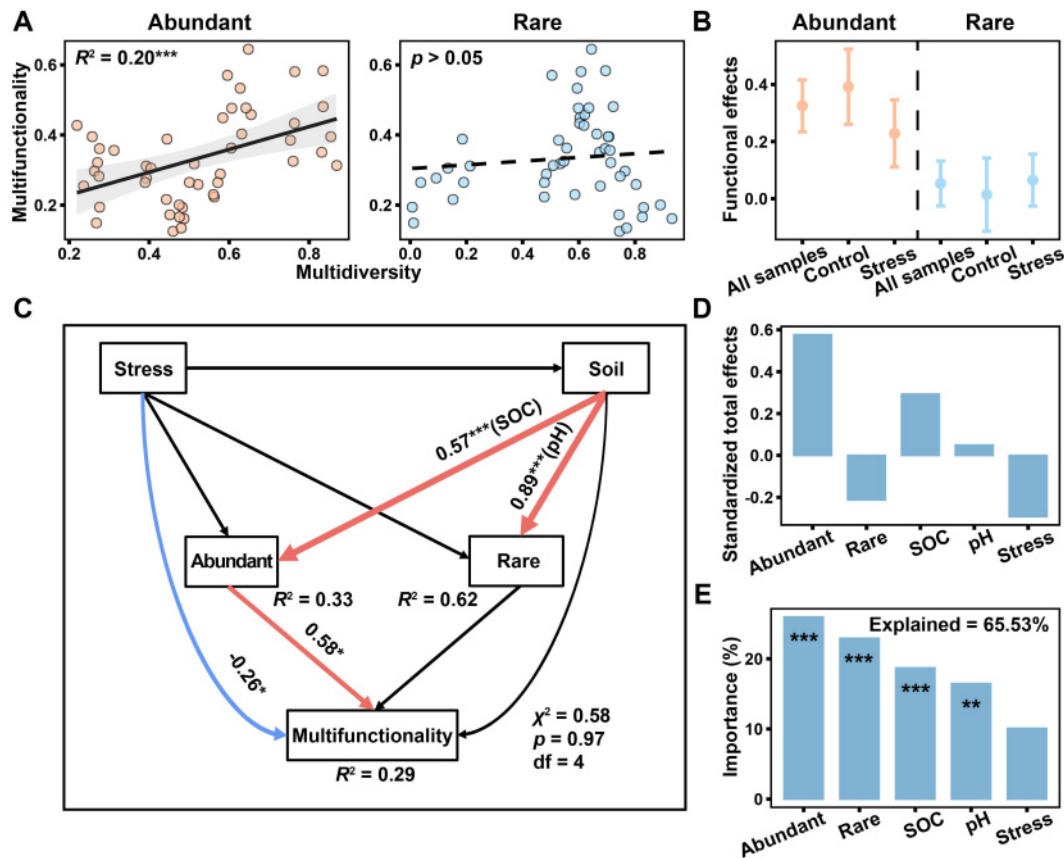
### 3.1 Relationships between biodiversity of soil abundant/rare taxa and multifunctionality under Hg stress

Abundant taxa accounted for only 4.8% of total OTUs but 45.7% of total reads, while rare taxa comprised 74.7% of total OTUs but only 21.0% of total reads (Fig. S1). In comparison with bacterial abundant taxa, fungal and protistan abundant taxa accounted for higher proportions of both total OTUs and reads (Fig. S1). We first estimated the effects of soil abundant and rare taxa biodiversity on multifunctionality under Hg stress. A strongly positive relationship between multidiversity and multifunctionality was observed for abundant taxa ( $p < 0.001$ ), but not for rare taxa (Fig. 1A). The biodiversity of soil abundant taxa exhibited higher functional effects (i.e., steeper slopes of the relationships between multifunctionality and multidiversity) than in the case of rare taxa (Fig. 1B). Hg stress decreased the functional effect of abundant taxa compared to the control, but slightly increased that of rare taxa (Figs. 1B and S2).

We then constructed a SEM to quantify the contributions of soil abundant and rare taxa multidiversity to multifunctionality, accounting for multiple factors simultaneously. Our SEM indicated that a positive and direct effect of soil abundant taxa diversity on multifunctionality was maintained under Hg stress, while the diversity of rare taxa had no significant effect on multifunctionality (Fig. 1C). After simultaneously judging from the standard total effects calculated by SEM and the importance of multiple factors on multifunctionality predicted by the random forest model, we found that biodiversity of soil abundant taxa, followed by biodiversity of soil rare taxa and chemical properties (i.e., SOC and pH), had the most important contribution to multifunctionality when Hg stress existed (Fig. 1D and E).

### 3.2 Supporting effects of soil abundant and rare taxa on functions

The diversity of multitrophic and most single groups of abundant taxa had significantly positive relationships with most single soil functions, which was not the case for the rare taxa (Fig. 2A). We further estimated the positive effects of individual phylotypes (i.e., supporting phylotypes) of abundant and rare taxa on single soil functions. Results showed that most of the supporting phylotypes for the single functions belonged to abundant taxa rather than rare taxa (Fig. 2B), with a higher average proportion of supporting phylotypes for abundant taxa than rare taxa (Fig. 2C). Both the proportions of supporting phylotypes of abundant and rare taxa showed decreasing trends with the increasing number of soil functions (Fig. 2D), with a higher average number of



**Fig. 1** Effects of soil abundant and rare taxa biodiversity on multifunctionality. (A) Linear relationships between multifunctionality and soil abundant taxa and rare taxa biodiversity. Statistical analysis was performed using ordinary least-squares linear regressions;  $p$  values are indicated by asterisks:  $***p < 0.001$ . (B) Functional effects (standardized slopes, i.e., mean  $\pm$  s.e.m.) of soil abundant taxa and rare taxa biodiversity on multifunctionality in all, control, and Hg stress samples, respectively. (C) Structural equation model showing the relationship between soil abundant/rare taxa biodiversity and multifunctionality. Red arrows represent significantly positive paths, and blue arrows represent significantly negative paths. Numbers adjacent to arrows are indicative of effect size of the relationship.  $R^2$  denotes the proportion of variance explained. Significance levels are shown as follows:  $*p < 0.05$ ,  $**p < 0.01$ , and  $***p < 0.001$ ; RMSEA = 0; GFI = 0.996; AIC = 34.584. Abbreviation: SOC, soil organic carbon. (D) Standardized total effects of soil abundant and rare taxa biodiversity, soil chemical properties (SOC and pH), and stress (control and Hg treatment) on multifunctionality. (E) Random forest predicting relative importance of soil abundant and rare taxa biodiversity, soil chemical properties, and stress for multifunctionality. Significance levels are shown as follows:  $**p < 0.01$ , and  $***p < 0.001$ .

supporting functions observed for abundant taxa than rare taxa (Fig. 2E).

