

# Soil Food Web Complexity Enhances the Link Between Soil Biodiversity and Ecosystem Multifunctionality in Agricultural Systems

**Shuo Jiao**

Northwest Agriculture and Forestry University <https://orcid.org/0000-0002-1228-1757>

**Yahai Lu** (✉ [luyh@pku.edu.cn](mailto:luyh@pku.edu.cn))

Peking University

**Gehong Wei**

Northwest A&F University: Northwest Agriculture and Forestry University

---

## Research

**Keywords:** Soil biodiversity, Multifunctionality, Soil food web, Agricultural ecosystem

**Posted Date:** September 4th, 2020

**DOI:** <https://doi.org/10.21203/rs.3.rs-70767/v1>

**License:**  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

# Abstract

**Background:** Belowground biodiversity supports multiple ecosystem functions and services that humans rely on. However, there is a dearth of studies conducted on a large spatial scale on the topic in intensely managed agricultural ecosystems. Existing studies have overlooked the fact that the functional diversity in other trophic groups within a food web could influence function of an individual in another trophic group. Here, we report significant and positive relationships between soil biodiversity (archaea, bacteria, fungi, protists, and invertebrates) and multiple ecosystem functions (nutrient provisioning, element cycling, and reduced pathogenicity potential) in 228 agricultural fields.

**Results:** The relationships were influenced by (I) the types of organisms with significant relationships in archaea, bacteria, and fungi and not in protists and invertebrates, and (II) the connectedness of dominant phylotypes across soil food webs, which generate different ecological clusters within soil networks to maintain multiple functions. In addition, we highlight the role of soil food web complexity, reflected by ecological networks comprising diverse organisms, which promote the multiple functions and enhance the link between soil biodiversity and ecosystem functions.

**Conclusions:** Overall, our results represent a significant advance in forecasting the impacts of belowground biodiversity within food webs on ecosystem functions in agricultural systems, and suggest that soil biodiversity, particularly soil food web complexity, should not be overlooked, but rather considered a key factor and integrated into policy and management activities aimed at enhancing and maintaining ecosystem productivity, stability, and sustainability under land-use intensification.

## Background

Belowground organisms are extremely complex and diverse, ranging from microscopic archaea, bacteria, fungi, and protists, to larger organisms such as invertebrates [1]. They represent a large fraction of soil biological communities, and participate in fundamental ecosystem functions, such as nutrient and hydrological cycling, decomposition, environmental remediation, pathogen control, and terrestrial primary production, which are closely linked to climate regulation, global food supply, and other ecosystem services [1–5]. Biodiversity influences ecosystem functioning [6]. Research on the links between biodiversity and ecosystem functions has focused mainly on aboveground organisms over the past two decades [7–10]. Considering the technical limitations of characterizing multiple belowground biodiversity [3], revealing the roles of soil multidiversity (archaea, bacteria, fungi, protists, and invertebrates) in driving multiple simultaneous functions and services (ecosystem multifunctionality) remains a challenge [2]. A poor understanding of the roles of soil multidiversity on ecosystem multifunctionality impairs our capacity to predict ecosystem function and stability following potential shifts in belowground diversity under current land intensification scenarios.

Although our understanding of the functional roles of belowground biodiversity is relatively poor [1, 2], there is mounting evidence that soil biological diversity could enhance ecosystem multifunctionality

based on controlled experiments[11, 12] and observational surveys over large spatial scales [2, 13, 14]. Such studies have focused on the biodiversity of a limited number of soil organism types and in natural terrestrial ecosystems. However, our understanding of the relationship between such multidiversity, including different groups of soil microbes (archaea, bacteria, fungi, protists) and invertebrates (nematodes, annelids, and arthropods), and multiple ecosystem function under human-managed conditions over large spatial scales, such agricultural fields, remains poor. Considering the importance of soil biodiversity for global food supply, understanding how soil biodiversity regulates multifunctionality in agricultural ecosystems is critical for the management of soil communities, which could be exploited for better provisioning of key ecosystem services that support sustainable crop yields.

Trophic interactions play important roles in stimulating ecosystem processes [1]. Soil organisms are structured, and form complex food webs, reflecting strong interrelationships within ecological networks [2, 15]. Soil food web characteristics, such as architecture and connectedness, could strongly predict and influence energy flux, food web stability, and carbon (C) and nitrogen (N) cycling processes [1, 15, 16]. In addition, the phylotypes within soil food webs with similar environmental and resource preferences, could form strongly connected ecological clusters in ecological networks, with major implications for ecosystem functioning[2, 17]. Soil physico-chemical properties and ecosystem processes may vary between agricultural and natural ecosystems, with distinct biodiversity patterns and food web architecture [16, 18, 19]. For example, intensified land use induces shifts from fungi-dominated to bacteria-dominated communities associated with increased N losses and reduced C sequestration [20–22], in turn, influencing consumers at higher trophic levels in the soil food web [16, 23]. However, it remains unknown whether changes in complexity of food webs, indicated by interconnectivity among co-occurring species networks [24], influence the role of biodiversity in ecosystem functioning in agricultural fields. Recent studies have demonstrated that the nature, variability, and intensity of competitive species interactions could shape biodiversity–ecosystem function relationships (BEF)[24, 25]. Competition for resources could reduce community functioning if unproductive species become dominant or productive competitors are inhibited [26]. Conversely, diverse communities would be more productive due to their more efficient exploitation of limited resources, in turn, improving community performance [26, 27]. However, the findings above are based on controlled experimental conditions [24, 26, 28], while most natural communities host highly complex taxonomic diversity [6, 29]. Currently, characterizing the influence of complexity of food webs on BEF relationships in complex real-world ecosystems at large spatial scales is challenging.

Here, our aim was to (I) evaluate the relationships between soil biodiversity and ecosystem functions across complex environmental gradients in agro-ecosystems; and (II) investigate whether food web complexity influences BEF relationships. We conducted a large-scale soil survey across 228 agricultural fields in China, and obtained diversity information on soil archaea, bacteria, fungi (mycorrhizal and saprotrophic fungi), protists, and invertebrates using high-throughput sequencing of 16S rRNA genes (for soil archaea and bacteria), ITS genes (for soil fungi), and 18S rRNA genes (for soil protists and invertebrates), comprising approximately 60,000 soil phylotypes. We also obtained data on a set of 15 ecosystem functions that are influenced by soil organisms and correspond to key ecosystem services,

such as nutrient provisioning, element cycling, and pathogen control (decreased relative abundance of potential fungal plant pathogens in soils), which represented core ecosystem functions that influence crop growth and health in agro-ecosystems, and were both fundamental and quantifiable. Food-web complexity was visualized based on ecological networks including phylotypes of all types of soil organisms. We applied the moving window approach to explore the consequences of changes in complexity in soil food webs on the strength of BEF relationships. Considering the intensified land-use activities in agricultural ecosystems, we explored the following two hypotheses: (I) soil biodiversity is positively correlated with multiple ecosystem functions, while larger soil phylotypes or phylotypes at higher trophic levels have weaker BEF relationships compared to smaller phylotypes or phylotypes at lower trophic levels; (II) an increase in soil food web complexity enhances BEF relationships, i.e., promotes the positive effects of soil biodiversity on ecosystem functions. By considering multiple aspects of soil biodiversity, our study could provide insights into the relationships between soil biodiversity and ecosystem functions in agricultural ecosystems and the potential impacts of shifts in food web complexity.

## Results

In agricultural fields across eastern China, the diversity of most single groups of soil organisms exhibited significantly positive relationships with averaging multifunctionality (Fig. 1A) and most single ecosystem functions (Fig. 1D), excluding in the cases of protists and invertebrates. This relationship was also observed when considering Shannon (Additional file 1: Fig. 3) and phylogenetic diversity (Additional file 1: Fig. 4). We further explored the relationships between the diversity of major phylotypes in different groups and ecosystem functions. Most of the protist and invertebrate phylotypes showed weaker or no relationships with averaging multifunctionality (Additional file 1: Fig. 5) and single ecosystem functions (Additional file 1: Fig. 6), when compared to those of archaea, bacteria and fungi. Notably, protists, including Chlorophyta and Ochrophyta, which are common phototrophs, exhibited positive biodiversity–ecosystem function relationships (BEF), while other consumers or predators exhibited negative (e.g. Cercozoa) or non-significant (e.g. Ciliophora and Lobosa) BEF relationships. In addition, all the nematode functional groups had negative or non-significant BEF relationships. The results suggest that the relationships between soil biodiversity and multiple functions in agricultural ecosystems depend on the type of organisms and the identities of the dominant phylotypes across soil food webs.

The multidiversity of all groups of soil organisms exhibited greater positive associations with averaging multifunctionality (Fig. 1B), with steeper slopes (Fig. 1C and Additional file 1: Fig. 7), and more explained variation when compared to the associations with individual soil phylotypes. Positive association between soil multidiversity and multifunctionality was also observed in both maize and rice fields when examined separately (Additional file 1: Fig. 8). Moreover, such positive relationships were maintained when applying an alternative multifunctionality index that was weighted by three groups of ecosystem services (nutrient provisioning, element cycling, and pathogen control), such that functions from each ecosystem service contributed equally to multifunctionality (Additional file 1: Fig. 7), and when applying multidimensional functionality (Additional file 1: Fig. 9). The consistent results of multiple approaches

validate the positive relationships between soil biodiversity and multiple ecosystem functions in agricultural soils.

