

## RESEARCH ARTICLE

# Soil enzyme profile analysis for indicating decomposer micro-food web

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## Abstract

Highly diverse exoenzymes mediate the energy flow from substrates to the multitrophic microbiota within the soil decomposer micro-food web. Here, we used a “soil enzyme profile analysis” approach to establish a series of enzyme profile indices; those indices were hypothesized to reflect micro-food web features. We systematically evaluated the shifts in enzyme profile indices in relation to the micro-food web features in the restoration of an abandoned cropland to a natural area. We found that enzymatic C:N stoichiometry and decomposability index were significantly associated with substrate availability. Furthermore, the higher Shannon diversity index in the exoenzyme profile, especially for the C-degrading hydrolase, corresponded to a greater microbiota community diversity. The increased complexity and stability of the exoenzyme network reflected similar changes with the micro-food web networks. In addition, the gross activity of the enzyme profile as a parameter for soil multifunctionality, effectively predicted the substrate content, microbiota community size, diversity, and network complexity. Ultimately, the proposed enzymic channel index was closely associated with the traditional decomposition channel indices

Wen Xing and Ning Hu contributed equally to this work.

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derived from microorganisms and nematodes. Our results showed that soil enzyme profile analysis reflected very well the decomposer food web features. Our study has important implications for projecting future climate change or anthropogenic disturbance impacts on soil decomposer micro-food web features by using soil enzyme profile analysis.

#### KEYWORDS

co-occurrence network, decomposition channel, food web, microbiome, nematode, protozoa, soil exoenzyme

#### Highlights

- Soil enzyme profile analysis for indicating decomposer micro-food web features was first proposed.
- Diversity indication utilities for contrasting enzyme profiles were different.
- Protozoa channel index and enzymatic channel index were proposed.

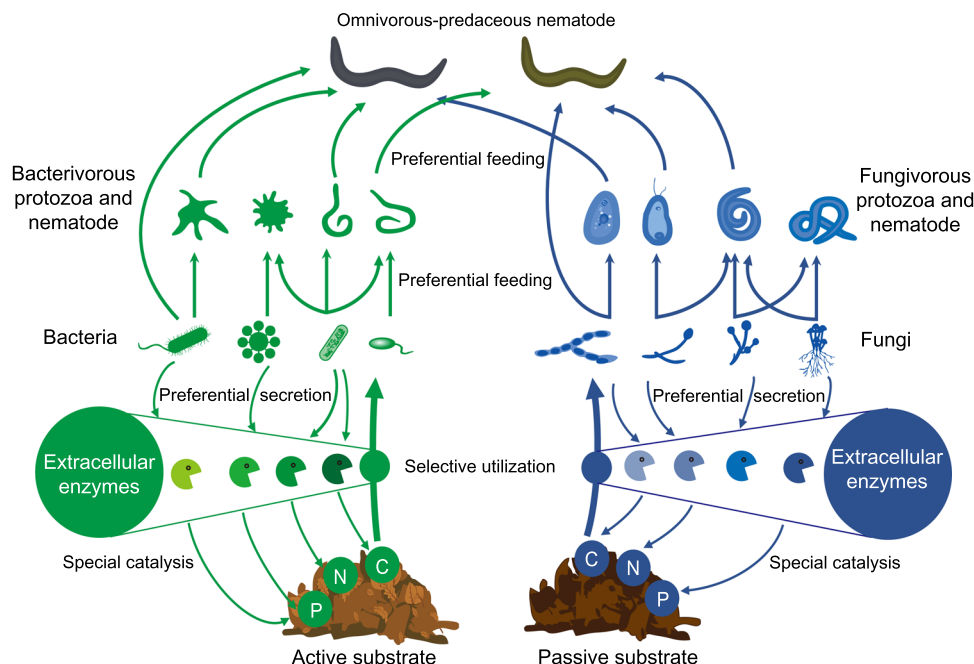
## INTRODUCTION

The soil decomposer food web plays a critical role in mediating biogeochemical cycling and sustaining ecosystem functions [1]. In the soil micro-food web, bacteria and fungi predominantly contribute to the decomposition of soil substrates [2]. Microfauna, represented mainly by protozoa and nematodes, prey on microbes and thus regulate microbial populations [3, 4]. Also, nematode communities are species-rich, multitrophic, and occupy a central position in the soil micro-food web [5, 6]. Ecological indices calculated using nematode community data are extensively used to characterize soil micro-food web [7]. Therefore, there is a growing interest in studying substrate-based micro-food web consisting of microbes and microfauna (Figure 1) [8]. Traditionally, the soil micro-food web is characterized by substrate quantity and quality (stoichiometry and decomposability), the abundance, diversity, and species composition of microbiota, and dominant energy channels (i.e., fungal- and bacterial-based channels). Additional interest has been focused on network complexity and stability, which represent major determinants of ecosystem functioning underpinned by the micro-food web [9, 10].

Exoenzymes are extracellular enzymes that play a major role in soil food webs [11]. At least 500 exoenzymes, which catalyze the rate-limiting steps of decomposition and mediate energy flow in soil food webs, are thought to primarily originate from soil microorganisms [12, 13]. Catalytic reactions of extracellular exoenzymes are substrate-specific, while soil substrates are highly variable spatially and temporary [14] (Figure 1). Therefore, the spatial and temporal

variabilities of soil substrates strongly influence exoenzyme activities. However, the enzymatic stoichiometry theory states that microorganisms can adapt to substrate changes by modifying the type of exoenzymes produced [15]. Consequently, ratios of exoenzyme activities can reflect substrate composition [14]. Additionally, the extracellular exoenzyme production of microorganisms is identity-specific (Figure 1). For instance, the production of peroxidase must be carried out by specialist saprotrophic fungi, for example, *Basidiomycota* [11, 16]. Therefore, shifts in soil microbial community composition may result in changes in exoenzyme production [16]. Moreover, the feeding of some microfauna on microbes is preferential (Figure 1). For example, the protozoa *Acanthamoeba castellanii* preferentially feeds on the fungi *Coprinus cinerea* [17] and the bacterivorous nematode *Caenorhabditis elegans* preferentially feeds on the bacterium *Escherichia coli* [18]. Hence, the exoenzyme production pattern may be modified by the species composition of microfauna.

Ecological indices that provide comprehensive information on decomposer micro-food web is lacking [5, 8]. Some of the key characteristics of the micro-food web as discussed above may be studied by a specific soil exoenzymatic profile analysis. Here, we first propose a methodology for soil exoenzyme profile analysis, which is expected to be an integrated measure of decomposer micro-food web features and to predict soil micro-food web status (Table 1). In this study, we assessed the application of soil exoenzyme profile analysis by using a case study based on the conversion of an arable system to a natural area. Multiple studies have indicated distinct differences in substrate status,



**FIGURE 1** Classic structure of soil decomposer micro-food web and its conceptual links to exoenzyme profile. The green and blue colors represent the bacteria- and fungal-based energy flow channels, respectively.

**TABLE 1** The proposed soil exoenzyme profile indices for indicating the decomposer micro-food web features.

| Exoenzyme profile index      | Calculation                                                                                                                                          | Implications for decomposer micro-food web features                                                                      |
|------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|
| Stoichiometry index          | Ratios of activities of exoenzymes acquiring elements                                                                                                | Linked to substrate stoichiometry and element constraint                                                                 |
| Carbon decomposability index | The ratio of carbon hydrolase activity to oxidase activity                                                                                           | Indicating carbon bioavailability of substrate                                                                           |
| Channel index                | The ratio of fungal- to bacterial-derived exoenzyme activities                                                                                       | Reflecting the dominance of fungal- versus bacterial-based decomposition channel                                         |
| Exoenzyme composition        | The first principal coordination score (PCo1) calculated by the Bray–Curtis dissimilarity matrix                                                     | Associated with the taxa composition of microbiota in micro-food web                                                     |
| Gross activity               | The average of standardized exoenzyme activity                                                                                                       | Greater gross exoenzyme activity indicates greater substrate quantity, community size, diversity, and network complexity |
| Diversity                    | Alpha diversity, incorporating species richness and evenness (Shannon Index and Pielou Evenness Index) for a specifically selected exoenzyme profile | Higher diversity of exoenzyme profile indicates a more diverse microbiota community                                      |
| Network complexity           | Complexity parameters (such as average degree, average clustering coefficient, etc.) derived from co-occurring network analysis                      | The exoenzyme profile with a greater network complexity corresponds to the more complex microbiota network               |
| Network stability            | Stability parameters (such as robustness and vulnerability) derived from co-occurring network analysis                                               | The exoenzyme profile with a higher network stability corresponds to the more stable microbiota network                  |

community size, species diversity, dominant decomposition channel type, and network properties of soil food web between arable and restored natural systems [19–21]. We hypothesized that a series of indices

derived from soil exoenzyme profile analysis would be strongly associated with the changes in decomposer micro-food web features (Table 1) with the conversion of cropland to a natural ecosystem.