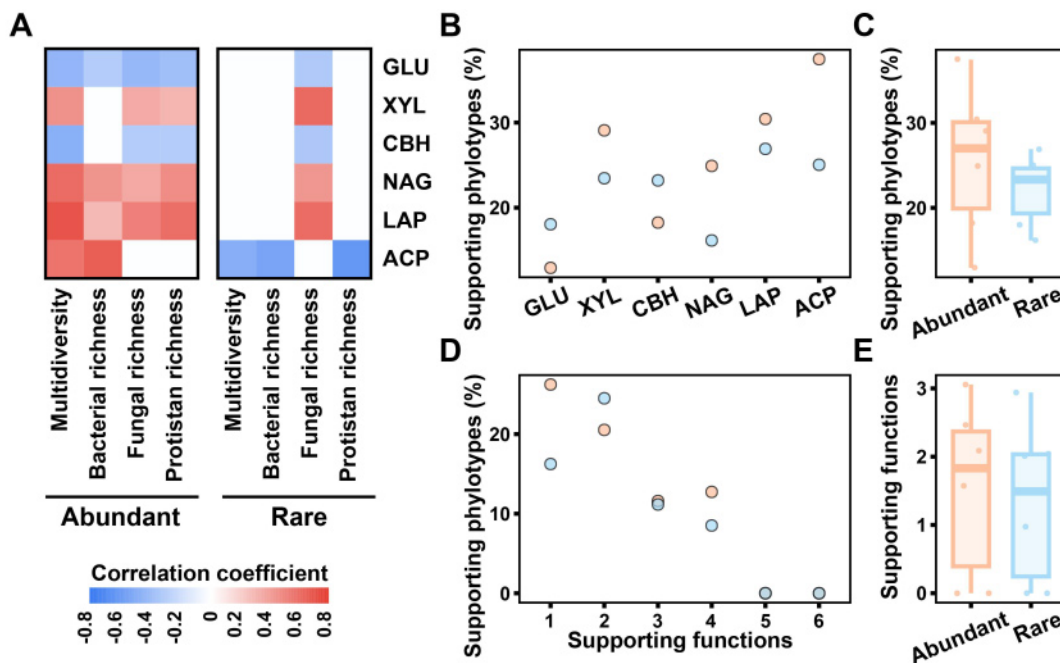
### 3.3 Relationships between soil multitrophic network, multidiversity, and multifunctionality

Soil multitrophic co-occurrence network was constructed for abundant taxa to explore the associations among multiple phylotypes (i.e., bacteria, fungi, and protists) and their contributions to multifunctionality (Fig. 3A). A strongly positive linear regression relationship was observed between the supporting multifunctionality and the degree of network for abundant taxa ( $p < 0.001$ , Fig. S3). Subsequently, we calculated network complexity index by averaging eight topological features extracted from subnetworks of individual samples. Soil network complexity was positively correlated with both multidiversity of abundant taxa and

multifunctionality ( $p < 0.001$ , Fig. 3B). Particularly, network complexity showed greater effects on multidiversity than multifunctionality (Fig. 3C). Hg stress decreased the network effects on both multidiversity and multifunctionality compared to the control (Figs. 3C and S4). The strength of SBF was determined by the explained variation of soil biodiversity on multifunctionality. Moreover, significant relationships were found between the strength of SBF and soil network complexity of abundant taxa ( $p < 0.001$ ), with first an increasing and then a decreasing trend of SBF strength along network complexity, using both 12 and 16 sample window sizes (Fig. 3D).

### 3.4 Relationships between community assembly processes, multidiversity, and multifunctionality

Normalized stochasticity ratio (NST) analyses revealed that



**Fig. 2** Functional roles of soil abundant and rare taxa biodiversity on multifunctionality. (A) Significant correlations (Spearman:  $p < 0.05$ ) between multidiversity as well as the diversity of single microorganism group and individual soil function. (B) Proportions of phylotypes supporting the individual soil function. Phylotypes which significantly and positively correlated with soil functions were defined as “supporting phylotypes”. (C) The average proportion of abundant and rare phylotypes supporting soil functions. (D) Proportions of phylotypes supporting the different numbers of soil functions. (E) The average number of supporting functions of abundant and rare phylotypes.

the stochastic community assembly ratio of the abundant subcommunity was decreased along the gradients of multidiversity and multifunctionality (Fig. 4A and B), but the normalized stochastic ratio of the rare subcommunity showed contrasting trends (Fig. S5A and B). Linear regression analyses then confirmed that multidiversity and multifunctionality were significantly and negatively correlated with the normalized stochastic ratio of the abundant subcommunity ( $p < 0.05$ , Fig. 4C), but these relationships were opposite for the rare subcommunity (Fig. S5C). The effect of community assembly on the relationship between multidiversity and multifunctionality was further quantified via the moving window approach. Significant and positive relationships were found between the strength of SBF and the normalized stochastic ratio of abundant subcommunity ( $p < 0.001$ , Fig. 4D), but these relationships were not observed for the rare subcommunity (Fig. S5D).

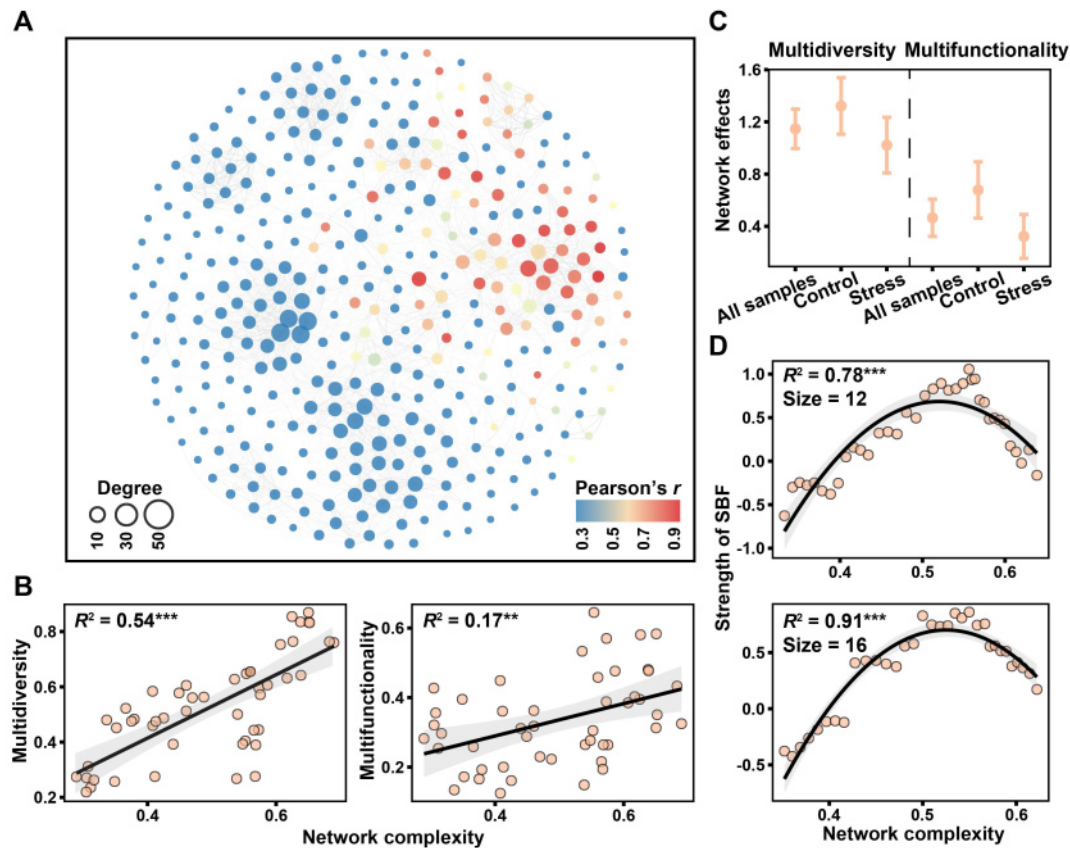
### 3.5 Effects of multiple factors on the relationships between soil biodiversity and multifunctionality under Hg stress

Comprehensively, we constructed SEMs to quantify the contributions of multiple factors including Hg stress, soil properties, network complexity, and community assembly on the strength of SBF for abundant taxa based on two random sampling sizes. Our SEMs suggested that positive and direct effects of stochastic ratio and network complexity on

the SBF strength were consistently maintained when accounting for the strongly negative effects of Hg stress simultaneously, using both 12 and 16 sampling sizes (Fig. 5A and B). After judging from the standard total effects calculated by SEMs and the importance of multiple factors on the strength of SBF predicted by the random forest model, the results indicated that stochastic community assembly process of abundant taxa was the most important predictor for the SBF strength when Hg stress existed, validated by the similar trends of both 12 and 16 random sampling sizes (Fig. 5C and D).