Subsequently, we performed structural equation modelling (SEM) to test whether the relationship between soil biodiversity and multifunctionality was maintained when accounting for multiple multifunctionality drivers simultaneously (such as climate [MAT] and soil properties [soil pH, total C and percentage of clay]; Additional file 1: Fig. 10, a priori model). The positive effect of soil biodiversity on multifunctionality was maintained after accounting for other key factors (Fig. 2A; Additional file 1: **Table 1**), verified based on the strong goodness-of-fit of our model. The Random Forest models indicated that soil biodiversity was a significant and important predictor, although Soil total C appeared to be the most significant parameter influencing multifunctionality (Fig. 2B), explaining 45% of the variation in multifunctionality (Fig. 2D). The spatial patterns of multifunctionality observed were consistent with the soil C spatial patterns (Fig. 2C and Additional file 1: Fig. 1). However, other environmental factors only had weaker or non-significant relationships with multifunctionality (Fig. 2D and Additional file 1: Fig. 11).

The soil correlation network was established to explore the strong associations (e.g. ecological clusters) among soil phylotypes of different organism groups, and their prediction of multifunctionality. Five major ecological clusters were identified, and they included > 91% of the soil phylotypes that strongly co-occurred within the network (Fig. 3A and Additional file 1: **Table 2**). The richness of four ecological clusters had positive correlations with multifunctionality (Fig. 3B and Additional file 1: **Fig. 12**) and most single ecosystem functions (Fig. 3C), indicating diversity of phylotypes with similar ecological preferences are also key predictors of multifunctionality. Subsequently, we calculated the topological features of the extracted sub-networks by preserving the phylotypes of individual soil samples to estimate the potential complexity of soil food webs. The number of nodes and edges, average degree, clustering coefficient, and graph density, reflecting the network complexity, were all positively correlated with multifunctionality; while average path length, denoting network sparsity, was negatively correlated with multifunctionality (Additional file 1: **Fig. 13**). The findings suggest that soil food web complexity can promote the multiple ecosystem functions.

We then applied the moving window approach to explore the determinants of BEF relationships. We selected two window sizes of 20 and 30 samples to validate our findings, generating 209 and 199 data subsets, respectively. The strength of BEF was reflected by the explained variation of soil biodiversity in relation to multifunctionality. The topological features of the soil networks exhibited significant correlations with BEF strength, with positive relationships of features reflecting network complexity, and negative relationships of features denoting network sparsity (Additional file 1: **Fig. 14**). Consequently, we calculated one index to reflect the potential soil food web complexity using the topological features of the soil networks via multidimensional scaling analysis (Additional file 1: **Tables 3 and 4**). We observed that soil food web complexity was positively correlated with BEF strength (Fig. 4A and 4B, Additional file 1: **Fig. 15 and Fig. 16**). The SEM (Additional file 1: Fig. 17, a priori model) results suggest that the positive effect of soil food web complexity on BEF strength was maintained when accounting for key driving factors simultaneously (such as climate [MAT] and soil properties [soil pH, total C and percentage of

clay]; Fig. 4C and 4D, Additional file 1: **Tables 5 and 6**). The Random Forest model results also indicated that soil biodiversity was a significant and important predictor (Additional file 1: **Fig. 18**). Soil pH was the most significant factor (Additional file 1: **Fig. 17**), and explained the most variation in BEF strength (Additional file 1: **Fig. 15** and **Fig. 16**). The results based on 20 and 30 sample window sizes exhibited similar trends, validating the positive effect of soil food web complexity on BEF strength.

## Discussion

Global change is causing biodiversity loss across many trophic groups [30, 31], with potential effects on ecosystem services<sup>6,29,32</sup>. Soil biodiversity is a key driver maintaining and promoting multiple ecosystem functions [2, 11–14]. Considering the high functional redundancy of soil microorganisms [33], most studies have focused on soil microbial diversity [12–15], while ignoring other soil biota, e.g., protists and invertebrates, which are key components of soil food webs in belowground ecosystems [1, 34, 35]. In addition, evidence for the link between soil biodiversity and multiple ecosystem functions is lacking in human-dominated agricultural ecosystems, and the factors influencing BEF relationships remain largely unknown in complex real-world ecosystems over large spatial scales.

Here, we provide evidence that soil biodiversity is essential for the maintenance of multiple ecosystem functions in agricultural ecosystems. By considering averaging multidiversity, the diversity of specific organism groups (archaea, bacteria, fungi, protists, and invertebrates), averaging multifunctionality, multiple individual functions, and the dominant phylotypes strongly co-occurring within soil ecological networks, we revealed the positive effect of soil biodiversity on ecosystem functions. Such relationships were influenced by the type of organisms and the identities of dominant phylotypes across soil food webs, so that soil phylotypes with larger sizes or at higher trophic levels, e.g., invertebrates or protist predators, appeared to exhibit weaker or no BEF relationships when compared to those with smaller sizes or at lower trophic levels, e.g. archaea, bacteria, fungi, and protist phototrophs. In particular, our study highlights the role of soil food web complexity in promoting not only the multiple functions but also enhancing the positive effects of soil biodiversity on ecosystem functions. Our findings suggest that management strategies should consider the value of soil biodiversity, particularly soil food web complexity, in the enhancement and maintenance of ecosystem productivity, stability, and sustainability, in agricultural systems under intensification.

The role of biodiversity in ecosystem functioning and the maintenance of multiple ecosystem functions could be attributed to a key feature, functional redundancy, which supports the ‘insurance’ [36] and ‘rivet-redundancy’ [37] hypotheses, in addition to the ‘portfolio’ effect [38]. For soil microbial communities confronted with a potentially high level of functional redundancy [33, 39], here, we demonstrate that greater soil biodiversity could also ensure greater performance in multiple ecosystem functions in agricultural systems. This was largely due to the effects of greater belowground biodiversity enhancing (1) the redundancy effect of the presence of more phylotypes present that support similar functions and (2) the presence of phylotypes that are predominantly associated with different ecosystem functions [15]. Nevertheless, we observed that not all the soil organisms participated equally in the maintenance of

multifunctionality. Our results indicate that protist and invertebrate diversity exhibited non-significant BEF relationships in general. In addition, all the nematode functional groups, common predators or parasites, exhibited negative or non-significant BEF relationships. The major protist phylotypes, including Cercozoa, Ciliophora, and Lobosa, known as consumers or predators [35], exhibited negative or non-significant BEF relationships; whereas phototroph protists [35], e.g., Chlorophyta and Ochrophyta, exhibited positive BEF relationships. The results could be explained by the distinct role of the diversity of different soil organisms in supporting multiple ecosystem functions at different levels of functioning [2]. For example, larger soil organisms are essential for the maintenance of high levels of functioning [2], e.g., acting as predators to regulate the flow of resources to organisms (microorganisms) at lower trophic levels; whereas, small organisms are essential for the fine-tuning of multifunctionality (for example, by nutrient recycling) [2].

Considering nutrient cycling is the most important agricultural ecosystem process influencing crop yield [1], the key functions in the major nutrient cycles could be performed by soil microorganisms, although the soil fauna have been demonstrated to have profound impacts on soil ecosystems and to regulate numerous essential soil processes [40]. Indeed, the intensification of human activity could reshape and simplify the architecture of soil food webs, leading to smaller-bodied organisms and fewer functional groups in agricultural ecosystems [16, 18, 19, 40], as well as shifts from fungal-dominated to more bacteria-dominated communities [20–22], in turn, influencing their consumers at higher trophic levels. The findings support our hypothesis (I) that soil biodiversity is positively associated with multiple ecosystem functions in agricultural ecosystems, while soil phylotypes with larger sizes or at higher trophic levels (e.g. protist predators and invertebrates) have weaker BEF relationships, when compared to smaller soil phylotypes or phylotypes at lower trophic levels (e.g., archaea, bacteria, fungi, and protist phototrophs).

Soil organisms live within complex soil food webs, forming ecological clusters of strongly co-occurring phylotypes within ecological networks [2, 15]. Here, we revealed that strongly co-occurring dominant taxa within the food web could also predict multiple ecosystem functions, with positive associations between soil phylotype richness and multifunctionality within four ecological clusters. The finding indicated that soils that had larger numbers of phylotypes belonging to these four ecological clusters had greater levels of multifunctionality, while those belonging to other ecological clusters might not contribute substantially to multifunctionality. The observation could be supported by the findings of a recent study [2]. Furthermore, the prediction of the network topological features with regard to the multifunctionality suggest that soil food web complexity could promote multiple ecosystem functions. Complex processes are driven by trophic interactions among soil organisms within food webs [1, 15]. For example, large soil invertebrates comminute large amounts of animal and plant litter to generate resources for fungi and bacteria [1]; positive interactions, induced by niche partitioning or facilitation, could promote microbial community functioning [28, 41]; microbial interkingdom associations could enhance ecosystem functioning [15] and promote plant health in the model plant *Arabidopsis* [42]. We also observed multiple potential associations among soil microbial biodiversity that could positively influence ecosystem multifunctionality. For example, the protist diversity was positively associated with archaeal, bacterial,

and fungal diversity, suggesting potential predator–prey associations (Additional file 1: Fig. 3) that could positively influence multifunctionality[2, 35]. The findings suggest that greater soil biodiversity also facilitates greater association complexity within soil food webs that are jointly required to support multiple ecosystem functions simultaneously.