## RESULTS

### Decomposer micro-food web features

Compared with the arable system, the restored natural area presented higher soil total organic carbon (TOC), dissolved organic carbon (DOC),  $\text{KMnO}_4$ -oxidized C ( $\text{C}_{\text{KMnO}_4}$ ), total nitrogen (TN), and dissolved nitrogen (DN) contents of 92.9%, 63.4%, 50.9%, 33.2%, and 26.7%, respectively (Figure 2A). Furthermore, TOC:TN and DOC:DN increased, while DOC:TOC and  $\text{C}_{\text{KMnO}_4}$ :TOC decreased, respectively. Significant differences across the two seasons were recorded for all these parameters and metrics.

The composition of each functional group and the whole microbiota community was significantly different under the restored natural area (Figure 2B). Specifically, the natural area had a 25.9% lower relative abundance of bacterial dominant taxa *Chloroflexi*, 31.8% higher fungal dominant taxa *Sordariomycetes*, 8.4% lower protozoa dominant taxa *Cerozoa*, and 9.1% lower nematode dominant taxa *Mesorhabditis* across two seasons, compared with the arable system (Figure S1).

The abundance of each functional group was significantly increased under the restored natural area in both seasons except for protozoa in September (Figure 2B). Across the two seasons, the abundances of bacterial, fungal, protozoan, and nematode communities were increased by 83.7%, 145.2%, 58.8%, and 140.1%, respectively. Also, the standardized abundance of the whole microbiota was significantly increased in the restored natural area in both seasons (Figure 2B).

Compared with the arable system, the restored natural area had 37.1%, 50.8%, 83.4%, and 50.1% higher richness of bacterial, fungal, protozoa, and nematode communities, respectively, across both seasons (Figure S2A). The evenness index of each functional group and the overall microbiota was higher in the natural area, and the difference was significant for bacteria, fungi, and nematode in June, and protozoa in September (Figure S2B). The Shannon Index of each functional group was significantly increased after the conversion of the arable system to a restored natural area in both seasons except for fungal diversity in September (Figure 2B). An average of 11.7%, 6.2%, 25.1%, and 30.2% increases across both seasons for bacterial, fungal, protozoa, and nematode communities, respectively, were found. Also, the standardized richness and Shannon Index of the whole microbiota community were significantly increased in both seasons (Figures S2B and 2B).

Compared with the arable system, the natural area had 33.2% and 48.9% higher fungi:bacteria (F:B) ratio

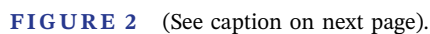
and nematode channel index (NCI), respectively, and 10.5% less nematode channel ratio (NCR) across the two seasons (Figure 2B). The protozoa channel index (PCI) was 37.3% lower in June, but was not different in September in the natural area, in comparison with the arable system.

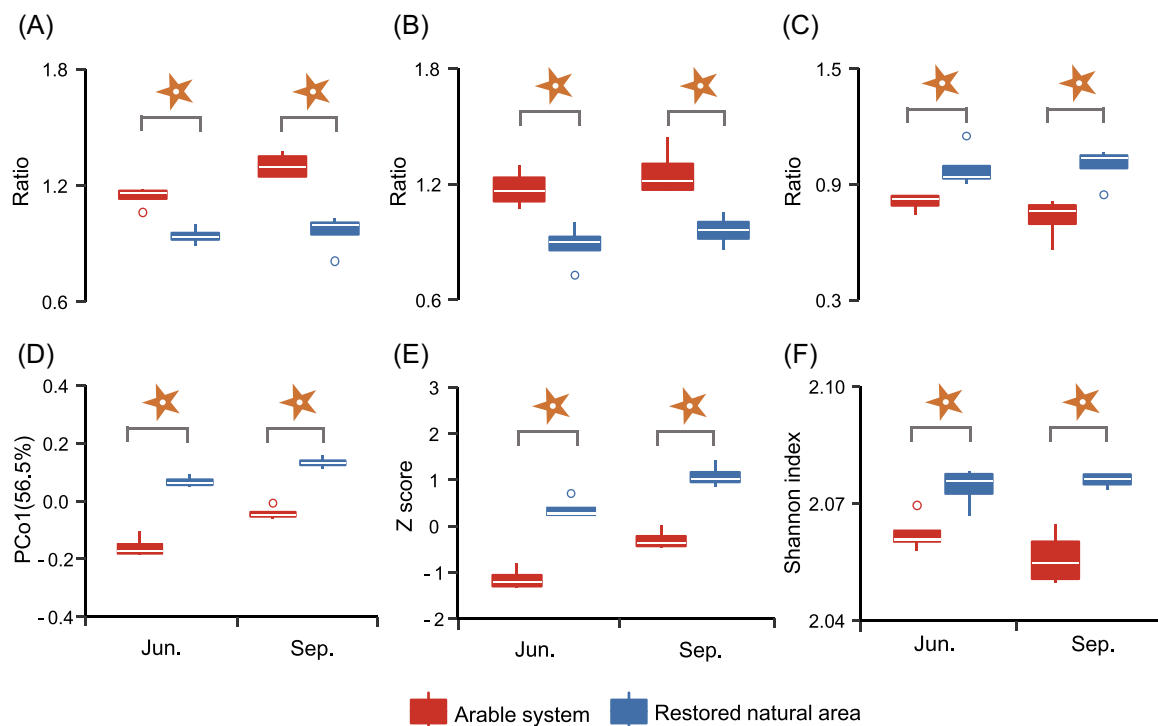
For the microbiota co-occurrence networks (Figure 2C), comparing the natural area to the arable system, specific network complexity indices such as the average degree (avgK) and average clustering coefficient (avgCC) values significantly increased for the bacterial network (Table S1). For the fungal network, the average path distance (GD) value significantly increased, while the values of the other four indices did not change. Values of avgK and GD significantly increased for both the nematode and the whole micro-food web networks. Robustness values of community networks of bacteria and nematode, as well as the whole micro-food web, significantly increased, while vulnerability values decreased for bacteria and whole micro-food web networks after the arable conversion to restored natural area (Table S1). Comparing the restored natural area to the arable system, more positive, negative links, and modules were detected for the microbiota co-occurrence networks (Table S2).

### Exoenzyme profile indices

Compared with the arable system,  $\beta$ -1,4-glucosidase:  $\beta$ -1,4-*N*-acetyl-glucosaminidase (BG:NAG) and  $\beta$ -1,4-glucosidase:phenol oxidase (BG:PHOX) were significantly decreased, while  $\beta$ -1,4-*N*-acetyl-glucosaminidase: leucine aminopeptidase (NAG:LAP) was significantly increased under the restored natural area, on average two seasons (Figure 3A–C). For the exoenzyme profile composition indices, the first principal coordination (PCo1) scores showed significant differences between treatments in both seasons (Figure 3D). Specifically, the relative activities of  $\alpha$ -1,4-glucosidase (AG), cellobiohydrolase (CB), and PHOX were increased by 2.2%, 3.4%, and 1.6% in the restored natural area, across both seasons, respectively (Figure S3). The gross exoenzyme activity and Shannon Index of the exoenzyme profile were also significantly elevated in the restored natural area (Figure 3E,F).

For the exoenzyme networks, greater values of exoenzyme network complexity parameters including avgK, avgCC, and connectedness (Con) were observed in the restored natural area than those in the arable system (Table S3). The robustness value of the exoenzyme network was significantly higher in the restored natural area.