## 4 Discussion

Previous studies have advanced our knowledge on the relationship between biodiversity and multifunctionality based on whole communities (Delgado-Baquerizo et al., 2020; Jiao et al., 2021), but less attention has been paid to the relative contributions of abundant and rare taxa to soil functions, let alone under an environmental stress. Here, we provide experimental evidence that the diversity of abundant taxa rather than rare taxa is necessary to maintain multiple soil functions under Hg pollution stress. Importantly, we found that soil multitrophic network complexity and stochastic assembly processes of the abundant subcommunity, but not of the rare subcommunity, can maintain the relationship



**Fig. 3** Effects of soil multitrophic network complexity on multidiversity of abundant taxa and multifunctionality. (A) Correlation networks for abundant taxa. Networks are colored based on the Pearson's correlation coefficients between each individual phylotype and multifunctionality; the coefficients were used to reflect their support for multifunctionality. (B) Relationships between soil multitrophic network complexity versus multidiversity and multifunctionality. The relationships were estimated by ordinary least-squares linear regressions. (C) Effects (standardized slopes, i.e., mean  $\pm$  s.e.m.) of multitrophic network complexity on multidiversity of soil abundant taxa and multifunctionality in all, control, and Hg stress samples. (D) Effects of network complexity on the relationships between soil abundant taxa biodiversity and multifunctionality. The variances of SBF strengths across network complexity gradients were based on moving window sizes of 12 and 16 samples. The relationships were estimated by ordinary least-squares linear regressions with second-order polynomial fits. Significance levels are shown as follows:  $**p < 0.01$ , and  $***p < 0.001$ .

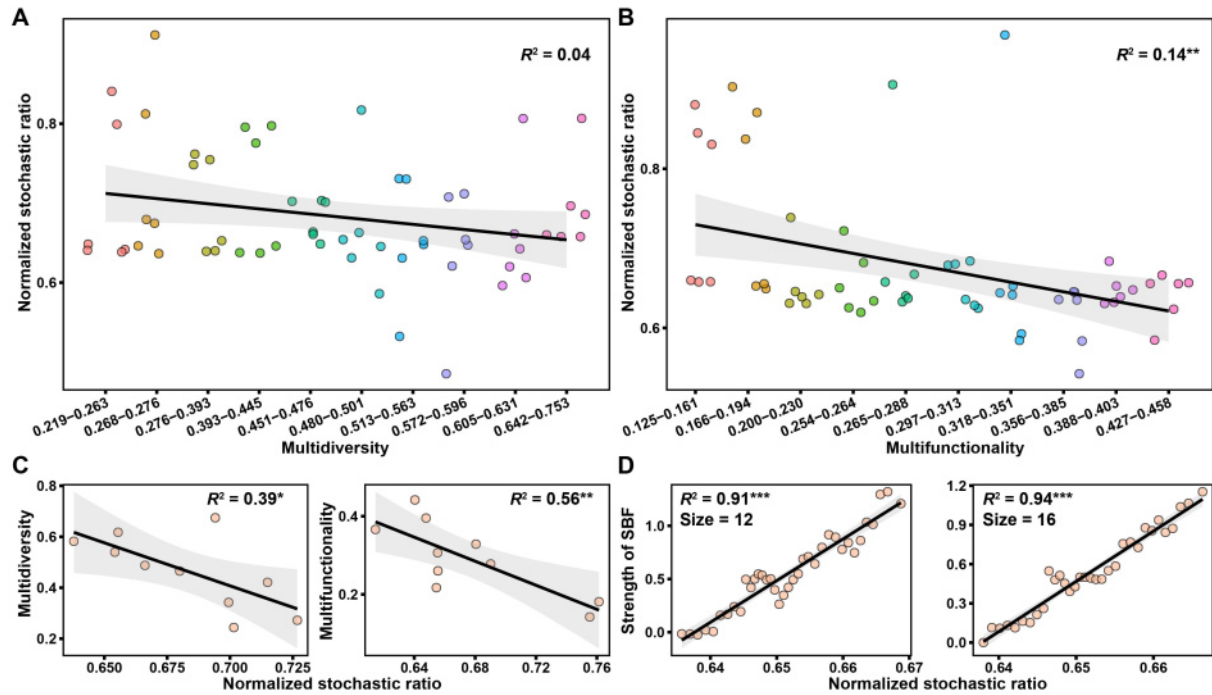
between soil biodiversity and multifunctionality. Our study highlights the significance of abundant taxa in sustaining soil multifunctionality and the relationship between community assembly, network complexity and SBF strength under environmental stresses.

#### 4.1 Effects of abundant/rare taxa diversity on multifunctionality

In the present study, we observed that Hg stress decreased the effect of abundant taxa biodiversity on soil multifunctionality. Heavy metal Hg is toxic and has adverse effect on most soil microorganisms, reducing biodiversity and soil functioning. Our previous study also indicated that legacy Hg pollution decreased the diversity of soil organisms including bacteria, fungi, and protists (Du et al., 2022). Importantly, our results revealed that the multidiversity of abundant taxa, rather than that of rare taxa, was positively

related to multifunctionality under Hg stress, suggesting that abundant taxa had a larger effect on multifunctionality. The abundant subcommunity was more stable than rare subcommunity in response to soil Hg pollution stress, which could be explained by the abundant community's wider breadth to environmental changes (Li et al., 2019b). Abundant taxa diversity can buffer the impacts of stress by maintaining functional redundancy and compensating for the loss of sensitive species (Balvanera et al., 2006; Gamfeldt et al., 2008). On the contrary, rare taxa diversity may be more susceptible to environmental stress due to their low abundance, making them more prone to extinction (Hughes et al., 1997; Gaston and Fuller, 2007). It has been reported that abundant species had broader environmental adaptation for ecological preferred traits compared to rare species in agricultural ecosystems (Jiao and Lu, 2020). Soil Hg stress filtered a larger number of rare species than abundant species, leading to a loss of biodiversity of rare species and





**Fig. 4** Effects of community assembly processes on multidiversity of abundant taxa and multifunctionality. (A and B) Patterns of normalized stochasticity ratio, estimated via null models, across different categories of multidiversity and multifunctionality. (C) Relationships between normalized stochasticity ratio versus multidiversity and multifunctionality. The relationships were estimated by ordinary least-squares linear regressions. (D) Effects of community assembly processes on the relationships between soil abundant taxa biodiversity and multifunctionality. The variances of SBF strengths across normalized stochasticity ratio gradients were based on moving window sizes of 12 and 16 samples. The relationships were estimated by ordinary least-squares linear regressions. Significance levels are shown as follows: \* $p < 0.05$ , \*\* $p < 0.01$ , and \*\*\* $p < 0.001$ .

a weak SBF relationship. Therefore, environmental stresses like soil pollution have the potential to affect the relative contributions of abundant and rare taxa to soil functions, as observed in our study.