What was the most intriguing about our findings was the positive effect of soil food web complexity, reflected by the ecological networks, including soil phylotypes from all types of organisms, based on BEF strength. Soil biodiversity would be a better predictor of multifunctionality when the associations among soil organisms are more complex. Although recent well-manipulated experimental studies have linked competitive species interactions to the direction of diversity-function relationship[24, 26], it is still challenging to characterize the factors influencing such relationships in complex natural ecosystems, largely due to the highly complex taxonomic diversity in natural communities [6, 29]. To the best of our knowledge, this is one of the first studies to link soil food web complexity to the strength of belowground biodiversity–function relationships. Previous studies have demonstrated that changes in soil food web structure could influence C and N cycling process[16, 22]. Species in highly competitive communities often grow less effectively due to intense competition for shared resources [26, 28, 43]. Weak-intransitive competition within communities made up of strong competitors exhibit negative diversity–function relationships[24]. Conversely, co-existing species, following niche partitioning based on distinct resources or facilitation, could positively interact, which can increase community functional performance [28, 41, 44]. Such cases support our findings that the soil food web complexity not only promotes multiple functions but also enhances the link between soil biodiversity and ecosystem functions. Considering intensive agricultural practices are considered to lead to simpler soil food webs [19, 40], and changes in the asymmetry of energy channels [22], our results reinforce the view that the elimination of complex belowground species associations can impair the biodiversity-driven ecosystem functioning in agricultural systems under intensification.

A few potential limitations should be considered within the context of the present study. First, the potential complexity of food webs was reflected by constructing ecological networks including soil phylotypes from all types of organisms, which was only based on correlation. Correlation network analyses are only a simplistic representation of a complex system. In addition, ecological networks that are based on correlations can yield spurious results, and associations between taxa within such networks cannot be automatically interpreted as interactions. Consequently, it may not be possible to comprehensively depict the architecture and connectedness of soil food webs in real-world conditions. However, ecological network information is still essential for estimating potential species interrelationships within complex soil food webs, and, in turn, for revealing the influence of soil food web complexity on biodiversity-driven ecosystem functioning [2]. Secondly, we highlighted the potential limitations of sequencing approaches for the quantifying of soil invertebrate biodiversity; larger soil organisms would be potentially underrepresented based on such an approach, although a few studies have applied the approach to estimate the soil invertebrate biodiversity[2, 3, 34].

# Conclusions

Our findings reveal the importance of soil biodiversity in supporting and maintaining ecosystem functioning in agricultural systems, by considering average multifunctionality, multiple individual functions, averaging multidiversity, the diversity of distinct groups (archaea, bacteria, fungi, protists and invertebrates), and the dominant phylotypes strongly co-occurring within the soil ecological network. Furthermore, our results indicate that soil phylotypes with larger sizes or at higher trophic levels (e.g. protist predators and invertebrates) exhibit weaker BEF relationships compared to those with smaller sizes or at lower trophic levels (e.g. archaea, bacteria, fungi and protist phototrophs), suggesting that such relationships vary based on the type of organisms involved, and the identities of the dominant phylotypes within soil food webs. Particularly, we highlight the role of soil food web complexity in promoting multiple ecosystem functions and enhancing our capacity to predict the effect of soil biodiversity on ecosystem function and stability. Overall, our results represent a considerable advancement in the capacity to forecast the effects of belowground biodiversity within food webs on ecosystem functions in agricultural systems. Our work further demonstrates that the potential role of soil food web complexity in the enhancement and maintenance of ecosystem productivity, stability, and sustainability should not be overlooked under land-use intensification scenarios. Conversely, it is a key factor that should be taken into account in policy development and soil management.

# Methods

## *Soil sampling*

Soil samples were collected between July and September 2017 from 114 locations in agricultural fields that had been under cultivation for at least 10 years. Maize and rice were selected as representative crops for dryland and wetland soils, respectively, and because they are globally important crops and are cultivated extensively across China. In each location, sampling sites were selected from two adjacent maize and rice fields less than 5 km apart, yielding 114 and 114 soil samples associated with maize and rice cultivation, respectively (see **Fig. 1** in supplemental material). The sampling sites extended from 18.30°N to 48.35°N and 87.61°E to 99.91°E across eastern China, covering tropical wet and dry climates, warm temperate climates, warm summer continental climates, and hot summer continental climates from south to north. Three 100-m<sup>2</sup> plots were sampled at each site, and five soil cores obtained per plot at a depth of 0–15 cm were combined. Standard testing methods were adopted for the measurement of soil pH, cation exchange capacity (CEC), total organic C, and percentage of clay, as previously described[45-47].

## *Soil biodiversity analysis*

In total, we observed approximately 60,894 taxa in the 228 agricultural fields. The diversity of soil archaea, bacteria, fungi, protists, and invertebrates were analyzed using high-throughput sequencing, by targeting the 16S rRNA region in archaea, the 16S rRNA region in bacteria, the internal transcribed spacer

1 region for fungi, and the 18S rRNA region in protists and invertebrates. Corresponding polymerase chain reaction assays were performed using the Arch519F/Arch915R, 515F/907R, ITS5-1737F/ITS2-2043R, TAREuk454FWD1/TAREukREV3 primer pairs, respectively [48, 49], and sequencing was performed on an Illumina HiSeq2500 platform (Illumina Inc., San Diego, CA, USA). The acquired sequences were filtered for quality control as previously described [50, 51]. Any chimeric sequences were removed using the USEARCH tool based on the UCHIME algorithm [52]. Subsequently, the sequences were split into operational taxonomic units (OTUs) at a 3% dissimilarity level using the UPARSE pipeline [52]. OTUs with fewer than two sequences were removed, and their representative sequences were assigned to taxonomic lineages using the RDP classifier within the SILVA database (release 128) for bacteria and archaea, UNITE+INSD (UNITE and the International Nucleotide Sequence Databases) for fungi, and PR2 (Protist Ribosomal Reference Database) for protists and invertebrates.

Before we calculated the soil organism diversity, the OTU tables were resampled to a minimum number of sequences from each sample, at 36,880 for archaea, 27,712 for bacteria, 30,369 for fungi, 5393 for protists, and 207 for invertebrates, which showed even sampling depth within each belowground group of organisms (Additional file 1: **Fig. 2**). Protists were defined as all eukaryotic taxa, excluding fungi, invertebrates (Metazoa), and vascular plants (Streptophyta), according previous studies [2, 35]. Here, we used richness (that is, number of soil phylotypes) as a metric of soil biodiversity, which is the most extensively used, as well as the simplest, metric of biodiversity [2]. We use the term soil biodiversity to refer to different types of richness in general terms. Overall, we calculated the richness of the most prevalent prokaryotic and eukaryotic organisms in our soil samples, including archaea, bacteria, mycorrhizal and saprophytic fungi, protists, and invertebrates. The identities of saprophytic and mycorrhizal fungi were determined using FUNguild [53]. We used only highly probable and probable guilds with an identified single trophic mode for the analysis.

To obtain a multidiversity index, we combined soil biodiversity characteristics by averaging the standardized scores of richness of archaea, bacteria, mycorrhizal and saprophytic fungi, protists and invertebrates. 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. The multidiversity index generally accepted and has been used extensively in the current biodiversity–function literature [9, 14, 30, 54, 55]. Particularly, the richness of each belowground group of organisms was highly correlated with their Shannon diversity (Pearson  $r=0.75-0.94$ ,  $P<0.001$ , Additional file 1: **Fig. 3**). The multidiversity calculated based on Shannon diversity was highly correlated with that calculated based on richness (Pearson  $r=0.67$ ,  $P<0.001$ ), and also showed positive associations with average multifunctionality (Additional file 1: **Fig. 3**). This trend was also observed when considering phylogenetic diversity (Additional file 1: **Fig. 4**). In addition, the soil biodiversity indices included in the averaging index were not highly multicollinear ( $r<0.8$ ). The results of the analyses suggest that the choice of diversity metric would not alter our results.

### ***Ecosystem functioning measures***

Fifteen ecosystem functions regulated by soil organisms under a broad range of ecosystem services were included in the present study: nutrient provisioning (extracellular enzyme activities related to sugar degradation [ $\beta$ -glucosidase and saccharase], chitin degradation [*N*-acetylglucosaminidase], P mineralization [phosphatase], soil dissolved organic C, N, and P availability, microbial C and N stocks), element cycling (soil Sulfur [S], iron [Fe], copper [Cu], zinc [Zn], and manganese [Mn] availability, involved in bio-electron transfer and energy exchange in the epigeosphere), and resistance to plant pathogens (reduced relative abundance of fungal plant pathogens in soils). Although we have included 15 ecosystem functions, some important functions are inevitably unmeasured, such as process rates including nitrification rates, denitrification rates, N mineralization, and decomposition rates, and future studies are encouraged to include more essential functions for comprehensive understanding of ecosystem functioning.