**FIGURE 3** Soil exoenzyme profile indices based on exoenzymes involved in carbon and nitrogen cycling under arable system and restored natural area in two seasons. Significance analysis was performed by the *T* test. ★,  $p < 0.05$ . (A) BG:NAG, (B) BG:PHOX, (C) NAG:LAP, (D) composition, (E) gross activity, and (F) diversity. BG,  $\beta$ -1,4-glucosidase; LAP, leucine aminopeptidase; NAG,  $\beta$ -1,4-*N*-acetyl-glucosaminidase; PCo1, the first principal coordination score; PHOX, phenol oxidase.

### Correlations between exoenzyme profile indices and decomposer micro-food web features

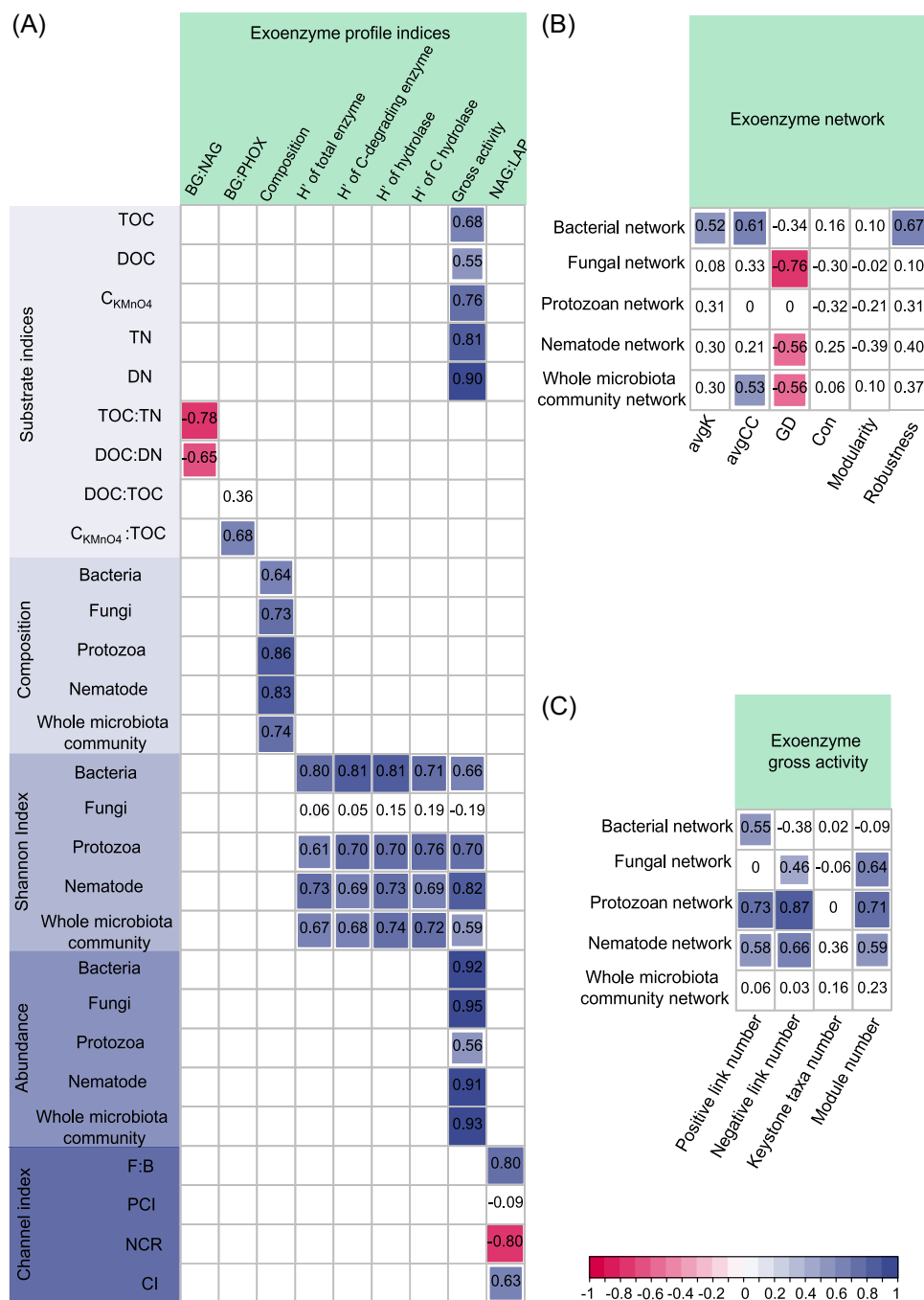
BG:NAG presented a significant negative correlation with the substrate C:N stoichiometry indices (i.e., TOC:TN and DOC:DN) (Figure 4A). BG:PHOX was significantly and positively correlated with the decomposability index of  $C_{KMnO_4}$ :TOC.

In terms of the PCo1 scores, the exoenzyme profile composition was significantly correlated with the composition of bacterial, fungal, protozoan, nematode groups, and the whole microbiota community (Figure 4A). The Shannon Index of exoenzyme profiles (comprising all eight of the selected exoenzymes) was significantly and positively correlated with the Shannon Index of each

functional group (except fungi) and the whole microbiota communities. Similarly, there were significantly positive correlations between Shannon indices of C-degrading exoenzyme, hydrolase, and C-degrading hydrolase profiles with those of bacteria, protozoa, and nematode groups, and the whole microbiota communities. The Shannon Index of C-degrading hydrolase showed the strongest correlations with microbiota communities among all types of reference exoenzyme profiles.

The avgK value of the exoenzyme network was positively correlated with the avgK value of the bacterial network (Figure 4B). The avgCC value of the exoenzyme network was positively correlated with the avgCC values of bacterial and whole microbiota networks. In addition, the robustness of the exoenzyme network was positively associated with the robustness of the bacterial network.

**FIGURE 2** Decomposer micro-food web features in the arable system and restored natural area. (A) Soil substrate quantity, substrate stoichiometry, and decomposability, (B) characteristics of soil microbiota community, and (C) co-occurrence networks of soil microbiota community. Significance analysis was performed using the *T* test. NS,  $p > 0.05$ ; ★,  $p < 0.05$ .  $C_{KMnO_4}$ ,  $KMnO_4$ -oxidized C; DN, dissolved nitrogen; DOC, dissolved organic carbon; F:B, fungi:bacteria; NCI, nematode channel index; NCR, nematode channel ratio; PCI, protozoa channel index; PCo1, the first principal coordination score; TN, total nitrogen; TOC, total organic carbon. The average of PCo1 scores of the four functional groups (average PCo1), the average standardized abundance, or Shannon Index of the four functional groups (average Z score).



**FIGURE 4** Correlations between soil exoenzyme profile indices and decomposer micro-food web features. (A) Correlations between soil exoenzyme profile indices and the characteristics of soil substrate and microbiota community, (B) between network complexity and stability indices of exoenzyme profile and microbiota community, (C) between exoenzyme gross activity and network indices of microbiota community ( $n = 16$ ) after the arable system converted to a natural system. Pearson correlation coefficients at  $p < 0.05$  are indicated in red (negative) or in blue (positive). The size of the square is proportional to the absolute value of the correlation coefficient. The blank squares indicate that no correlation analysis was conducted. avgCC, average clustering coefficient; avgK, average degree; BG,  $\beta$ -1,4-glucosidase; C<sub>KMnO<sub>4</sub></sub>, KMnO<sub>4</sub>-oxidized C; Con, connectedness; DN, dissolved nitrogen; DOC, dissolved organic carbon; F:B, fungi:bacteria; GD, average path distance; H', Shannon Index; LAP, leucine aminopeptidase; NAG,  $\beta$ -1,4-*N*-acetyl-glucosaminidase; NCI, nematode channel index; NCR, nematode channel ratio; PCI, protozoa channel index; PHOX, phenol oxidase; TN, total nitrogen; TOC, total organic carbon.

Gross activity of the exoenzyme profile was positively correlated with substrate quantity indices (i.e., TOC, DOC, C<sub>KMnO<sub>4</sub></sub>, TN, and DN contents), the abundance of each functional group and the whole microbiota

community, and the Shannon Index of each functional group (except fungi) and the whole microbiota community (Figure 4A). The gross activity of the exoenzyme profile was also positively correlated with the number of

positive links, negative links, and modules of bacterial, fungal, and protozoan and nematode networks (Figure 4C). NAG:LAP was positively correlated with F:B and NCI, and negatively with NCR, while exhibiting no association with PCI (Figure 4A).