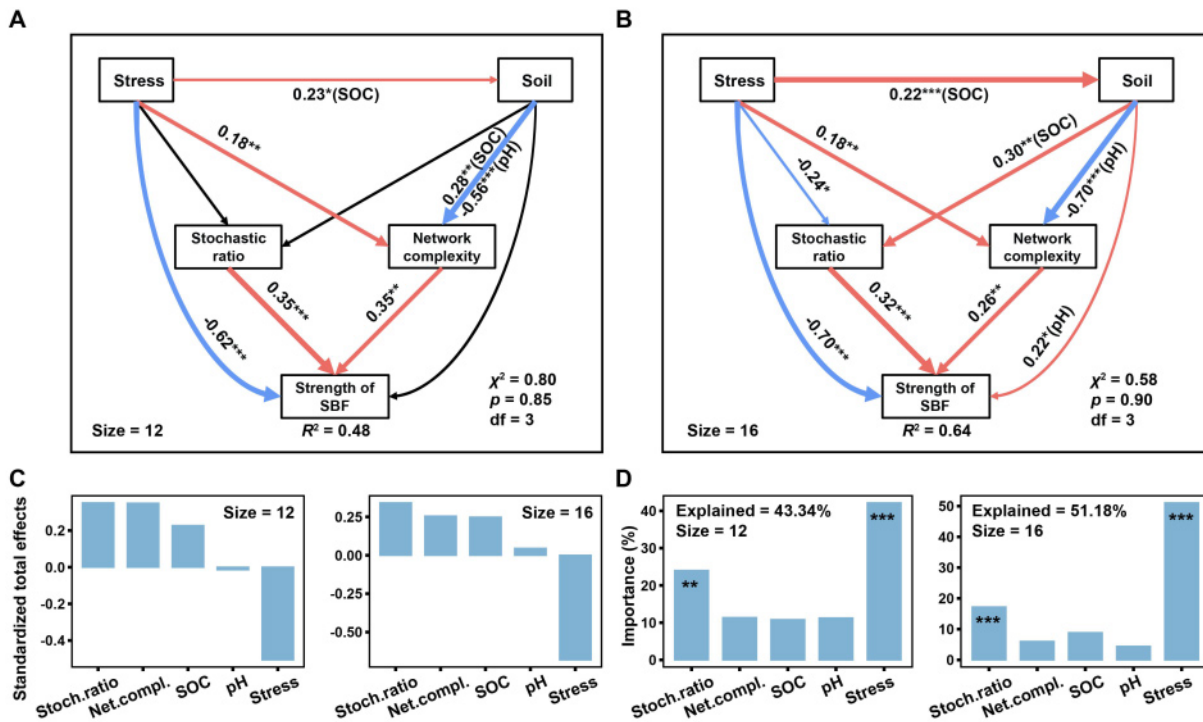
Moreover, we found that abundant subcommunity contributed more supporting phylotypes with respect to soil functions than was the case for the rare subcommunity, and this could explain the stronger positive effect of abundant taxa diversity on multifunctionality. In particular, abundant phylotypes exhibited higher supporting effects of individual soil enzyme activities related to the degradation of sugar, chitin, and protein. One possible reason could be that abundant taxa occupy diverse niches, utilize wide resources, and drive broad functions such as nutrient cycling, primary production, and decomposition (Jiao et al., 2017; Rivett and Bell, 2018). Abundant taxa are also more likely to possess redundant functional traits, meaning that if one species is negatively affected by environmental stress, others can compensate for their functions, thus maintaining ecosystem processes (Maestre et al., 2012; Balvanera et al., 2014). Conversely, rare taxa generally have narrow functional roles, because they are restricted by habitat specificity (Barberán et al., 2014; Jousset et al., 2017). The above findings highlight the importance of abundant species for maintaining soil multifunctionality under environmental stress, and will be

beneficial in guiding future sustainable management in agricultural ecosystems.

#### 4.2 Linking multitrophic network complexity to SBF relationship

Microbial interactions play critical roles in supporting community functional performance (Bardgett and Van Der Putten, 2014). For example, species coexisting could promote positive interactions via niche partitioning based on distinct resources (Li et al., 2019a; Yu et al., 2019). In this study, we observed that abundant taxa were strongly correlated with multifunctionality in the correlation network, suggesting that abundant taxa could support multifunctionality at a relatively high network connectivity. We further revealed that network complexity of soil abundant subcommunity could support multidiversity and multifunctionality. This can be partly attributed to the strong connections among abundant taxa, sharing similar habitats and environmental preferences for executing high functional performance (Newman, 2003, 2006). A previous study also demonstrated that microbial interkingdom interactions could support ecosystem functioning related to nutrient cycling in grassland soils (Wagg et al., 2019).

Multitrophic network complexity, including the presence of



**Fig. 5** Effects of multiple factors on the relationships between biodiversity of abundant taxa and multifunctionality. (A and B) Structural equation models showing the effects of stochastic community assembly, multitrophic network complexity, soil properties, and Hg stress on the relationships between soil abundant taxa biodiversity and multifunctionality. The variances of SBF strengths were based on random sampling method with sizes of 12 and 16 samples. Red arrows represent significantly positive paths, and blue arrows represent significantly negative paths. Numbers adjacent to arrows are indicative of effect size of the relationship.  $R^2$  denotes the proportion of variance explained. Significance levels are shown as follows: \* $p < 0.05$ , \*\* $p < 0.01$ , and \*\*\* $p < 0.001$ ; RMSEA = 0; GFI = 0.997; AIC = 36.795 for sampling size = 12, and RMSEA = 0; GFI = 0.998; AIC = 36.580 for sampling size = 16, respectively. (C) Standardized total effects of stochastic ratio, network complexity, soil chemical properties (SOC and pH), and stress (control and Hg treatment) on the strength of SBF with sampling size of 12 and 16. (D) Random forest predicting relative importance of stochastic ratio, network complexity, soil chemical properties, and stress for the strength of SBF with sampling size of 12 and 16. Significance levels are shown as follows: \*\* $p < 0.01$ , and \*\*\* $p < 0.001$ . Abbreviations: Stoch.ratio, stochastic ratio; Net.compl., network complexity.

abundant and rare taxa, can contribute to the resistance of ecosystems in face of environmental stress (Pocock et al., 2012; Zhao et al., 2023). In this study, Hg pollution stress decreased the effects of network complexity on multidiversity and multifunctionality, and these effects could be attributed to the adverse effects of Hg on biological interactions. Intensive environmental disturbances are considered to lead to the simplification of soil multitrophic networks (Tsiafouli et al., 2015; Bender et al., 2016). The reduction of the complexity of microbial interactions can damage the biodiversity-driven ecosystem functioning (Jiao et al., 2021; Wang et al., 2022b). Abundant taxa may show higher resistance to environmental stress due to their broad ecological tolerances or adaptability in comparison with rare taxa (Isbell et al., 2015), therefore they may play an important role in stabilizing ecosystem functioning (Balvanera et al., 2006; Li et al., 2021). Moreover, our results suggest that moderate biological interactions have the greatest supporting Hg effects on the strength of SBF under Hg stress. Microbial cooperation and competition could support the SBF relationship at a range of

network complexity. Relative high network complexity provides more alternative pathways and interactions, which can buffer the impacts of environmental stress on soil functioning (Bardgett and Van Der Putten, 2014; Wagg et al., 2014). For example, soil multitrophic network complexity has been shown to enhance the link between biodiversity and multifunctionality in agricultural systems (Jiao et al., 2021). Overall, our findings suggest that soil multitrophic network complexity is a key factor in maintaining the diversity-multifunction relationship.