All of these are state variables rather than rates of changes.

In all the soil samples, the extracellular enzyme activities were measured using fluorometry as described previously [56]. The soil dissolved organic C, available N, available phosphorus, microbial biomass C, microbial biomass N, available S, available Fe, available Cu, available Mn, and available Zn were all measured using standard soil testing procedures[45-47]. The relative abundance of potential fungal plant pathogens in soils was obtained from the sequencing analyses and was inferred by parsing the soil phylotypes using FUNguild [53]. Similarly, we applied only highly probable and probable guilds with an identified single trophic mode for the analysis. The inverse abundance (reduced relative abundance) of potential fungal plant pathogens was obtained by calculating the inverse of the variable (total relative abundance of fungal plant pathogens $\times$ -1). Because the values for ecosystem functions varied widely, we standardized all variables to a common scale ranging from 0 to 1 (as described above).

To derive a quantitative multifunctionality index for each sample, we used three independent multifunctionality approaches[57]: (1) multiple single functions[2], (2) averaging multifunctionality index[9], and (3) principal coordinate multifunctionality index[58]. To obtain an averaging ecosystem multifunctionality index, we averaged the standardized scores (a common scale ranging from 0 to 1) of all individual ecosystem functions. We also used principal coordinate analysis to examine the different dimensions of multifunctionality[58]. Note that we do not argue that one is better or more appropriate than the other.

### ***Statistical analyses***

*Linking soil biodiversity to multifunctionality.* We conducted ordinary least squares linear regressions between soil multidiversity (standardized averaged of the diversity of soil organisms) and single soil organisms with multifunctionality and multidimensional functioning (axes of a principal coordinate analysis including 15 functions), and performed Spearman correlation analyses between the diversity of single soil organisms and single functions. In addition, linear regression relationships between the diversity of major phylotypes were estimated in different groups and ecosystem functions. We selected the dominant phylotypes of different groups, accounting for >85% sequences in total. The dominant

phylotypes were: archaeal (Thaumarchaeota and Euryarchaeota), bacterial (Proteobacteria, Acidobacteria, Chloroflexi, Actinobacteria, Bacteroidetes, Firmicutes, Gemmatimonadetes, Planctomycetes, and Nitrospirae), mycorrhizal fungi (Ascomycota, Basidiomycota and Glomeromycota), saprotrophic fungi (Ascomycota, Basidiomycota, Chytridiomycota and Zygomycota), protists (Cercozoa, Ciliophora, Chlorophyta, Ochrophyta, Lobosa and Stramenopiles), invertebrates (Nematoda, Arthropoda, Rotifera, Annelida, Gastrotricha, and Platyhelminthes) and a Nematoda functional group (Herbivore, Bacterivore, Animal-Predator, Fungivore, Animal-Parasite and Omnivore).

*Structural equation model and random forest analyses.* We used structural equation modelling (SEM) to evaluate the direct link between soil biodiversity and multifunctionality (averaging), and between complexity of soil food webs and strength of BEF (explained below), after accounting for multiple drivers such as climate (mean annual temperature) and soil properties (soil pH, total C, and percentage of clay) simultaneously (Additional file 1: **Fig. 9** and **13**, priori models). We obtained climatic data including mean annual temperature (MAT) for all sampling sites from the Worldclim database ([www.worldclim.org](http://www.worldclim.org)). Here, we did not consider mean annual precipitation since the source of water in the agricultural fields was partly from artificial irrigation. All the variables were included as independent observable variables. The goodness of fit of SEM models was checked using the following procedures: the  $\chi^2$  test, the root mean square error of approximation (RMSEA) and Comparative Fit Index (CFI), the model had a good fit when the CFI value was close to 1 and the *P* values of the statistics were high (traditionally >0.05) [59]. With a good model fit, we were able to interpret the path coefficients of the model and their associated *P* values. A path coefficient is analogous to the partial correlation coefficient, and describes the strength and sign of the relationship between two variables. SEM was conducted using the “lavaan” package in R environment (v3.5.1; <http://www.r-project.org/>) [60]. In addition, random forest (RF) analysis was performed to identify the major driving factors. To estimate the importance of the variables, we used percentage increases in MSE (mean squared error) of variables: higher MSE% values imply more important variables. The analysis was conducted using the “rfPermute” package in R [61]. Significance of the model was assessed with 5000 permutations of the response variable using the “A3” package in R [62].

*Co-occurrence networks.* To explore the species interrelationships within complex soil food webs, ecological co-occurrence networks consisting of dominant soil phylotypes from all types of organisms were constructed. We focused on the most dominant phylotypes—those that were both abundant (top 10% of all identified prokaryotes and eukaryotes in terms of relative abundance) and ubiquitous (>50% for prokaryotic and >10% for eukaryotic organisms of all locations) across all soil samples, and identified ecological clusters of strongly co-occurring soil phylotypes within the networks. Using such a filtering approach, our aim was to ensure that we identified dominant phylotypes in agricultural fields and minimize potential spurious correlations from the rare taxa [2, 17, 63]. We focused on the dominant soil phylotypes because they are expected to have a disproportionate functional importance in their ecosystems, and are distributed widely. Although many bacterial taxa are widely distributed, it is unlikely

to be the case for eukaryotic organisms. Therefore, we applied a ubiquity threshold of >50% for prokaryotic and >10% for eukaryotic organisms in all locations.

Robust correlations with Spearman's correlation coefficients ( $\rho$ ) >0.65 and false discovery rate (FDR)-corrected  $P$ -values <0.001 were used to construct networks. We expected that the cut-off, which has been extensively used in literature and is comparable across studies[2], would have both a mathematical and biological meaning and reveal the organisms that are strongly correlated with each other. Nevertheless, we acknowledge that correlation network analyses can yield spurious results, and associations between taxa within such networks cannot be directly interpreted as interactions, and might not fully represent the architecture and connectedness of soil food webs in real-world conditions. However, the information derived from the networks is essential for generating novel hypothesis and ecological frameworks (to be tested in future experiments) on the role of strongly co-occurring phylotypes within food webs in controlling multifunctionality and the link between soil biodiversity and ecosystem functions.

Our network included 1,513 dominant and strongly co-occurring soil phylotypes. The soil phylotypes were dominated by 263 archaea, 1,077 bacteria, 114 fungi, 58 protists, and 1 invertebrate. We identified the ecological clusters within our ecological network using the "igraph" package, and calculated the richness of soil organisms within each ecological cluster across all samples. In addition, we extracted sub-networks by preserving the phylotypes of individual soil samples using the `induced_subgraph` function in "igraph" package in R [64]. The topological features of the sub-networks in each sample were calculated to estimate the potential complexity of soil food webs, including the number of nodes and edges, average degree, clustering coefficient, average path length, network diameter, and graph density. Average path length refers to the average network distance between all pairs of nodes; network diameter refers to the greatest distance between the nodes that exist in the network; average degree refers to the average connections of each node with another unique node in the network; clustering coefficient represents the degree to which the nodes tend to cluster together; and graph density refers to the intensity of connections among nodes [65, 66]. Therefore, higher numbers of nodes and edges, average degree, clustering coefficient, and graph density, and lower average path lengths and diameters suggest a more connected network, reflecting more potential complexity of soil food webs [45, 67]. Networks were visualized using the interactive Gephi platform (<https://gephi.org>).

*Linking the complexity of soil food webs to the strength of BEF.* We applied the moving window approach to explore the determinants of BEF relationships. The technique facilitates the analysis of multivariate data along an ecological gradient [68, 69]. To ensure adequate data amounts, we selected two window sizes of 20 and 30 consecutive samples across sites, generating 209 (e.g. 1-20, 2-21 ... 209-228) and 199 (e.g. 1-30, 2-31 ... 199-228) data points, respectively. The window was advanced across the sampling sites after reordering along the latitude gradient, generating adjacent sampling subsets to better estimate the complexity of soil food webs and BEF relationships. Based on each sub-dataset, we estimated the amount of variance in multifunctionality explained ( $R^2$  of the ordinary least squares linear regressions) by soil biodiversity to reflect the strength of BEF, and constructed soil networks as described above. We then calculated one index to reflect the potential complexity of soil food webs using the topological features of

the soil networks via multidimensional scaling analysis[58] (Additional file 1: **Table 3** and **4**). Note that average path length and diameter, denoting the network sparsity, was calculated as the inverse of the variables ( $\times -1$ ) before the calculation of the index. Subsequently, SEM and RF analysis were performed to evaluate the effect of complexity of soil food webs on BEF strength (as explained above).

## Declarations

### *Ethics approval and consent to participate*

Not applicable

### *Consent for publication*

Not applicable

### *Availability of data and materials*

The raw sequence data reported in this paper are available in the NCBI Sequence Read Archive under BioProject PRJNA544819

### *Competing interests*

The authors declare that they have no competing interests.