## DISCUSSION

### Exoenzymatic stoichiometry and decomposability indices were linked to substrate quality of the micro-food web

The stoichiometry and decomposability of substrates are key drivers of micro-food web function [11]. In our case study, after the arable system was converted to a natural system, TOC:TN and DOC:DN were both increased, indicating the increased C availability but decreased substrate N availability for soil micro-food web. Furthermore,  $C_{KMnO_4}$ :TOC and DOC:TOC decreased, suggesting decreased substrate C decomposability. We found that BG:NAG was decreased under the restored natural area, and presented negative correlations with TOC:TN and DOC:DN. This indicated the usefulness of BG:NAG in evaluating the substrate C:N stoichiometry. Our results strongly supported the enzymatic stoichiometry theory [15] that soil microorganisms can adapt to nutrient deficiency by shifting the type of exoenzymes produced in favor of nutrient cycling and therefore boosting nutrient turnover. While only C:N stoichiometry was investigated in this study, numerous studies have also shown the close association between enzymatic C:P or N:P with substrate C:P or N:P [14, 22, 23]. Also, we observed that BG:PHOX was significantly decreased after the arable system was converted to the natural area and it exhibited significant positive correlations with  $C_{KMnO_4}$ :TOC and DOC:TOC. This suggests that the enzymatic C decomposability index can indicate substrate C decomposability and is consistent with the result of previous studies reporting that microorganisms can modulate the relative allocation of resources into exoenzymes that target labile versus recalcitrant C in response to the change of substrate C decomposability [24].

Note that these exoenzymes were chosen for calculating the corresponding indices of the soil micro-food web, because their catalyzed substrate components are thought to be the most abundant in respective relevant substrate complexes in most study cases [11, 15]. However, the utility of these indices may depend on the specific substrate background. For example, a previous study found that in the study with cellulose as the dominant C source in the soil food web, BG:NAG can effectively reflect the C versus N requirements of microorganisms, while the

study focused on chitin, peptidoglycan, and protein as the main C source, BG:NAG cannot be used to indicate the microbial C versus N requirements [25]. As a result, appropriately selecting specific exoenzymes according to the specific substrates present is critical for improving the utility of these enzymatic indices for indicating changes in the substrate features of soil food web.

### Composition and diversity of exoenzymatic profile associated with the micro-food web

Consistent with the results of multiple previous studies [19–21], we found that each functional group and the whole microbiota community exhibited greater diversity in terms of richness, evenness, and the Shannon Index in the restored natural area compared with the arable system. This is possibly due to increased substrate amount (e.g., increased TOC and TN as presented in this study), substrate complexity, and/or improved habitats (e.g., improved soil pH and aggregation) in the restored natural area benefit coexistence of multiple microbes [26, 27]. However, the exoenzyme richness did not differ between the arable system and the restored natural area for all the studied exoenzymes detected in both treatments. This possibly resulted from a study limitation with only eight exoenzymes included, all of which are common. Selecting a larger number of exoenzymes, also including rare ones, can be important for improving the indication utility of the exoenzyme profile. As we expected, the exoenzyme profile composition was significantly altered and the Shannon Index was higher in the restored natural area. There were also significant correlations between the composition and Shannon Index of the exoenzyme profile and the composition and Shannon Index of microbiota functional groups and the whole microbiota community. This showed the diversity-indication utility of exoenzyme profile, which can firstly be attributed to different species of microorganisms performing distinct metabolic functions and producing distinct exoenzyme profiles [28]. Additionally, the specific trophic interaction of microbe–microfauna (protozoa and nematodes) accounted for the diversity correlation between exoenzymes and microfauna. In the present case study, there were significant positive correlations between microbe and microfauna diversities (Figure S4). On the one hand, microbial community structure can modify microfauna structure via bottom-up effects [29]. Therefore, exoenzyme profile structure can be indirectly linked to microfauna community structure. On the other hand, microfauna community structure can regulate microbial

community structure via top-down effects, and in turn indirectly control the microbe-derived exoenzyme profile structure [30].

As discussed previously, in our case study, the changed substrate stoichiometry and decomposability resulted in significant shifts in the structure of specific exoenzymes under the restored natural area. This can lead to confusing in the utility of exoenzyme profile evenness for indicating the microbiota diversity. To minimize this effect, we further selected specific subsets of the exoenzyme profile to assess the diversity indications of microbiota. We found a strong positive correlation between the Shannon Index of the C-degrading hydrolase with the Shannon Index of most microbiota communities and the whole micro-food web. This was possibly due to that compared with the C-degrading hydrolase profile, the studied total enzyme, C-degrading enzyme, hydrolase profiles were comprised of more types of exoenzymes, and thus the C-degrading hydrolase profile was the least influenced by the substrate stoichiometry and decomposability among the studied enzyme profile [28]. These results indicate that when using exoenzyme evenness to evaluate microbiota diversity, selecting the appropriate exoenzyme sub-profile is important with regard to the study area and research question(s). Alternatively, selecting a larger number of exoenzymes may be useful to increase the effectiveness of this approach.

### Network complexity and stability of exoenzymatic profile corresponding to those of the micro-food web

Soil food web complexity is traditionally described based on trophic interactions among microbiota functional groups. The network with species as nodes and species-species relationships as links, is generally used to characterize complex ecological interactions such as predation, competition, and mutualism within the soil food web [20, 31, 32]. Using this network analysis, a series of parameters quantifying ecological network complexity and stability have been developed.

Because exoenzymatic secretion patterns can be distinct among different microbiota taxa, interactions between taxa may normally induce certain correlations of corresponding exoenzymes. For instance, assuming exoenzyme A1 (random naming) specifically originates from microbiota taxa B1 and exoenzyme A2 from taxa B2, then the interaction between B1 and B2 (assumed as a positive link) would cause a statistically positive correlation between A1 and A2. Higher biodiversity of soil food webs often supports a more complex ecological

network [10], and a more complicated network usually shows greater stability. This is probably because the complexity indices such as connectivity and modularity are positively associated with the robustness and stability of complex systems [10]. In the present case study, we also found that compared with the arable system, as microbiota diversity increased under the restored natural area, the network complexity and stability of the soil micro-food web also increased, as indicated by its greater avgK, avgCC, and GD, strengthened robustness, and weakened vulnerability. Additionally, although only eight types of exoenzymes were included in the co-occurrence network analysis, higher avgK, avgCC, and Con values, and greater robustness were observed. This indicates that more complex and stable exoenzyme networks were present in the restored natural system compared with the arable system. Furthermore, we found avgK and avgCC of the exoenzyme network were positively correlated with avgK and avgCC of the biota network, respectively. Seemingly, there was a corresponding relationship between exoenzyme profile and micro-food web structure in terms of their network properties.

### Exoenzymatic gross activity in relation to substrate quantity, community size, diversity, and network complexity of the micro-food web

Soil microorganisms are known to acquire energy, carbon, and multiple nutrients for their growth through secreting exoenzymes to catalyze soil substrate (Figure 1). Typically, enzyme activity represents a key indicator of soil fertility and microbial function. In our case study, the increased soil organic carbon and nitrogen contents stimulated microbial growth, which in turn elevated the gross activity of exoenzymes after the conversion of the arable system to a restored natural area. Additionally, the stimulated microbial growth further promoted the growth of their predators (i.e., protozoa and nematodes) via the bottom-up effects of trophic cascading [29]. This resulted in a significant increase in the abundance of protozoa and nematodes, and caused positive correlations between microbiota and their predators' abundances (Figure S4). As a result, the gross activity of the exoenzyme profile was positively correlated not only with soil carbon and nitrogen contents, but also with microbial, protozoa, and nematode abundances. This indicates that the gross activity of the exoenzyme profile can be used as an indicator to reflect substrate quantity and community size of the micro-food web.