#### 4.3 Linking community assembly processes to SBF relationship

Community assembly processes refer to the mechanisms that shape the composition and structure of ecological communities, including the factors that determine the abundance and distribution of abundant and rare taxa (Leibold et al., 2004; Chase and Myers, 2011). Therefore, it is critical to differentiate the stochastic or deterministic assembly

processes of soil abundant and rare subcommunities and their consequences for ecosystem functioning (Mokany et al., 2013; Mori et al., 2018). In this study, we unraveled that stochastic assembly processes promoted the positive effect of the biodiversity of abundant taxa, rather than rare taxa, on multifunctionality under Hg stress. Abundant taxa are well-adapted to environmental disturbances due to their broader niches and competitive advantage; thus, they potentially influence SBF relationship (Mouillot et al., 2013). In contrast, rare taxa with unique or specialized traits may be filtered out if their adaptations are not advantageous under the stress (Kraft et al., 2015). We have demonstrated that the dominant taxa drove the resistance of soil microbial community to Hg stress and maintained the functional stability in our previous study (Du et al., 2023). A recent study also claimed that there were more co-occurrence associations within the soil microbiome when stochastic processes predominantly drove community assembly (Jiao et al., 2020).

In addition, stochastic processes-driven community assembly could increase the effect of biodiversity on functions via random sampling effect (Knelman and Nemerbut, 2014). This effect means that some communities may contain a higher number or different combinations of species compared to others by chance, and can enhance ecosystem functioning by promoting a greater variety of functional traits, resource utilization, and ecological interactions (Adler et al., 2007; Vellend, 2010). On the contrary, a larger contribution of deterministic assembly processes can decrease the biodiversity effect on ecosystem functions via dilution effect (Zhang et al., 2022). These processes can limit the diversity of species within a community, potentially diluting the positive effects of biodiversity on ecosystem functions (Duffy et al., 2007; Byrnes et al., 2014). We also observed that the stochastic assembly ratio of abundant subcommunity was negatively correlated with biodiversity and multifunctionality. A possible reason could be that stochastic assembly processes lead to more species competition, thus reduce microbial diversity and functional redundancy (Zhang et al., 2022). Collectively, our results suggest that stochastic assembly ratio of abundant taxa is the most important factor for maintaining biodiversity-multifunctionality relationship under Hg stress.

## 5 Conclusions

To the best of our knowledge, this is the first study to link soil network complexity and community assembly processes of abundant and rare species to the strength of SBF relationship under soil Hg pollution stress. Our findings provide empirical evidence that the diversity of abundant taxa is more critical than that of rare taxa for supporting soil

multifunctionality under pollution stress. Moreover, our results indicate that soil multitrophic network complexity and stochastic assembly processes of the abundant subcommunity can maintain the relationships between soil biodiversity and multifunctionality, but this is not the case for the rare subcommunity. Overall, we highlight that stochastic assembly ratio of abundant taxa is the most important factor in sustaining the effects of biodiversity on multiple functions under soil pollution stress after accounting for multiple factors. These results can help in forecasting functional roles and consequences of compositional changes within soil microbial communities, across multitrophic groups, those are affected by environmental disturbances including pollution stress in an ecosystem.

## Data availability

The raw bacterial, fungal, and protistan sequencing data reported in this paper are available in the NCBI Sequence Read Archive under BioProject PRJNA914639, PRJNA803336, and PRJNA803337, respectively.

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## Author contributions

Shuai Du: Conceptualization, Methodology, Software, Formal analysis, Investigation, Writing-original draft, Writing-review and editing, Visualization. Xin-Qi Li: Data curation, Software, Formal analysis, Writing-review and editing, Visualization. Li Bi: Data curation, Software, Formal analysis, Writing-review and editing, Visualization. Dong Zhu: Formal analysis, Investigation, Writing-review and editing, Visualization. Hang-Wei Hu: Formal analysis, Investigation, Writing-review and editing, Visualization. Xiuli Hao: Formal analysis, Investigation, Writing-review and editing, Visualization. Jiao Feng: Formal analysis, Investigation, Writing-review and editing, Visualization. Qiaoyun Huang: Formal analysis, Investigation, Writing-review and editing, Visualization. Yu-Rong Liu: Conceptualization, Investigation, Writing-review and editing, Supervision, Resources, Funding acquisition.

## Ethics declarations

The authors declare that they have no competing interests.