### *Funding*

This work was supported by the National Science Foundation of China (Grant Nos. 41830755 and 41807030), and National Key Research and Development Program of China (Grant No. 2016YFD0200306).

### *Authors' contributions*

S.J. conceived and designed the study with the help of Y.L. and G.W.; S.J. performed the experiments, analyzed the data, and drafted the manuscript; Y.L. and G.W. reviewed the manuscript.

### *Acknowledgements*

Not applicable

## References

1. Bardgett RD, Van Der Putten WH: **Belowground biodiversity and ecosystem functioning**. *Nature* 2014, **515**(7528):505-511.
2. Delgado-Baquerizo M, Reich PB, Trivedi C, Eldridge DJ, Abades S, Alfaro FD, Bastida F, Berhe AA, Cutler NA, Gallardo A: **Multiple elements of soil biodiversity drive ecosystem functions across**

- biomes.** *Nature Ecology & Evolution* 2020, **4**(2):210-220.
3. George PB, Lallias D, Creer S, Seaton FM, Kenny JG, Eccles RM, Griffiths RI, Lebron I, Emmett BA, Robinson DA: **Divergent national-scale trends of microbial and animal biodiversity revealed across diverse temperate soil ecosystems.** *Nat Commun* 2019, **10**(1):1-11.
  4. Wall DH, Nielsen UN, Six J: **Soil biodiversity and human health.** *Nature* 2015, **528**(7580):69-76.
  5. van Elsas JD, Chiurazzi M, Mallon CA, Elhottová D, Křišťůfek V, Salles JF: **Microbial diversity determines the invasion of soil by a bacterial pathogen.** *Proc Natl Acad Sci USA* 2012, **109**(4):1159-1164.
  6. Cardinale BJ: **Biodiversity improves water quality through niche partitioning.** *Nature* 2011, **472**(7341):86-U113.
  7. Lefcheck JS, Byrnes JEK, Isbell F, Gamfeldt L, Griffin JN, Eisenhauer N, Hensel MJS, Hector A, Cardinale BJ, Duffy JE: **Biodiversity enhances ecosystem multifunctionality across trophic levels and habitats.** *Nat Commun* 2015, **6**.
  8. Tilman D, Lehman CL, Thomson KT: **Plant diversity and ecosystem productivity: Theoretical considerations.** *Proc Natl Acad Sci USA* 1997, **94**(5):1857-1861.
  9. Maestre FT, Quero JL, Gotelli NJ, Escudero A, Ochoa V, Delgado-Baquerizo M, Garcia-Gomez M, Bowker MA, Soliveres S, Escolar C *et al*: **Plant species richness and ecosystem multifunctionality in global drylands.** *Science* 2012, **335**(6065):214-218.
  10. Meyer ST, Ptacnik R, Hillebrand H, Bessler H, Buchmann N, Ebeling A, Eisenhauer N, Engels C, Fischer M, Halle S *et al*: **Biodiversity-multifunctionality relationships depend on identity and number of measured functions.** *Nat Ecol Evol* 2018, **2**(1):44-49.
  11. Kardol P, Wardle DA: **How understanding aboveground-belowground linkages can assist restoration ecology.** *Trends in Ecology & Evolution* 2010, **25**(11):670-679.
  12. Wagg C, Bender SF, Widmer F, van der Heijden MG: **Soil biodiversity and soil community composition determine ecosystem multifunctionality.** *Proc Natl Acad Sci USA* 2014, **111**(14):5266-5270.
  13. Delgado-Baquerizo M, Maestre FT, Reich PB, Jeffries TC, Gaitan JJ, Encinar D, Berdugo M, Campbell CD, Singh BK: **Microbial diversity drives multifunctionality in terrestrial ecosystems.** *Nat Commun* 2016, **7**.
  14. Jing X, Sanders NJ, Shi Y, Chu HY, Classen AT, Zhao K, Chen LT, Shi Y, Jiang YX, He JS: **The links between ecosystem multifunctionality and above- and belowground biodiversity are mediated by climate.** *Nat Commun* 2015, **6**.
  15. Wagg C, Schlaeppi K, Banerjee S, Kuramae EE, van der Heijden MG: **Fungal-bacterial diversity and microbiome complexity predict ecosystem functioning.** *Nat Commun* 2019, **10**(1):1-10.
  16. de Vries FT, Thébault E, Liiri M, Birkhofer K, Tsiafouli MA, Bjørnlund L, Jørgensen HB, Brady MV, Christensen S, de Ruiter PC: **Soil food web properties explain ecosystem services across European land use systems.** *Proc Natl Acad Sci USA* 2013, **110**(35):14296-14301.

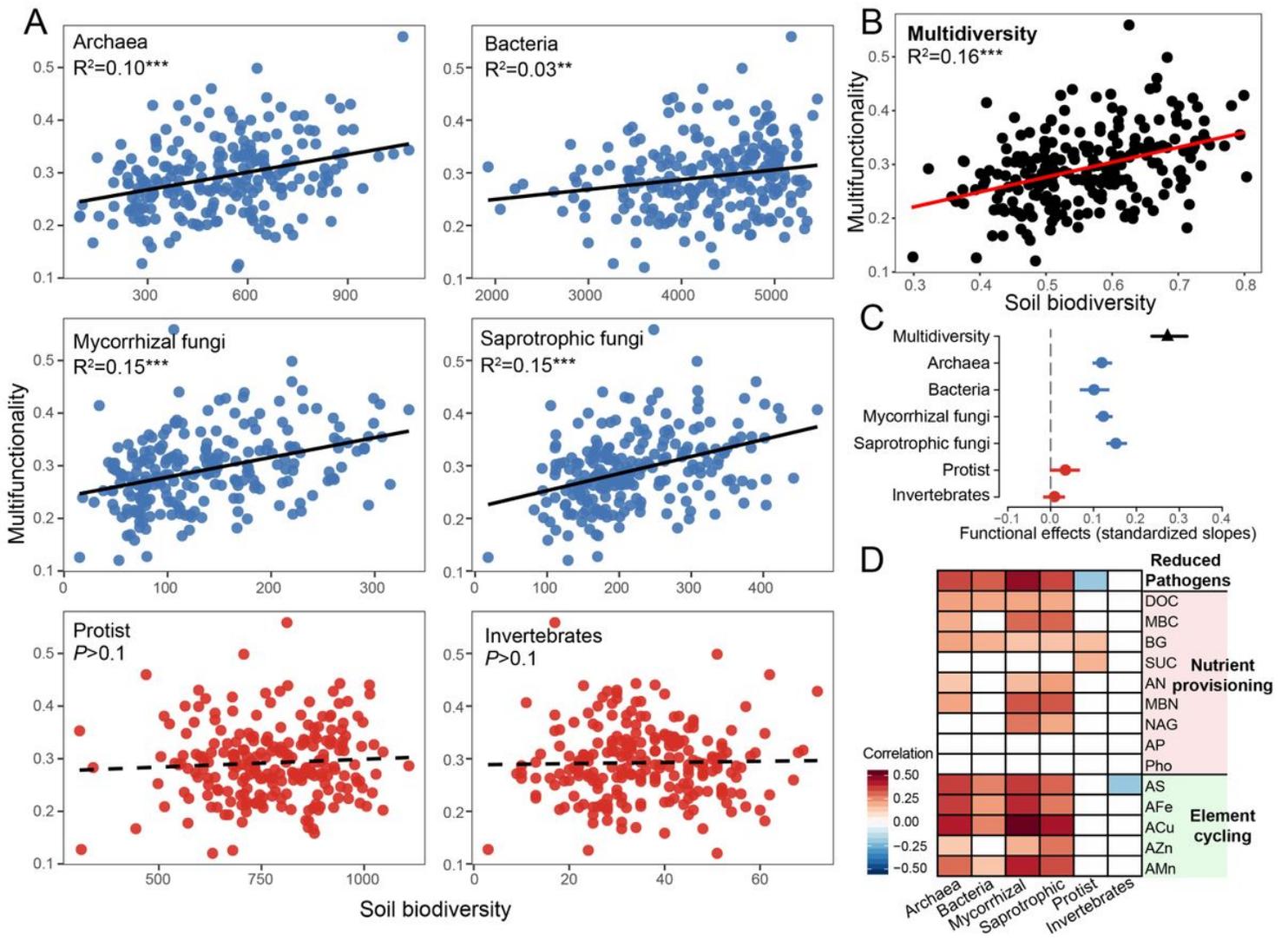
17. Delgado-Baquerizo M, Oliverio AM, Brewer TE, Benavent-Gonzalez A, Eldridge DJ, Bardgett RD, Maestre FT, Singh BK, Fierer N: **A global atlas of the dominant bacteria found in soil.** *Science* 2018, **359**(6373):320-325.
18. Bartley TJ, McCann KS, Bieg C, Cazelles K, Granados M, Guzzo MM, MacDougall AS, Tunney TD, McMeans BC: **Food web rewiring in a changing world.** *Nature ecology & evolution* 2019, **3**(3):345-354.
19. Tsiafouli MA, Thébault E, Sgardelis SP, De Ruiter PC, Van Der Putten WH, Birkhofer K, Hemerik L, De Vries FT, Bardgett RD, Brady MV: **Intensive agriculture reduces soil biodiversity across Europe.** *Global Change Biol* 2015, **21**(2):973-985.
20. De Vries FT, Van Groenigen JW, Hoffland E, Bloem J: **Nitrogen losses from two grassland soils with different fungal biomass.** *Soil Biol Biochem* 2011, **43**(5):997-1005.
21. Six J, Frey S, Thiet R, Batten K: **Bacterial and fungal contributions to carbon sequestration in agroecosystems.** *Soil Sci Soc Am J* 2006, **70**(2):555-569.
22. De Vries FT, Liiri ME, Bjørnlund L, Bowker MA, Christensen Sr, Setälä HM, Bardgett RD: **Land use alters the resistance and resilience of soil food webs to drought.** *Nat Clim Change* 2012, **2**(4):276-280.
23. De Vries FT, Liiri ME, Bjørnlund L, Bowker MA, Christensen S, Setälä HM, Bardgett RD: **Land use alters the resistance and resilience of soil food webs to drought.** *Nat Clim Change* 2012, **2**(4):276-280.
24. Maynard DS, Crowther TW, Bradford MA: **Competitive network determines the direction of the diversity-function relationship.** *Proc Natl Acad Sci U S A* 2017, **114**(43):11464-11469.
25. Lehman CL, Tilman D: **Biodiversity, stability, and productivity in competitive communities.** *Am Nat* 2000, **156**(5):534-552.
26. Becker J, Eisenhauer N, Scheu S, Jousset A: **Increasing antagonistic interactions cause bacterial communities to collapse at high diversity.** *Ecol Lett* 2012, **15**(5):468-474.
27. Loreau M, Hector A: **Partitioning selection and complementarity in biodiversity experiments.** *Nature* 2001, **412**(6842):72-76.
28. Yu X, Polz MF, Alm EJ: **Interactions in self-assembled microbial communities saturate with diversity.** *ISME J* 2019, **13**(6):1602-1617.
29. Cardinale BJ, Duffy JE, Gonzalez A, Hooper DU, Perrings C, Venail P, Narwani A, Mace GM, Tilman D, Wardle DA: **Biodiversity loss and its impact on humanity.** *Nature* 2012, **489**(7401):59-67.
30. Allan E, Bossdorf O, Dormann CF, Prati D, Gossner MM, Tscharntke T, Blüthgen N, Bellach M, Birkhofer K, Boch S *et al.*: **Interannual variation in land-use intensity enhances grassland multidiversity.** *Proc Natl Acad Sci USA* 2014, **111**(1):308-313.
31. Newbold T, Hudson LN, Hill SLL, Contu S, Lysenko I, Senior RA, Borger L, Bennett DJ, Choimes A, Collen B *et al.*: **Global effects of land use on local terrestrial biodiversity.** *Nature* 2015, **520**(7545):45-+.
32. Naeem S, Duffy JE, Zavaleta E: **The Functions of Biological Diversity in an Age of Extinction.** *Science* 2012, **336**(6087):1401-1406.