Because the gross activity of exoenzyme profile represents multiple metabolic functions (i.e., soil multifunctionality) of soil microorganisms [33], and that the capacity of soil biotic communities maintaining multifunctionality is strongly dependent on soil biodiversity [34–37], we expected that there would be a strong association between exoenzyme gross activity and soil biodiversity. Indeed, we observed positive correlations between the gross activity of exoenzyme profile and the Shannon Index of multiple soil organisms (i.e., bacteria, protozoa, nematode, and whole microbiota community). This can be explained by the classic assumptions of “complementarity effect” and “selection effect” [38]. The complementarity effect is defined as the ability of different species to each utilize a different subset of the available niches, with the consequence that a multi-species assemblage will exploit a greater part of the resource than any singular species [39]. Therefore, a combination of different species may not only coexist, but also outperform any of them surviving in isolation [39]. Alternatively, the selection effect suggests that dominant species with particular traits can overperform specific functions in a community [40]. Apart from richness, evenness is also believed to favor the multifunctionality of a community. In an uneven community dominated by a few taxa, any organism is likely to interact with its kin [39]. Conversely, in a community with high species evenness, an organism will most likely interact with other functionally dissimilar organisms, which is helpful to promote synergistic interactions and thus boost soil multifunctionality [36].

The association complexity among biotic taxa provides a new dimension for understanding the positive effects of biodiversity on multifunctionality [9]. The gross activity of the exoenzyme profile thus can also reflect the network complexity of the micro-food web, as indicated by our case study. This can be explained by the following three aspects. Firstly, the positive (e.g., facilitation) and negative (e.g., competition) interactions among different taxa drive ecosystem functions [9]. As presented in this case study, a more complex network may possess more numbers of positive and/or negative links (Table S1), suggesting more taxonomic interactions, which may exert positive influences on soil multifunctionality in terms of enzymatic gross activity. In addition, keystone taxa are highly connected taxonomic groups conferring greater biotic connectivity to a community [41]. In our study, more numbers of keystone taxa were identified in the restored natural area networks. These keystone taxa are important for maintaining network structure and thus multiple functions, which could positively affect soil multifunctionality [42]. Finally, a module in the network is a group of taxa which are highly connected among

themselves, but less connected with taxa outside the group [31]. In the food web, functionally dissimilar groups often form different network modules, so that a community with more modular architectures may have more functional group diversity and stronger ability to provide multiple ecosystem functions [43]. Indeed, more numbers of modules were detected for the microbiota co-occurrence networks after the conversion of the arable system to a restored natural area in our case study.

### Exoenzymatic channel index for reflecting decomposition channel structure of the micro-food web

The energy flow in a soil food web consists mainly of bacterial- and fungal-based channels [2]. The relative importance of these two channels represents a crucial parameter for predicting the functioning and sustainability of the food web [1]. As we expected, the decomposition channel of the restored natural area shifted towards fungal dominance relative to the arable system. This was indicated by enhanced F:B and NCI, and reduced NCR, which is consistent with previous reports [20, 44]. Traditionally, protozoa are thought to prey mainly on bacteria [17, 45]. However, recent studies pointed out that some protozoa (e.g., *A. castellanii*) prefer to feed on fungi [17]. On the basis of this information, here we first tried to propose the PCI for indicating the decomposition channel structure among different ecosystems. However, in the present study, the PCI value did not increase under the restored natural area when compared with the arable system. This indicated that the above-mentioned view of some protozoa preferentially feeding on fungi may be questionable, and so the PCI calculation needs to be further improved in future studies. Alternatively, this can be explained by the limited sampling time points in this study. With respect to the PCI as an instant indicator, its response to the restored natural area might be seasonally varied and the response might be ineffective in indicating the structural change of gross decomposition at some specific sampling time points. Hence, further testing should be done on the PCI utility including additional sampling time points.

Considering that NAG is known to originate mainly from fungi [11], here we first proposed the enzymatic channel index calculated by the ratio of NAG to LAP. These two enzymes are involved in nitrogen cycling [15]. In the present study, NAG:LAP was observed to significantly increase after the conversion of arable land to a restored natural area, and to show strong positive correlations with F:B and NCI, and a negative correlation with NCR. This indicated the usefulness of NAG:LAP in

reflecting the decomposition channel structure. Higher NAG:LAP means greater relative importance of fungal-based decomposition pathways in the soil micro-food web. Among the microbiota, microorganisms are known to dominantly contribute to the energy flow of the soil food web, and thus the channel indices derived from microorganisms such as F:B normally represent the basic criterion for indicating decomposition channel structure. Due to the longer reproductive period of higher trophic-level organisms (e.g., nematodes), there may be a lag of several days to weeks before changes in faunal channel indices in response to microbial changes [5]. In contrast, soil exoenzymes produced by microorganisms can rapidly shift in response to environmental change. Consequently, the enzymatic channel index may be more effective in predicting the instant change in decomposition channel structure than other faunal channel indices.

## CONCLUSION

In this case study on the restored natural area of an arable system, we found close associations between the proposed soil enzyme profile indices and the corresponding parameters of the decomposer micro-food web. Our data indicated that “soil enzyme profile analysis” can be a useful tool to comprehensively reflect decomposer micro-food web features. However, its general utility needs to be further examined. Moreover, it is recommended that a sufficient number and appropriate type collection of enzymes should be considered based on the outcomes of the study.

To optimize and develop enzyme profile indices and to promote the application of the “soil enzyme profile analysis” for indicating changes in soil micro-food web, the following two study directions need to be further strengthened in future work: (i) specific sources of soil exoenzymes: although many soil exoenzymes have been found and they are known to originate mainly from microbial secretion, our knowledge of the unique sources of exoenzymes remains limited. It will be helpful for us to better understand theoretical links between exoenzymes and the micro-food web. For example, if some exoenzyme(s) are exclusively derived from soil microfauna, the relative activity of such exoenzyme(s) would be expected to indicate a vertical trophic structure of the micro-food web; (ii) high-efficient measurement of exoenzyme activities: the fluorescence detection technology has greatly enhanced measurement efficiency of soil exoenzyme activities, but the types of enzymes that can be measured by this technology remains limited. As mentioned above, the more enzymatic identities included in the reference

“soil enzyme profile,” the better the predictive ability of diversity and network parameters related to the enzyme profile as part of the micro-food web.

## METHODS

### Study site

A long-term field experiment with an “arable system” and “a restored natural system” was selected for this study. The experiment was established in 1990 at the National Soil Quality Observation Experimental Station at Qiyang (26°45' N, 119°52' E) in Hunan Province, Southern China. This region belongs to a subtropical monsoon climate with a mean annual precipitation and temperature of 1250 mm and 18°C, respectively. The soil is classified as a Ferralic Cambisol (WRB Classification). The field experiment includes a series of treatments on soil use and nutrient management strategies with each plot area of 200 m<sup>2</sup>. Before initiating the field experiment, topsoil (0–20 cm) chemical properties were as follows: TOC = 6.7 g kg<sup>-1</sup>, TN = 1.07 g kg<sup>-1</sup>, available N (AN) = 79 mg kg<sup>-1</sup>, available phosphorus (AP) = 10.8 mg kg<sup>-1</sup>, and pH = 5.70.

### Experiment design

For this study, two treatments were selected, namely, a conventional cropping (i.e., arable) system and a restored natural area. There were four plots (25 m<sup>2</sup> each) as replicates for each treatment. The arable system followed a rainfed wheat–maize rotation with conventional fertilization. Urea, superphosphate, and potassium chloride were applied at rates of 188 kg N ha<sup>-1</sup> year<sup>-1</sup>, 41 kg P ha<sup>-1</sup> year<sup>-1</sup>, and 78 kg K ha<sup>-1</sup> year<sup>-1</sup>, respectively, for maize, and at rates of 165 kg N ha<sup>-1</sup> year<sup>-1</sup>, 36 kg P ha<sup>-1</sup> year<sup>-1</sup>, and 68 kg K ha<sup>-1</sup> year<sup>-1</sup>, respectively, for wheat. Crop straw was removed after harvest. The vegetation was dominated by herbaceous plants, shrubs, and trees in the restored natural area.

### Soil sampling

Soil samples were collected in June and September 2021. At each sampling, six topsoil (0–15 cm) samples were collected from each subplot to form one composite sample. This composite sample was divided into three parts and sieved using the required mesh sizes. One part was stored at –80°C for soil genomic DNA extraction, while another part was air-dried for soil property measurements. Finally, the remaining part was stored

at 4°C for less than 1 week for soil moisture and exoenzyme activity measurements, as well as protozoa and nematode extractions.