## Electronic supplementary material

Supplementary material is available in the online version of this

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## References

- Adler, P.B., HilleRisLambers, J., Levine, J.M., 2007. A niche for neutrality. *Ecology Letters* 10, 95–104.
- Archer, E., 2016. rfPermute: estimate permutation  $p$ -values for random forest importance metrics. R package version 1.
- Balvanera, P., Pfisterer, A.B., Buchmann, N., He, J.S., Nakashizuka, T., Raffaelli, D., Schmid, B., 2006. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. *Ecology Letters* 9, 1146–1156.
- Balvanera, P., Siddique, I., Dee, L., Paquette, A., Isbell, F., Gonzalez, A., Byrnes, J., O'Connor, M.I., Hungate, B.A., Griffin, J.N., 2014. Linking biodiversity and ecosystem services: current uncertainties and the necessary next steps. *Bioscience* 64, 49–57.
- Barberán, A., Bates, S.T., Casamayor, E.O., Fierer, N., 2012. Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME Journal* 6, 343–351.
- Barberán, A., Ramirez, K.S., Leff, J.W., Bradford, M.A., Wall, D.H., Fierer, N., 2014. Why are some microbes more ubiquitous than others? Predicting the habitat breadth of soil bacteria. *Ecology Letters* 17, 794–802.
- Bardgett, R.D., Van Der Putten, W.H., 2014. Belowground biodiversity and ecosystem functioning. *Nature* 515, 505–511.
- Bastian, M., Heymann, S., Jacomy, M., 2009. Gephi: an open source software for exploring and manipulating networks. *Proceedings of the International AAAI Conference on Web and Social Media* 3, 361–362.
- Bell, C.W., Fricks, B.E., Rocca, J.D., Steinweg, J.M., McMahon, S.K., Wallenstein, M.D., 2013. High-throughput fluorometric measurement of potential soil extracellular enzyme activities. *Journal of Visualized Experiments* 81, e50961.
- Bender, S.F., Wagg, C., van der Heijden, M.G., 2016. An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. *Trends in Ecology & Evolution* 31, 440–452.
- Breiman, L., 2001. Random forests. *Machine Learning* 45, 5–32.
- Byrnes, J.E., Gamfeldt, L., Isbell, F., Lefcheck, J.S., Griffin, J.N., Hector, A., Cardinale, B.J., Hooper, D.U., Dee, L.E., Emmett Duffy, J., 2014. Investigating the relationship between biodiversity and ecosystem multifunctionality: challenges and solutions. *Methods in Ecology and Evolution* 5, 111–124.
- Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Tumbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7, 335–336.
- Cardinale, B.J., 2011. Biodiversity improves water quality through niche partitioning. *Nature* 472, 86–89.
- Carlson, M.L., Flagstad, L.A., Gillet, F., Mitchell, E.A., 2010. Community development along a proglacial chronosequence: are above-ground and below-ground community structure controlled more by biotic than abiotic factors? *Journal of Ecology* 98, 1084–1095.
- Chase, J.M., Myers, J.A., 2011. Disentangling the importance of ecological niches from stochastic processes across scales. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 366, 2351–2363.
- Csardi, G., Nepusz, T., 2006. The igraph software package for complex network research. *InterJournal, Complex Systems* 1695, 1–9.
- De Vries, F.T., Thébault, E., Liiri, M., Birkhofer, K., Tsiafouli, M.A., Bjørnlund, L., Bracht Jørgensen, H., Brady, M.V., Christensen, S., De Ruiter, P.C., d'Hertefeldt, T., Frouz, J., Hedlund, K., Hemerik, L., Hol, W.H.G., Hotes, S., Mortimer, S.R., Setälä, H., Sgardelis, S.P., Uteseny, K., van der Putten, W.H., Wolters, V., Bardgett, R.D., 2013. Soil food web properties explain ecosystem services across European land use systems. *Proceedings of the National Academy of Sciences of the United States of America* 110, 14296–14301.
- Delgado-Baquerizo, M., Maestre, F.T., Reich, P.B., Jeffries, T.C., Gaitan, J.J., Encinar, D., Berdugo, M., Campbell, C.D., Singh, B.K., 2016. Microbial diversity drives multifunctionality in terrestrial ecosystems. *Nature Communications* 7, 1–8.
- Delgado-Baquerizo, M., Reich, P.B., Trivedi, C., Eldridge, D.J., Abades, S., Alfaro, F.D., Bastida, F., Berhe, A.A., Cutler, N.A., Gallardo, A., Garcia-Velazquez, L., Hart, S.C., Hayes, P.E., He, J.Z., Hseu, Z.Y., Hu, H.W., Kirchmair, M., Neuhauser, S., Perez, C.A., Reed, S.C., Santos, F., Sullivan, B.W., Trivedi, P., Wang, J.T., Weber-Grullon, L., Williams, M.A., Singh, B.K., 2020. Multiple elements of soil biodiversity drive ecosystem functions across biomes. *Nature Ecology & Evolution* 4, 210–220.
- Du, S., Li, X.Q., Feng, J., Huang, Q., Liu, Y.R., 2023. Soil core microbiota drive community resistance to mercury stress and maintain functional stability. *Science of the Total Environment* 894, 165056.
- Du, S., Li, X.Q., Hao, X., Hu, H.W., Feng, J., Huang, Q., Liu, Y.R., 2022. Stronger responses of soil protistan communities to legacy mercury pollution than bacterial and fungal communities in agricultural systems. *ISME Communications* 2, 1–12.
- Duffy, J.E., Cardinale, B.J., France, K.E., McIntyre, P.B., Thébault, E., Loreau, M., 2007. The functional role of biodiversity in ecosystems: incorporating trophic complexity. *Ecology Letters* 10, 522–538.
- Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature Methods* 10, 996–998.
- Fajardo, C., Costa, G., Nande, M., Botías, P., García-Cantalejo, J., Martín, M., 2019. Pb, Cd, and Zn soil contamination: monitoring functional and structural impacts on the microbiome. *Applied Soil Ecology* 135, 56–64.
- Fortmann-Roe, S., 2015. Consistent and clear reporting of results from diverse modeling techniques: the A3 method. *Journal of Statistical Software* 66, 1–23.
- Gamfeldt, L., Hillebrand, H., Jonsson, P.R., 2008. Multiple functions increase the importance of biodiversity for overall ecosystem