33. Delgado-Baquerizo M, Giaramida L, Reich PB, Khachane AN, Hamonts K, Edwards C, Lawton LA, Singh BK: **Lack of functional redundancy in the relationship between microbial diversity and ecosystem functioning.** *J Ecol* 2016, **104**.
34. Bastida F, Eldridge DJ, Abades S, Alfaro FD, Gallardo A, García-Velázquez L, García C, Hart SC, Pérez CA, Santos F *et al*: **Climatic vulnerabilities and ecological preferences of soil invertebrates across biomes.** *Mol Ecol* 2019, **29**(4):752-761.
35. Oliverio AM, Geisen S, Delgado-Baquerizo M, Maestre FT, Turner BL, Fierer N: **The global-scale distributions of soil protists and their contributions to belowground systems.** *Science Advances* 2020, **6**(4):eaax8787.
36. Yachi S, Loreau M: **Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis.** *Proc Natl Acad Sci USA* 1999, **96**(4):1463-1468.
37. Cardinale BJ, Matulich KL, Hooper DU, Byrnes JE, Duffy E, Gamfeldt L, Balvanera P, O'connor MI, Gonzalez A: **The functional role of producer diversity in ecosystems.** *Am J Bot* 2011, **98**(3):572-592.
38. Doak DF, Bigger D, Harding E, Marvier M, O'malley R, Thomson D: **The statistical inevitability of stability-diversity relationships in community ecology.** *The American Naturalist* 1998, **151**(3):264-276.
39. Allison SD, Martiny JBH: **Resistance, resilience, and redundancy in microbial communities.** *Proc Natl Acad Sci USA* 2008, **105**:11512-11519.
40. Bender SF, Wagg C, van der Heijden MGA: **An Underground Revolution: Biodiversity and Soil Ecological Engineering for Agricultural Sustainability.** *Trends in Ecology & Evolution* 2016, **31**(6):440-452.
41. Li M, Wei Z, Wang J, Jousset A, Friman V-P, Xu Y, Shen Q, Pommier T: **Facilitation promotes invasions in plant-associated microbial communities.** *Ecol Lett* 2019, **22**(1):149-158.
42. Durán P, Thiergart T, Garrido-Oter R, Agler M, Kemen E, Schulze-Lefert P, Hacquard S: **Microbial interkingdom interactions in roots promote Arabidopsis survival.** *Cell* 2018, **175**(4):973-983. e914.
43. Xavier JB, Foster KR: **Cooperation and conflict in microbial biofilms.** *Proc Natl Acad Sci USA* 2007, **104**(3):876-881.
44. Bruno JF, Stachowicz JJ, Bertness MD: **Inclusion of facilitation into ecological theory.** *Trends in Ecology & Evolution* 2003, **18**(3):119-125.
45. Ma B, Wang H, Dsouza M, Lou J, He Y, Dai Z, Brookes PC, Xu J, Gilbert JA: **Geographic patterns of co-occurrence network topological features for soil microbiota at continental scale in eastern China.** *ISME J* 2016, **10**:1891-1901.
46. Shi Y, Li Y, Xiang X, Sun R, Yang T, He D, Zhang K, Ni Y, Zhu YG, Adams JM *et al*: **Spatial scale affects the relative role of stochasticity versus determinism in soil bacterial communities in wheat fields across the North China Plain.** *Microbiome* 2018, **6**(1):27.
47. Bao SD: **Soil and agricultural chemistry analysis.** *Agriculture Publication, Beijing* 2000:355-356.

48. Jiao S, Chen W, Wang J, Du N, Li Q, Wei G: **Soil microbiomes with distinct assemblies through vertical soil profiles drive the cycling of multiple nutrients in reforested ecosystems.** *Microbiome* 2018, **6**(1):146.
49. Stoeck T, Bass D, Nebel M, Christen R, Jones MDM, Breiner H-W, Richards TA: **Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water.** *Mol Ecol* 2010, **19**(s1):21-31.
50. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R: **Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample.** *Proc Natl Acad Sci USA* 2011, **108**:4516-4522.
51. Dissanayake AJ, Purahong W, Wubet T, Hyde KD, Zhang W, Xu H, Zhang G, Fu C, Liu M, Xing Q *et al*: **Direct comparison of culture-dependent and culture-independent molecular approaches reveal the diversity of fungal endophytic communities in stems of grapevine (*Vitis vinifera*).** *Fungal Diversity* 2018, **90**(1):85-107.
52. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R: **UCHIME improves sensitivity and speed of chimera detection.** *Bioinformatics* 2011, **27**(16):2194-2200.
53. Nguyen NH, Song ZW, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG: **FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild.** *Fungal Ecol* 2016, **20**:241-248.
54. Wang L, Delgado-Baquerizo M, Wang D, Isbell F, Liu J, Feng C, Liu J, Zhong Z, Zhu H, Yuan X *et al*: **Diversifying livestock promotes multidiversity and multifunctionality in managed grasslands.** *Proc Natl Acad Sci U S A* 2019, **116**(13):6187-6192.
55. Soliveres S, van der Plas F, Manning P, Prati D, Gossner MM, Renner SC, Alt F, Arndt H, Baumgartner V, Binkenstein J *et al*: **Biodiversity at multiple trophic levels is needed for ecosystem multifunctionality.** *Nature* 2016, **536**(7617):456-459.
56. Bell CW, Fricks BE, Rocca JD, Steinweg JM, McMahon SK, Wallenstein MD: **High-throughput fluorometric measurement of potential soil extracellular enzyme activities.** *Journal of Visualized Experiments* 2013, **15**(81):e50961.
57. Byrnes JEK, Gamfeldt L, Isbell F, Lefcheck JS, Griffin JN, Hector A, Cardinale BJ, Hooper DU, Dee LE, Emmett Duffy J: **Investigating the relationship between biodiversity and ecosystem multifunctionality: challenges and solutions.** *Methods in Ecology and Evolution* 2014, **5**(2):111-124.
58. Manning P, van der Plas F, Soliveres S, Allan E, Maestre FT, Mace G, Whittingham MJ, Fischer M: **Redefining ecosystem multifunctionality.** *Nature Ecology & Evolution* 2018, **2**(3):427-436.
59. Schermelleh-Engel K, Moosbrugger H, Müller H: **Evaluating the Fit of Structural Equation Models: Tests of Significance and Descriptive Goodness-of-Fit Measures.** *Methods Psychol Res Online* 2003, **8**(8):23–74.
60. Rosseel Y: **Lavaan: An R package for structural equation modeling and more. Version 0.5–12 (BETA).** *Journal of statistical software* 2012, **48**(2):1-36.