## Substrate measurements

Soil TOC and TN contents were measured using an elemental analyzer (Vario EL V, Elementar). Since the soil was acidic and its carbonate content was neglected, we used total C content as an approximation of TOC. The  $\text{KMnO}_4$ -oxidized C was determined following the methods described by the previous study [46]. Soil DOC and DN were extracted using a 0.5 M  $\text{K}_2\text{SO}_4$  solution. The supernatant was filtered through a 0.45  $\mu\text{m}$  filter and the C and N concentrations in the filtrate were determined using an automated TOC/TN analyzer (multi-N/C 3000, Analytik Jena).

The ratios of TOC to TN (TOC:TN) and DOC to DN (DOC:DN) were calculated as the substrate C:N stoichiometry. The SOC decomposability was assessed considering the proportion (%) of DOC to TOC and  $\text{KMnO}_4$ -oxidized C to TOC.

## Bacterial and fungal community analysis

Abundances of bacteria and fungi were assessed using their gene copy numbers. Soil genome DNA was extracted from homogenized 0.5 g subsamples using the FastDNA SPIN Kit for Soil (MP Biomedicals) following the manufacturer's instructions. Bacterial and fungal ribosomal RNA (rRNA) gene copy numbers were quantified by quantitative polymerase chain reaction (PCR) using a StepOnePlus Real-Time PCR System (Applied Biosystems). Primer set 27F/519R [47] and ITS1/ITS4 [48] were used to target 16S and ITS rRNA genes, respectively. Standard curves were generated using 10-fold serial dilutions of linearized recombinant plasmids for each quantitative PCR (qPCR) run. The qPCR assay was performed in triplicate in a 20- $\mu\text{L}$  reaction volume containing 10  $\mu\text{L}$  of 2 $\times$  concentration SYBR Premix Ex Taq (premix of dNTP Mixture, SYBR Green I,  $\text{Mg}^{2+}$ , Tli RNaseH, Takara Ex Taq HS), 0.4  $\mu\text{L}$  of forward and reverse primers, 0.4  $\mu\text{L}$  of ROX Reference Dye (50 $\times$ ), 2  $\mu\text{L}$  of template DNA, and 6.8  $\mu\text{L}$  of autoclaved distilled water. The ratio of fungal gene to bacterial gene copy numbers (F:B) was calculated as a decomposition channel index.

High-throughput sequencing was performed for bacterial and fungal community composition analysis. We amplified the bacterial 16S rRNA gene with the targeting primer set 338F/806R [49] and the fungal ITS1

rRNA gene with the targeting primer set ITS1F/ITS2R [48]. Purified amplicons were sequenced on an Illumina MiSeq platform (2  $\times$  300, pair end). Sequencing data were processed using the open-source Quantitative Insights into Microbial Ecology 2 (QIIME2, version 2021.4) pipeline [50]. Sequences were quality filtered, denoised, and the chimeras removed using the DADA2 plugin [51]. Next, the sequences were clustered into amplicon sequence variants (ASVs). Taxonomic identities were assigned to ASVs by the classify-sklearn naïve Bayes taxonomy classifier in the feature-classifier plugin [52] using the SILVA version 132 database for bacteria [53] and the UNITE version 8.3 database for fungi [54]. Alpha-diversity metrics (i.e., richness, Pielou Evenness Index, and Shannon Index) were calculated based on the rarefied data at a minimum number of sequences [55, 56].

## Protozoan community analysis

The abundances of major protozoa groups including flagellates, amoebae, and ciliates were determined using the most-probable number method [57]. Briefly, 2 g fresh soil was suspended in 18 mL sterile water and shaken at 180 rpm for 30 min. A threefold dilution series was added into 96-well microtiter plates in quadruplicates. The plates were incubated at 25°C in the dark. After 4, 7, and 11 days, the wells were examined for the presence of protozoa using a Nikon Eclipse TS100 inverted optical microscope. The abundances of the major protozoa groups were determined and expressed as the number of individuals  $\text{g}^{-1}$  dry soil.

High-throughput sequencing was used to characterize the protozoan community. We amplified the 18S rRNA gene with the targeting primer set TAR-euk454FWD1/TAReukREV3 [58]. The sequencing data process for the protozoa community was similar to that of the bacteria and fungi. ASVs were taxonomically classified by blasting against the Protist Ribosomal Reference database (version 4.14) [59]. The protozoa community was semi-quantitatively assayed based on identified protozoan sequences [60, 61], which were selected from the obtained ASV table. We assigned protozoa ASVs to functional groups of bacterivores and fungivores according to their feeding habits [60, 62]. The same alpha-diversity metrics used previously were applied to estimated protozoan diversity based on the rarefied data at a minimum number of sequences. This study is the first to propose the PCI, calculated as the ratio of the relative abundance of fungivorous to bacterivorous protozoa, as a new decomposition channel index.

## Nematode community analysis

Nematodes were extracted from 100 g fresh soil (field condition) using the shallow dish method [63]. Next, nematodes were killed and fixed by adding a 4% formaldehyde solution heated to 65°C. In each sample all extracted nematodes were counted and identified to genus level using macro slides and an optical microscope (Olympus BX50). The nematode community was classified into the following four trophic groups: bacterivores, fungivores, omnivores–predators, and herbivores. The first three trophic groups (i.e., free living nematodes) included in the decomposer food web were used for the nematode community analysis, from which a minimum of 100 nematodes were chosen to calculate diversity indices (i.e., richness, Pielou Evenness Index, and Shannon Index). The rarefied data were further used to calculate the NCR as follows:  $NCR = B/(B + F)$ , where B and F are the abundance of bacterivores and fungivores, respectively [6]. Additionally, nematodes were assigned into functional guilds that are based on life history characteristics [5]. More information on the functional guild classification of nematodes can be found at <http://nemaplex.ucdavis.edu/>. The NCI, which is a measure of the primary decomposition pathway (bacterial vs. fungal), was calculated as follows:  $NCI = 100 \times [0.8 \times Fu_2 / (3.2 \times Ba_1 + 0.8 \times Fu_2)]$ , where  $Ba_1$  and  $Fu_2$  are the abundances of bacterivorous colonizer–persister (cp) group 1 and fungivorous cp group 2, respectively [5].

## Overall microbiota characterization

On the basis of the relative abundance of taxa, we calculated Bray–Curtis dissimilarity matrices for bacteria, fungi, and protozoa using ASV data, and for nematodes using genus level data with the *vegdist* function in R (version 4.1.1). Next, a principal coordinate analysis (PCoA) was conducted, and the PCo1 score was used as the indicator of community composition. The average of PCo1 scores of the four functional groups was used as an index of the whole microbiota community composition. Then, the *z* score approach [64] and the average standardized abundance, richness, Pielou Evenness Index, and Shannon Index of the four groups were used to reflect on the whole microbiota community abundance and diversity, respectively.

## Microbiota co-occurrence network analysis

To visualize potential interactions among all individual members of bacteria, fungi, protozoa, nematode, and of

the whole microbiota community, we constructed co-occurrence networks for the corresponding communities. For the whole microbiota community, the relative abundance table was established by combining bacterial, fungal, and protozoa ASVs and nematode genera. Pairwise Spearman correlation matrixes were calculated, with cutoffs for the absolute value of correlation coefficient  $>0.6$ . *p* Values had a 0.05 cutoff. Networks for the arable system and the restored nature area were constructed independently. To evaluate the network complexity, we calculated five topological parameters: avgK, avgCC, GD, Con, and modularity in the subgraph function via the *igraph* package in R (version 4.1.1). Furthermore, robustness and vulnerability were used to evaluate network stability. Robustness is defined as the proportion of the remaining members in this network after random or targeted nodes removal [65]. Vulnerability is defined as the relative contribution of each node to the global efficiency which measures how fast information spreads within the network [41]. We measured the robustness of each network when 50% of random nodes were removed, and calculated the maximal vulnerability of nodes in the network [10]. Moreover, we calculated the number of positive or negative links for different networks. Finally, the topological roles of individual nodes in the network were determined by the threshold values of  $Z_i$  and  $P_i$ . Nodes were classified into four categories: module hubs ( $Z_i > 2.5$ ), network hubs ( $Z_i > 2.5$  and  $P_i > 0.62$ ), connectors, and peripherals ( $Z_i < 2.5$  and  $P_i < 0.62$ ). The connectors, module hubs, and network hubs were categorized as keystone nodes [31]. In theory, greater avgK indicates greater number of positive or negative links between species in community; greater avgCC suggests that species were more extensively divided into several different components; greater GD means greater overall independence among species; higher Con suggests greater proportion of positive (or negative) links from all possible links in the network; greater modularity implies greater separation strength of species clusters within the network; strengthening robustness, and weakening vulnerability indicates a stabilizing network [10, 66].