- functioning. *Ecology* 89, 1223–1231.
- Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2, 113–118.
- Gaston, K.J., Fuller, R.A., 2007. Biodiversity and extinction: losing the common and the widespread. *Progress in Physical Geography* 31, 213–225.
- Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., Boutte, C., Burgaud, G., de Vargas, C., Decelle, J., Del Campo, J., Dolan, J.R., Dunthorn, M., Edvardsen, B., Holzmann, M., Kooistra, W.H., Lara, E., Le Bescot, N., Logares, R., Mahe, F., Massana, R., Montresor, M., Morard, R., Not, F., Pawlowski, J., Probert, I., Sauvadet, A.L., Siano, R., Stoeck, T., Vaultot, D., Zimmermann, P., Christen, R., 2013. The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy. *Nucleic Acids Research* 41, D597–D604.
- Hao, X., Zhu, J., Rensing, C., Liu, Y., Gao, S., Chen, W., Huang, Q., Liu, Y.R., 2021. Recent advances in exploring the heavy metal (loid) resistant microbiome. *Computational and Structural Biotechnology Journal* 19, 94–109.
- Hughes, J.B., Daily, G.C., Ehrlich, P.R., 1997. Population diversity: its extent and extinction. *Science* 278, 689–692.
- Isbell, F., Craven, D., Connolly, J., Loreau, M., Schmid, B., Beierkuhnlein, C., Bezemer, T.M., Bonin, C., Bruelheide, H., De Luca, E., Ebeling, A., Griffin, J.N., Guo, Q., Hautier, Y., Hector, A., Jentsch, A., Kreyling, J., Lanta, V., Manning, P., Meyer, S.T., Mori, A.S., Naeem, S., Niklaus, P.A., Polley, H.W., Reich, P.B., Roscher, C., Seabloom, E.W., Smith, M.D., Thakur, M.P., Tilman, D., Tracy, B.F., van der Putten, W.H., van Ruijven, J., Weigelt, A., Weisser, W.W., Wilsey, B., Eisenhauer, N., 2015. Biodiversity increases the resistance of ecosystem productivity to climate extremes. *Nature* 526, 574–577.
- Jassey, V.E., Lamentowicz, Ł., Robroek, B.J., Gąbka, M., Rusińska, A., Lamentowicz, M., 2014. Plant functional diversity drives niche - size - structure of dominant microbial consumers along a poor to extremely rich fen gradient. *Journal of Ecology* 102, 1150–1162.
- Jiao, S., Chen, W., Wei, G., 2017. Biogeography and ecological diversity patterns of rare and abundant bacteria in oil-contaminated soils. *Molecular Ecology* 26, 5305–5317.
- Jiao, S., Lu, Y., 2020. Abundant fungi adapt to broader environmental gradients than rare fungi in agricultural fields. *Global Change Biology* 26, 4506–4520.
- Jiao, S., Lu, Y., Wei, G., 2021. Soil multitrophic network complexity enhances the link between biodiversity and multifunctionality in agricultural systems. *Global Change Biology* 28, 140–153.
- Jiao, S., Wang, J., Wei, G., Chen, W., Lu, Y., 2019. Dominant role of abundant rather than rare bacterial taxa in maintaining agro-soil microbiomes under environmental disturbances. *Chemosphere* 235, 248–259.
- Jiao, S., Yang, Y., Xu, Y., Zhang, J., Lu, Y., 2020. Balance between community assembly processes mediates species coexistence in agricultural soil microbiomes across eastern China. *ISME Journal* 14, 202–216.
- Jousset, A., Bienhold, C., Chatzinotas, A., Gallien, L., Gobet, A., Kurm, V., Küsel, K., Rillig, M.C., Rivett, D.W., Salles, J.F., van der Heijden, M.G.A., Youssef, N.H., Zhang, X., Wei, Z., Hol, W.H.G., 2017. Where less may be more: how the rare biosphere pulls ecosystems strings. *ISME Journal* 11, 853–862.
- Kardol, P., Wardle, D.A., 2010. How understanding aboveground-belowground linkages can assist restoration ecology. *Trends in Ecology & Evolution* 25, 670–679.
- Knelman, J.E., Nemergut, D.R., 2014. Changes in community assembly may shift the relationship between biodiversity and ecosystem function. *Frontiers Media SA*, p. 424.
- Kraft, N.J., Adler, P.B., Godoy, O., James, E.C., Fuller, S., Levine, J.M., 2015. Community assembly, coexistence and the environmental filtering metaphor. *Functional Ecology* 29, 592–599.
- Langfelder, P., Horvath, S., 2012. Fast R functions for robust correlations and hierarchical clustering. *Journal of Statistical Software* 46, 1–17.
- Leibold, M.A., Holyoak, M., Mouquet, N., Amarasekare, P., Chase, J.M., Hoopes, M.F., Holt, R.D., Shurin, J.B., Law, R., Tilman, D., Loreau, M., Gonzalez, A., 2004. The metacommunity concept: a framework for multi-scale community ecology. *Ecology Letters* 7, 601–613.
- Lennon, J.T., Jones, S.E., 2011. Microbial seed banks: the ecological and evolutionary implications of dormancy. *Nature Reviews Microbiology* 9, 119–130.
- Li, H.Z., Zhu, D., Lindhardt, J.H., Lin, S.M., Ke, X., Cui, L., 2021. Long-term fertilization history alters effects of microplastics on soil properties, microbial communities, and functions in diverse farmland ecosystem. *Environmental Science & Technology* 55, 4658–4668.
- Li, M., Wei, Z., Wang, J., Jousset, A., Friman, V.P., Xu, Y., Shen, Q., Pommier, T., 2019a. Facilitation promotes invasions in plant-associated microbial communities. *Ecology Letters* 22, 149–158.
- Li, P., Liu, J., Jiang, C., Wu, M., Liu, M., Li, Z., 2019b. Distinct Successions of common and rare bacteria in soil under humic acid amendment – a microcosm study. *Frontiers in Microbiology* 10, 2271.
- Liu, L., Yang, J., Yu, Z., Wilkinson, D.M., 2015. The biogeography of abundant and rare bacterioplankton in the lakes and reservoirs of China. *ISME Journal* 9, 2068–2077.
- Liu, Y.R., Delgado-Baquerizo, M., Bi, L., Zhu, J., He, J.Z., 2018. Consistent responses of soil microbial taxonomic and functional attributes to mercury pollution across China. *Microbiome* 6, 183.
- Liu, Y.R., van der Heijden, M.G., Riedo, J., Sanz-Lazaro, C., Eldridge, D.J., Bastida, F., Moreno-Jiménez, E., Zhou, X.Q., Hu, H.W., He, J.Z., Moreno, J.L., Abades, S., Alfaro, F., Bamigboye, A.R., Berdugo, M., Blanco-Pastor, J.L., de los Ríos, A., Duran, J., Grebenc, T., Illán, J.G., Makhalyane, T.P., Molina-Montenegro, M.A., Nahberger, T.U., Peñalosa-Bojacá, G.F., Plaza, C., Rey, A., Rodríguez, A., Siebe, C., Teixido, A.L., Casado-Coy, N., Trivedi, P., Torres-Díaz, C., Verma, J.P., Mukherjee, A., Zeng, X.M., Wang, L., Wang, J., Zaady, E., Zhou, X., Huang, Q., Tan, W., Zhu, Y.G., Rillig, M.C., Delgado-Baquerizo, M., 2023. Soil contamination in nearby natural areas mirrors that in urban greenspaces worldwide. *Nature Communications* 14, 1706.
- Lu, T., Xu, N., Lei, C., Zhang, Q., Zhang, Z., Sun, L., He, F., Zhou, N.Y., Peñuelas, J., Zhu, Y.G., Qian, H., 2023. Bacterial biogeog-