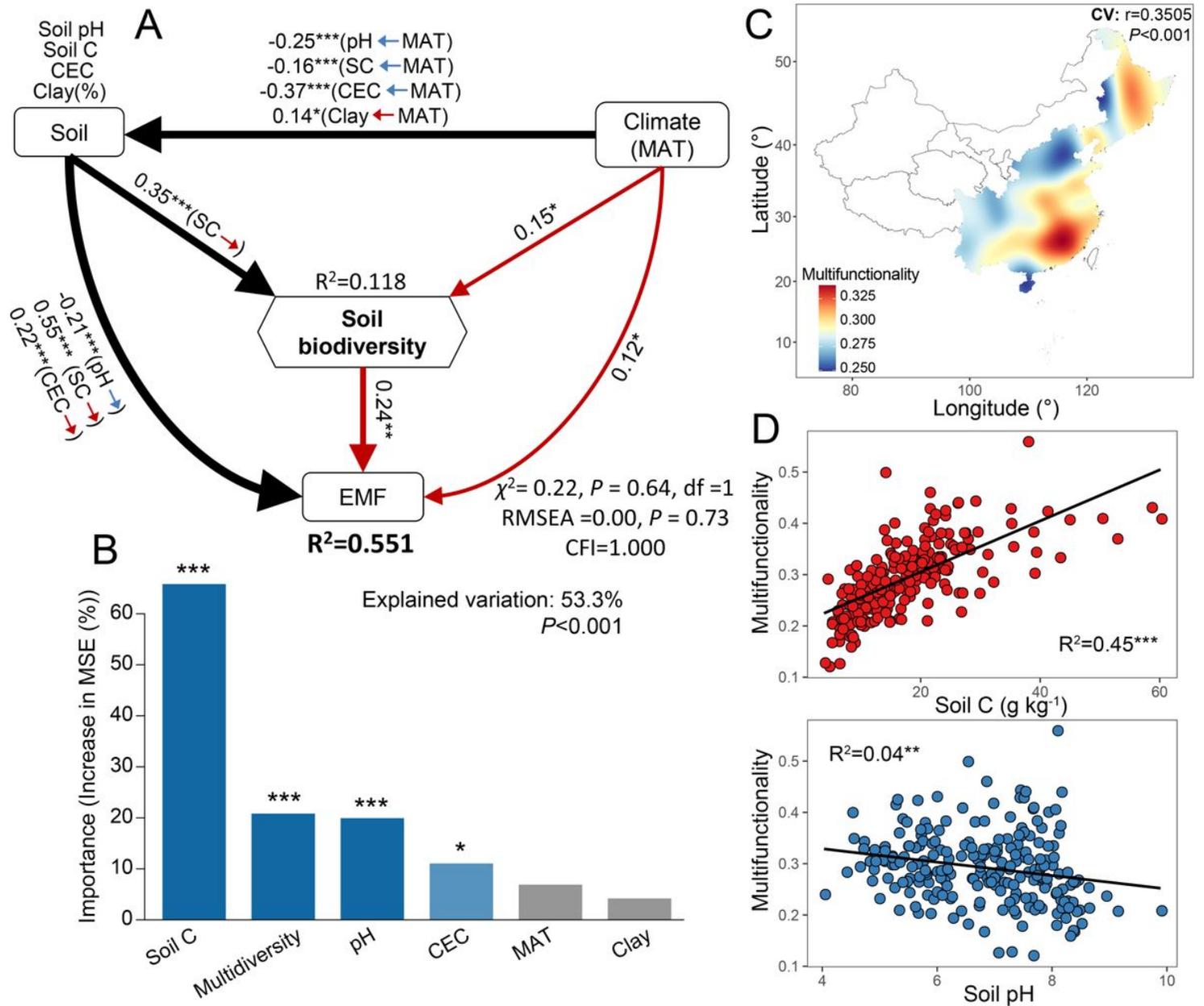
61. Archer E: **rfPermute: Estimate permutation p-values for Random Forest importance metrics. R package version 1.5. 2.** In.; 2013.
62. Fortmann-Roe S: **Consistent and clear reporting of results from diverse modeling techniques: the A3 method.** *Journal of Statistical Software* 2015, **66**(7):1-23.
63. Jiao S, Xu Y, Zhang J, Hao X, Lu Y: **Core Microbiota in Agricultural Soils and Their Potential Associations with Nutrient Cycling.** *Msystems* 2019, **4**(2):e00313-00318.
64. Csardi G, Nepusz T: **The igraph software package for complex network research.** *InterJournal, Complex Systems* 2006, **1695**(5):1-9.
65. Newman ME: **The structure and function of complex networks.** *Siam Rev* 2003, **45**(2):167-256.
66. Newman ME: **Modularity and community structure in networks.** *Proc Natl Acad Sci USA* 2006, **103**(23):8577-8582.
67. Barberán A, Bates ST, Casamayor EO, Fierer N: **Using network analysis to explore co-occurrence patterns in soil microbial communities.** *ISME J* 2012, **6**(2):343-351.
68. Jassey V, Lamentowicz Ł, Robroek B, Gąbka M, Rusińska A, Lamentowicz M: **Plant functional diversity drives niche-size structure of dominant microbial consumers along a poor to extremely rich fen gradient.** *J Ecol* 2014, **102**:1150–1162.
69. Carlson ML, Flagstad LA, Gillet Fo, Mitchell EAD: **Community development along a proglacial chronosequence: are above-ground and below-ground community structure controlled more by biotic than abiotic factors?** *J Ecol* 2010, **98**(5):1084-1095.

## Figures



**Figure 1**

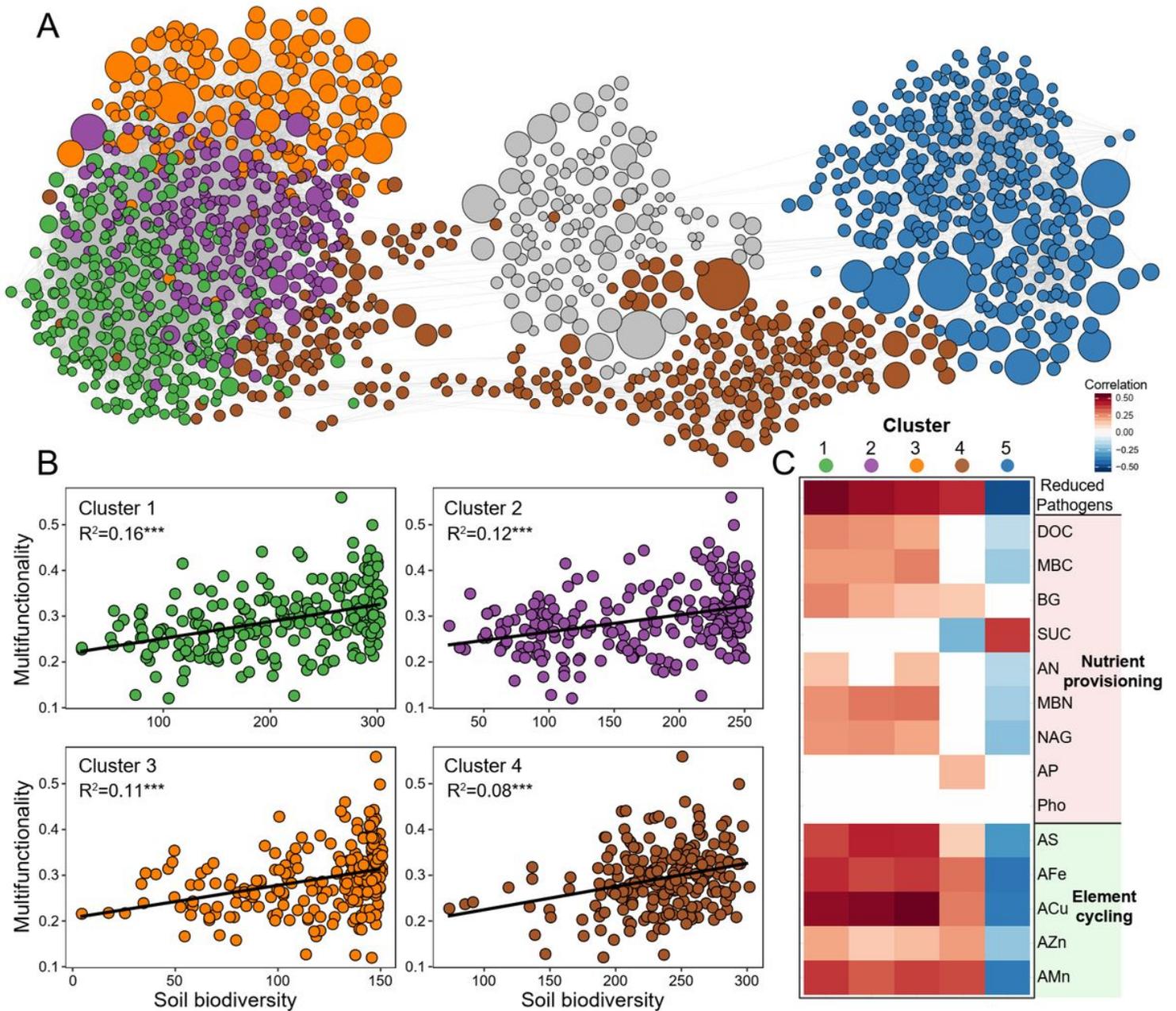
The relationships between multifunctionality and biodiversity of organisms in agricultural ecosystems. The linear relationships between multifunctionality and the biodiversity of selected groups of soil organisms (A; number of species, richness) or multidiversity (B; averaged standardized between 0 and 1). Statistical analysis was performed using ordinary least squares linear regressions; P values were indicated by asterisks: \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$ . (C) Functional effects (standardized slopes (mean $\pm$ s.e.m.) of biodiversity of multiple (triangle) and individual soil organisms (circle) on ecosystem multifunctionality. (D) Significant correlations (Spearman;  $P < 0.05$ ) between the diversity of single groups of organisms and single ecosystem functions.