## Exoenzyme activity measurements

Four hydrolytic C-acquiring exoenzymes (AG, BG, CB, and xylosidase [XS]), two hydrolytic N-acquiring exoenzymes (NAG and LAP), and two oxidative exoenzymes (PHOX and peroxidases [PEOX]) were selected to form a reference exoenzyme profile. Hydrolytic exoenzyme activities were assayed using standard fluorometric techniques [67]. The soil suspension was supplemented with 7-amino-4-

methylcoumarin (MUC for LAP) or 4-methylumbelliferone (MUB for other hydrolytic exoenzymes) to establish standard curves. Each sample was assayed with six replicates in 96-well microplates and incubated in the dark at 25°C for 3 h. Then the fluorescence was measured using a fluorescence spectrometer (Beckman Coulter DTX 880) with 365 nm excitation and 450 nm emission filters. Oxidase activities were measured spectrophotometrically using the substrate L-3,4-dihydroxy-phenylalanine. For PEOX, 0.3% H<sub>2</sub>O<sub>2</sub> was added. The microplates were incubated in the dark at 25°C for 20 h and then the absorbance was quantified at 450 nm. The units for all exoenzyme activities were nmol g<sup>-1</sup> soil h<sup>-1</sup>.

## Exoenzyme profile analysis

We calculated a series of exoenzyme profile indices as follows: the ratio of BG to NAG (BG:NAG) was calculated as an enzymatic stoichiometry index to study substrate C:N stoichiometry; the ratio of BG to PHOX (BG:PHOX) was calculated as the enzymatic C decomposability index; the ratio of NAG to LAP was calculated (NAG:LAP) as the enzymatic channel index to reflect the dominance of fungal- versus bacterial-based decomposition channels in the soil decomposer micro-food web. This is possible since NAG is thought to be mainly secreted by fungi, while bacteria are a major source of soil LAP activity [11]. Furthermore, the mean of all standardized exoenzyme activities was used as the gross activity index of the whole exoenzyme profile. Since large differences in the order of magnitude of specific activities existed among the eight exoenzymes, we first conducted a simple mathematical transformation by dividing the original data of each exoenzyme by its maximum value observed in the data set of samples, and then multiplying it by 100 [21]. Thereafter, based on the proportions of the eight exoenzymes, the PCoA was conducted and the PCo1 scores were used as the indicator of exoenzyme composition. Since all eight exoenzymes were detected in every sample, we did not calculate richness, but instead calculated Shannon diversity of the whole exoenzyme profile (eight exoenzymes), C-degrading exoenzymes (four C hydrolases and two oxidases), hydrolase (four C hydrolases and two N hydrolases), and C hydrolase (four exoenzymes), using the formula  $H' = (-\sum P_i [\ln P_i])$ , where  $P_i$  was the proportion of a particular exoenzyme activity to the sum of activities of all exoenzymes [21]. As described above, a correlation network approach was used to visualize potential interactions among all individual members of the specific exoenzyme profile, and then the network complexity and stability parameters were calculated in both treatments.

## Statistical analysis

All statistical analyses were performed in R version 4.1.1 (R Development Core Team 2021). A *T* test was used to evaluate effects of the restoration of cropland to the natural area on soil decomposer micro-food web characteristics and exoenzyme profile indices in comparison to the arable system. Relationships between exoenzyme profile indices and corresponding micro-food web characteristics, between soil microbes (bacteria and fungi) with substrate and microfauna (protozoa and nematode) after the conversion of an arable system to a natural area were evaluated by a Pearson correlation analysis. Statistical significance was set at  $\alpha = 0.05$ .

## AUTHOR CONTRIBUTIONS

Yilai Lou conceived the ideas and designed the research. Weng Xing, Ning Hu, Gerhard Du Preez, Scott X. Chang, and Yilai Lou drafted the manuscript. Zhongfang Li, Liangshan Feng, Weidong Zhang, Huimin Zhang, Dongchu Li, and Shunbao Lu performed the experiments. All the authors read and approved the final manuscript.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive in BIG Data Center, Beijing Institute of Genomics (BIG), Chinese Academy of Sciences, under accession numbers CRA009970, CRA009972, and CRA009974 that are publicly accessible at <https://ngdc.cnca.ac.cn/gsa>. The data and scripts used are saved in GitHub <https://github.com/spinagexingwen/Xing2023iMeta>. Supplementary materials (figures, tables, scripts, graphical abstract, slides, videos, Chinese translated version, and update materials) may be found in the online DOI or iMeta Science <http://www.imeta.science/>.