- raphy in China and its association to land use and soil organic carbon. *Soil Ecology Letters* 5.
- Lynch, M.D., Neufeld, J.D., 2015. Ecology and exploration of the rare biosphere. *Nature Reviews Microbiology* 13, 217–229.
- Ma, B., Wang, H., Dsouza, M., Lou, J., He, Y., Dai, Z., Brookes, P. C., Xu, J., Gilbert, J.A., 2016. Geographic patterns of co-occurrence network topological features for soil microbiota at continental scale in eastern China. *ISME Journal* 10, 1891–1901.
- Maestre, F.T., Quero, J.L., Gotelli, N.J., Escudero, A., Ochoa, V., Delgado-Baquerizo, M., García-Gómez, M., Bowker, M.A., Soliveres, S., Escolar, C., García-Palacios, P., Berdugo, M., Valencia, E., Gozalo, B., Gallardo, A., Aguilera, L., Arredondo, T., Blones, J., Boeken, B., Bran, D., Conceição, A.A., Cabrera, O., Chaieb, M., Derak, M., Eldridge, D.J., Espinosa, C.I., Florentino, A., Gaitán, J., Gatica, M.G., Ghiloufi, W., Gómez-González, S., Gutiérrez, J.R., Hernández, R.M., Huang, X., Huber-Sannwald, E., Jankju, M., Miriti, M., Moneris, J., Mau, R.L., Morici, E., Naseri, K., Ospina, A., Polo, V., Prina, A., Pucheta, E., Ramírez-Collantes, D.A., Romão, R., Tighe, M., Torres-Díaz, C., Val, J., Veiga, J.P., Wang, D., Zaady, E., 2012. Plant species richness and ecosystem multifunctionality in global drylands. *Science* 335, 214–218.
- Mahbub, K.R., Krishnan, K., Naidu, R., Andrews, S., Megharaj, M., 2017. Mercury toxicity to terrestrial biota. *Ecological Indicators* 74, 451–462.
- Meyer, S.T., Ptacnik, R., Hillebrand, H., Bessler, H., Buchmann, N., Ebeling, A., Eisenhauer, N., Engels, C., Fischer, M., Halle, S., Klein, A.M., Oelmann, Y., Roscher, C., Rottstock, T., Scherber, C., Scheu, S., Schmid, B., Schulze, E.D., Temperton, V.M., Tschamntke, T., Voigt, W., Weigelt, A., Wilcke, W., Weisser, W. W., 2018. Biodiversity–multifunctionality relationships depend on identity and number of measured functions. *Nature Ecology & Evolution* 2, 44–49.
- Mokany, K., Burley, H.M., Paini, D.R., 2013.  $\beta$  Diversity contributes to ecosystem processes more than by simply summing the parts. *Proceedings of the National Academy of Sciences of the United States of America* 110, E4057–E4057.
- Mori, A.S., Isbell, F., Seidl, R., 2018.  $\beta$ -diversity, community assembly, and ecosystem functioning. *Trends in Ecology & Evolution* 33, 549–564.
- Mouillot, D., Graham, N.A., Villéger, S., Mason, N.W., Bellwood, D. R., 2013. A functional approach reveals community responses to disturbances. *Trends in Ecology & Evolution* 28, 167–177.
- Newman, M.E., 2003. The structure and function of complex networks. *SIAM Review* 45, 167–256.
- Newman, M.E., 2006. Modularity and community structure in networks. *Proceedings of the National Academy of Sciences of the United States of America* 103, 8577–8582.
- Nilsson, R.H., Larsson, K.H., Taylor, A.F.S., Bengtsson-Palme, J., Jeppesen, T.S., Schigel, D., Kennedy, P., Picard, K., Glöckner, F. O., Tedersoo, L., Saar, I., Kõljalg, U., Abarenkov, K., 2019. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Research* 47, D259–D264.
- Ning, D., Deng, Y., Tiedje, J.M., Zhou, J., 2019. A general framework for quantitatively assessing ecological stochasticity. *Proceedings of the National Academy of Sciences of the United States of America* 116, 16892–16898.
- Pedrés-Alió, C., 2012. The rare bacterial biosphere. *Annual Review of Marine Science* 4, 449–466.
- Pocock, M.J., Evans, D.M., Memmott, J., 2012. The robustness and restoration of a network of ecological networks. *Science* 335, 973–977.
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glockner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* 41, D590–D596.
- Rillig, M.C., Ryo, M., Lehmann, A., 2021. Classifying human influences on terrestrial ecosystems. *Global Change Biology* 27, 2273–2278.
- Rillig, M.C., Ryo, M., Lehmann, A., Aguilar-Trigueros, C.A., Buchert, S., Wulf, A., Iwasaki, A., Roy, J., Yang, G., 2019. The role of multiple global change factors in driving soil functions and microbial biodiversity. *Science* 366, 886–890.
- Rivett, D.W., Bell, T., 2018. Abundance determines the functional role of bacterial phylotypes in complex communities. *Nature Microbiology* 3, 767–772.
- Schermele-Engel, K., Moosbrugger, H., Müller, H., 2003. Evaluating the fit of structural equation models: Tests of significance and descriptive goodness-of-fit measures. *Methods of Psychological Research Online* 8, 23–74.
- Soliveres, S., Manning, P., Prati, D., Gossner, M.M., Alt, F., Arndt, H., Baumgartner, V., Binkenstein, J., Birkhofer, K., Blaser, S., Blüthgen, N., Boch, S., Böhm, S., Börschig, C., Buscot, F., Diekötter, T., Heinze, J., Hölzel, N., Jung, K., Klaus, V.H., Klein, A.M., Kleinebecker, T., Klemmer, S., Krauss, J., Lange, M., Morris, E.K., Müller, J., Oelmann, Y., Overmann, J., Pašalić, E., Renner, S.C., Rillig, M.C., Schaefer, H.M., Schloter, M., Schmitt, B., Schöning, I., Schrupp, M., Sikorski, J., Socher, S.A., Solly, E.F., Sonnemann, I., Sorkau, E., Steckel, J., Steffan-Dewenter, I., Stempfhuber, B., Tschapka, M., Türke, M., Venter, P., Weiner, C. N., Weisser, W.W., Werner, M., Westphal, C., Wilcke, W., Wolters, V., Wubet, T., Wurst, S., Fischer, M., Allan, E., 2016. Locally rare species influence grassland ecosystem multifunctionality. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 371, 20150269.
- Stegen, J.C., Lin, X., Fredrickson, J.K., Chen, X., Kennedy, D.W., Murray, C.J., Rockhold, M.L., Konopka, A., 2013. Quantifying community assembly processes and identifying features that impose them. *ISME Journal* 7, 2069–2079.
- Stoeck, T., Bass, D., Nebel, M., Christen, R., Jones, M.D., Breiner, H.W., Richards, T.A., 2010. Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. *Molecular Ecology* 19, 21–31.
- Trivedi, P., Delgado-Baquerizo, M., Trivedi, C., Hu, H., Anderson, I. C., Jeffries, T.C., Zhou, J., Singh, B.K., 2016. Microbial regulation of the soil carbon cycle: evidence from gene–enzyme relationships. *ISME Journal* 10, 2593–2604.
- Tsiafouli, M.A., Thébault, E., Sgardelis, S.P., De Ruiter, P.C., Van Der Putten, W.H., Birkhofer, K., Hemerik, L., De Vries, F.T., Bardgett, R.D., Brady, M.V., Bjornlund, L., Jørgensen, H.B.,

- Christensen, S., Hertefeldt, T.D., Hotes, S., Gera Hol, W.H., Frouz, J., Liiri, M., Mortimer, S.R., Setälä, H., Tzanopoulos, J., Uteseny, K., Pižl, V., Stary, J., Wolters, V., Hedlund, K., 2015. Intensive agriculture reduces soil biodiversity across Europe. *Global Change Biology* 21, 973–985.
- Van Elsas, J.D., Chiurazzi, M., Mallon, C.A., Elhottová, D., Křišťůfek, V., Salles, J.F., 2012. Microbial diversity determines the invasion of soil by a bacterial pathogen. *Proceedings of the National Academy of Sciences of the United States of America* 109, 1159–1164.
- Vellend, M., 2010. Conceptual synthesis in community ecology. *Quarterly Review of Biology* 85, 183–206.
- Wagg, C., Bender, S.F., Widmer, F., Van Der Heijden, M.G., 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences of the United States of America* 111, 5266–5270.
- Wagg, C., Schlaeppi, K., Banerjee, S., Kuramae, E.E., van der Heijden, M.G., 2019. Fungal-bacterial diversity and microbiome complexity predict ecosystem functioning. *Nature Communications* 10, 4841.
- Walkley, A., Black, I.A., 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science* 37, 29–38.
- Wang, Y.F., Chen, P., Wang, F.H., Han, W.X., Qiao, M., Dong, W.X., Hu, C.S., Zhu, D., Chu, H.Y., Zhu, Y.G., 2022b. The ecological clusters of soil organisms drive the ecosystem multifunctionality under long-term fertilization. *Environment International* 161, 107133.
- Wu, W., Logares, R., Huang, B., Hsieh, C., 2017. Abundant and rare picoeukaryotic sub-communities present contrasting patterns in the epipelagic waters of marginal seas in the northwestern Pacific Ocean. *Environmental Microbiology* 19, 287–300.
- Xun, W., Li, W., Xiong, W., Ren, Y., Liu, Y., Miao, Y., Xu, Z., Zhang, N., Shen, Q., Zhang, R., 2019. Diversity-triggered deterministic bacterial assembly constrains community functions. *Nature Communications* 10, 3833.
- Xun, W., Liu, Y., Li, W., Ren, Y., Xiong, W., Xu, Z., Zhang, N., Miao, Y., Shen, Q., Zhang, R., 2021. Specialized metabolic functions of keystone taxa sustain soil microbiome stability. *Microbiome* 9, 35.
- Yu, X., Polz, M.F., Alm, E.J., 2019. Interactions in self-assembled microbial communities saturate with diversity. *ISME Journal* 13, 1602–1617.
- Zhang, Z., Lu, Y., Wei, G., Jiao, S., Zambrano, M.M., 2022. Rare species-driven diversity-ecosystem multifunctionality relationships are promoted by stochastic community assembly. *mBio* 0, e00449–e00422.
- Zhao, J., Duan, G., Zhu, Y., Zhu, D., 2023. Gut microbiota and transcriptome response of earthworms (*Metaphire guillelmi*) to polymyxin B exposure. *Journal of Environmental Sciences* 133, 37–47.
- Zhao, Z.B., He, J.Z., Geisen, S., Han, L.L., Wang, J.T., Shen, J.P., Wei, W.X., Fang, Y.T., Li, P.P., Zhang, L.M., 2019. Protist communities are more sensitive to nitrogen fertilization than other microorganisms in diverse agricultural soils. *Microbiome* 7, 33.