**Figure 2**

Links between soil biodiversity and multifunctionality in agricultural ecosystems. (A) Structural equation model describing the direct relationship between the multidiversity of soil organisms and averaging ecosystem multifunctionality (EMF). We grouped the edaphic properties into the same box in the model for graphical simplicity, which did not represent latent variables. Numbers adjacent to arrows were indicative of the effect size of the relationship.  $R^2$  denotes the proportion of variance explained. Red arrows represented positive paths, and blue arrows represented negative paths.  $R^2$  denoted the proportion of variance explained. Significance levels were as follows:  $*P < 0.05$ ,  $**P < 0.01$  and  $***P < 0.001$ . RMSEA: root mean square error of approximation; CFI: Comparative Fit Index; MAT: mean annual temperature; CEC: cation exchange capacity. Information about our a priori model was provided in Additional file 1: Fig. 10 and Additional file 1: Table 1. (B) Random Forest mean predictor importance of influencing factors for multifunctionality. The accuracy importance measure was computed for each tree and

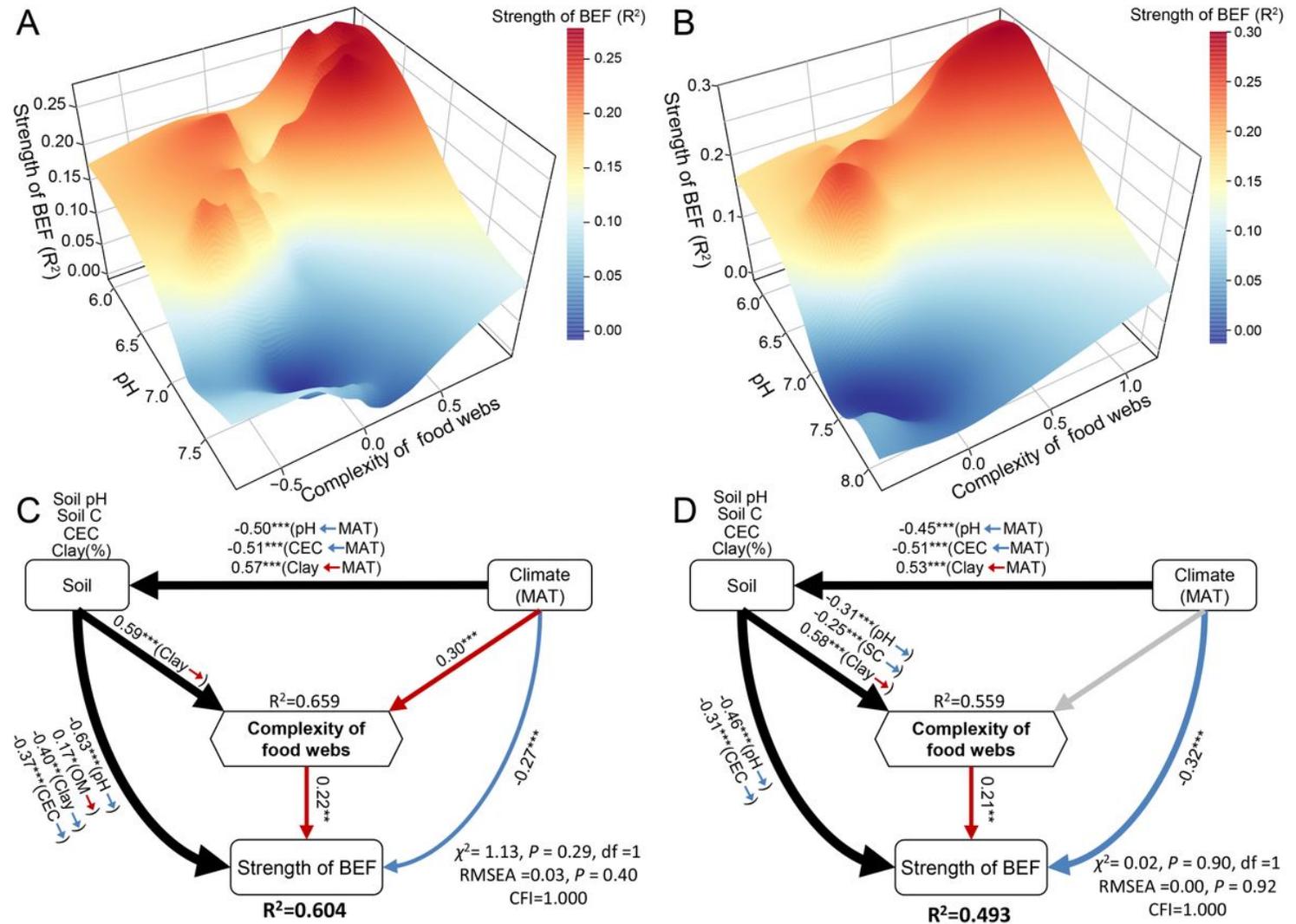
averaged over the forest (5000 trees). Percentage increases in the MSE (mean squared error) of variables was used to estimate the importance of these predictors, and higher MSE% values implied more important predictors. (C) The predicted spatial distributions of multifunctionality. The cross-validation (“CV”) of the maps was based on Pearson correlation between the predicted and observed values in each sampling site. (D) The linear relationships between multifunctionality and soil C and pH.



**Figure 3**

Links between the soil biodiversity within ecological network and multifunctionality. (A) Correlation network including multiple ecological clusters. A connection indicates a strong (Spearman’s  $p > 0.65$ ) and significant (FDR-corrected  $p < 0.001$ ) correlation. The colors of the nodes represent different ecological clusters; the size of each node is proportional to the relative abundance of the OTUs; the thickness of a connection between two nodes (i.e., an edge) is proportional to the value of the Spearman’s correlation

coefficient. (B) The linear relationships between average multifunctionality and the diversity (number of phylotypes) of soil phylotypes within the main ecological clusters. Statistical analysis was performed using ordinary least squares linear regressions; P values were indicated by asterisks: \*P < 0.05, \*\*P < 0.01 and \*\*\*P < 0.001. (C) Significant correlations (Spearman; P < 0.05) between the diversity of soil phylotypes within the main ecological clusters and single ecosystem functions.



**Figure 4**

Effect of potential complexity of soil food webs on the links between soil biodiversity and ecosystem multifunctionality based on moving-window analysis. The variances of the BEF strengths across complexity of soil food webs and soil pH gradients based on window sizes of 30 (A) and 20 (B) samples. The strengths of biodiversity-multifunctionality relationships (BEF) were reflected by the explained variances ( $R^2$  of the ordinary least squares linear regressions) of soil biodiversity to multifunctionality. The potential complexity of soil food webs was estimated using the topological features of the soil networks via multidimensional scaling analysis. Structural equation model describing the direct relationship between the complexity of soil food webs and the BEF strengths based on window sizes of 30 (C) and 20 (D) samples. We grouped the edaphic properties into the same box in the model for graphical simplicity, which did not represent latent variables. Numbers adjacent to arrows were indicative

of the effect size of the relationship. R<sup>2</sup> denotes the proportion of variance explained. Red arrows represented positive paths, and blue arrows represented negative paths. R<sup>2</sup> denoted the proportion of variance explained. Significance levels were as follows: \*P < 0.05, \*\*P < 0.01 and \*\*\*P < 0.001. RMSEA: root mean square error of approximation; CFI: Comparative Fit Index; MAT: mean annual temperature; CEC: cation exchange capacity. Information about our a priori model is provided in Additional file 1: Fig. 17 and Additional file 1: Table 5 and 6.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Additionalfiles.docx](#)
- [TableS2.xlsx](#)
- [Additionalfile1.docx](#)