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## REFERENCES

- Moore, John C., and H. William Hunt. 1988. "Resource Compartmentation and the Stability of Real Ecosystems." *Nature* 333: 261–3. <https://doi.org/10.1038/333261a0>
- Moore, John C., Eric L. Berlow, David C. Coleman, Peter C. de Ruiter, Quan Dong, Alan Hastings, Nancy Collins Johnson, et al. 2004. "Detritus, Trophic Dynamics and Biodiversity." *Ecology Letters* 7: 584–600. <https://doi.org/10.1111/j.1461-0248.2004.00606.x>
- Geisen, Stefan, Edward A. D. Mitchell, Sina Adl, Michael Bonkowski, Micah Dunthorn, Flemming Ekelund, Leonardo D. Fernández, et al. 2018. "Soil Protists: A Fertile Frontier in Soil Biology Research." *FEMS Microbiology Reviews* 42: 293–323. <https://doi.org/10.1093/femsre/fuy006>
- Zhao, Jie, Xunyang He, Yunpeng Nie, Wei Zhang, Zhiyong Fu, and Kelin Wang. 2015. "Unusual Soil Nematode Communities on Karst Mountain Peaks in Southwest China." *Soil Biology and Biochemistry* 88: 414–9. <https://doi.org/10.1016/j.soilbio.2015.06.020>
- Ferris, Howard, Tom Bongers, and Ron G. M. de Goede. 2001. "A Framework for Soil Food Web Diagnostics: Extension of the Nematode Faunal Analysis Concept." *Applied Soil Ecology* 18: 13–29. [https://doi.org/10.1016/S0929-1393\(01\)00152-4](https://doi.org/10.1016/S0929-1393(01)00152-4)
- Yeates, Gregor W. 2003. "Nematodes as Soil Indicators: Functional and Biodiversity Aspects." *Biology and Fertility of Soils* 37: 199–210. <https://doi.org/10.1007/s00374-003-0586-5>
- Du Preez, Gerhard, Mieke Daneel, Ron De Goede, Marié Joey Du Toit, Howard Ferris, Hendrika Fourie, Stefan Geisen, et al. 2022. "Nematode-based Indices in Soil Ecology: Application, Utility, and Future Directions." *Soil Biology and Biochemistry* 169: 108640. <https://doi.org/10.1016/j.soilbio.2022.108640>
- Du, Xiaofang, Yingbin Li, Fang Liu, Xiaolin Su, and Qi Li. 2018. "Structure and Ecological Functions of Soil Micro-Food Web." *Chinese Journal of Applied Ecology* 29: 403–11. <https://doi.org/10.13287/j.1001-9332.201802.033>
- Wagg, Cameron, Klaus Schlaeppi, Samiran Banerjee, Eiko E. Kuramae, and Marcel G. A. van der Heijden. 2019. "Fungal–Bacterial Diversity and Microbiome Complexity Predict Ecosystem Functioning." *Nature Communications* 10: 4841. <https://doi.org/10.1038/s41467-019-12798-y>
- Yuan, Mengting Maggie, Xue Guo, Linwei Wu, Ya Zhang, Najia Xiao, Daliang Ning, Zhou Shi, et al. 2021. "Climate Warming Enhances Microbial Network Complexity and Stability." *Nature Climate Change* 11: 343–8. <https://doi.org/10.1038/s41558-021-00989-9>
- Caldwell, Bruce A. 2005. "Enzyme Activities as a Component of Soil Biodiversity: A Review." *Pedobiologia* 49: 637–44. <https://doi.org/10.1016/j.pedobi.2005.06.003>
- Allison, Steven D., and Peter M. Vitousek. 2005. "Responses of Extracellular Enzymes to Simple and Complex Nutrient Inputs." *Soil Biology and Biochemistry* 37: 937–44. <https://doi.org/10.1016/j.soilbio.2004.09.014>
- Paz-Ferreiro, Jorge, and Shenglei Fu. 2016. "Biological Indices for Soil Quality Evaluation: Perspectives and Limitations." *Land Degradation & Development* 27: 14–25. <https://doi.org/10.1002/ldr.2262>
- Sinsabaugh, Robert L., Brian H. Hill, and Jennifer J. Follstad Shah. 2009. "Ecoenzymatic Stoichiometry of Microbial Organic Nutrient Acquisition in Soil and Sediment." *Nature* 462: 795–8. <https://doi.org/10.1038/nature08632>
- Sinsabaugh, Robert L., and Jennifer J. Follstad Shah. 2012. "Ecoenzymatic Stoichiometry and Ecological Theory." *Annual Review of Ecology, Evolution, and Systematics* 43: 313–43. <https://doi.org/10.1146/annurev-ecolsys-071112-124414>
- Kaiser, Christina, Marianne Koranda, Barbara Kitzler, Lucia Fuchslueger, Jörg Schneckner, Peter Schweiger, Frank Rasche, Sophie Zechmeister-Boltenstern, Angela Sessitsch, and Andreas Richter. 2010. "Belowground Carbon Allocation by Trees Drives Seasonal Patterns of Extracellular Enzyme Activities by Altering Microbial Community Composition in a Beech Forest Soil." *New Phytologist* 187: 843–58. <https://doi.org/10.1111/j.1469-8137.2010.03321.x>
- Geisen, Stefan, Robert Koller, Maike Hünninghaus, Kenneth Dumack, Tim Urich, and Michael Bonkowski. 2016. "The Soil Food Web Revisited: Diverse and Widespread Mycophagous Soil Protists." *Soil Biology and Biochemistry* 94: 10–8. <https://doi.org/10.1016/j.soilbio.2015.11.010>
- Coolon, Joseph D., Kenneth L. Jones, Timothy C. Todd, Bryanua C. Carr, and Michael A. Herman. 2009. "Caenorhabditis elegans Genomic Response to Soil Bacteria Predicts Environment-Specific Genetic Effects on Life History Traits." *PLoS Genetics* 5: e1000503. <https://doi.org/10.1371/journal.pgen.1000503>
- Gilbert, Kadeem J., Timothy J. Fahey, John C. Maerz, Ruth E. Sherman, Patrick Bohlen, Jason J. Dombroskie, Peter M. Groffman, and Joseph B. Yavitt. 2014. "Exploring Carbon Flow Through the Root Channel in a Temperate Forest Soil Food Web." *Soil Biology and Biochemistry* 76: 45–52. <https://doi.org/10.1016/j.soilbio.2014.05.005>
- Morriën, Elly, S. Emilia Hannula, L. Basten Snoek, Nico R. Helmsing, Hans Zweers, Mattias de Hollander, Raquel Luján Soto, et al. 2017. "Soil Networks Become More Connected and Take Up More Carbon as Nature Restoration Progresses." *Nature Communications* 8: 14349. <https://doi.org/10.1038/ncomms14349>
- Rodríguez-Loinaz, Gloria, Miren Onaindia, Ibone Amezcaga, Iker Mijangos, and Carlos Garbisu. 2008. "Relationship Between Vegetation Diversity and Soil Functional Diversity in Native Mixed-oak Forests." *Soil Biology and Biochemistry* 40: 49–60. <https://doi.org/10.1016/j.soilbio.2007.04.015>
- Shen, Fangfang, Jianping Wu, Houbao Fan, Wenfei Liu, Xiaomin Guo, Honglang Duan, Liang Hu, Xueming Lei, and Xiaohua Wei. 2019. "Soil N/P and C/P Ratio Regulate the Responses of Soil Microbial Community Composition and Enzyme Activities in a Long-Term Nitrogen Loaded Chinese Fir Forest." *Plant and Soil* 436: 91–107. <https://doi.org/10.1007/s11104-018-03912-y>
- Zhao, Yingzhi, Chenfei Liang, Shuai Shao, Junhui Chen, Hua Qin, and Qiufang Xu. 2021. "Linkages of Litter and Soil C:N:P Stoichiometry With Soil Microbial Resource Limitation and Community Structure in a Subtropical Broadleaf Forest Invaded by Moso bamboo." *Plant and Soil* 465: 473–90. <https://doi.org/10.1007/s11104-021-05028-2>
- Herold, Nadine, Ingo Schöning, Doreen Berner, Heike Haslswimmer, Ellen Kandeler, Beate Michalzik, and Marion Schrumpf. 2014. "Vertical Gradients of Potential Enzyme Activities in Soil Profiles of European Beech, Norway

- Spruce and Scots Pine Dominated Forest Sites." *Pedobiologia* 57: 181–9. <https://doi.org/10.1016/j.pedobi.2014.03.003>
25. Mori, Taiki, Ryota Aoyagi, Kanehiro Kitayama, and Jiangming Mo. 2021. "Does the Ratio of  $\beta$ -1,4-Glucosidase to  $\beta$ -1,4-N-Acetylglucosaminidase Indicate the Relative Resource Allocation of Soil Microbes to C and N Acquisition?" *Soil Biology and Biochemistry* 160: 108363. <https://doi.org/10.1016/j.soilbio.2021.108363>
  26. Wan, Xiaohua, Zhiqun Huang, Zongming He, Zaipeng Yu, Minhuang Wang, Murray R. Davis, and Yusheng Yang. 2015. "Soil C:N Ratio is the Major Determinant of Soil Microbial Community Structure in Subtropical Coniferous and Broad-leaf Forest Plantations." *Plant and Soil* 387: 103–16. <https://doi.org/10.1007/s11104-014-2277-4>
  27. Ritz, Karl, and Iain M. Young. 2004. "Interactions Between Soil Structure and Fungi." *Mycologist* 18: 52–9. <https://doi.org/10.1017/s0269915x04002010>
  28. Yuan, Meng, Wen Xing, Meiling Luo, Yufeng Wang, Xuejia Gu, Jiqing Song, and Yilai Lou. 2021. "Analysis of Soil Enzymes Under Organic Manure Partial Substitution for Nitrogen Fertilizer in Paddy Field of Northeast China." *Chinese Journal of Ecology* 40: 123–30. <https://doi.org/10.1016/j.apsoil.2010.03.011>
  29. Thakur, Madhav P., and Stefan Geisen. 2019. "Trophic Regulations of the Soil Microbiome." *Trends in Microbiology* 27: 771–80. <https://doi.org/10.1016/j.tim.2019.04.008>
  30. Saleem, Muhammad, Ingo Fetzer, Carsten F. Dormann, Hauke Harms, and Antonis Chatzinotas. 2012. "Predator Richness Increases the Effect of Prey Diversity on Prey Yield." *Nature Communications* 3: 1305. <https://doi.org/10.1038/ncomms2287>
  31. Deng, Ye, Yihuei Jiang, Yunfeng Yang, Zhili He, Feng Luo, and Jizhong Zhou. 2012. "Molecular Ecological Network Analyses." *BMC Bioinformatics* 13: 113. <https://doi.org/10.1186/1471-2105-13-113>
  32. Jiao, Shuo, Haiyan Chu, Baogang Zhang, Xiaorong Wei, Weimin Chen, and Gehong Wei. 2022. "Linking Soil Fungi to Bacterial Community Assembly in Arid Ecosystems." *iMeta* 1: e2. <https://doi.org/10.1002/imt2.2>
  33. Trivedi, Pankaj, Manuel Delgado-Baquerizo, Chanda Trivedi, Hangwei Hu, Ian C. Anderson, Thomas C. Jeffries, Jizhong Zhou, and Brajesh K. Singh. 2016. "Microbial Regulation of the Soil Carbon Cycle: Evidence from Gene–Enzyme Relationships." *The ISME Journal* 10: 2593–604. <https://doi.org/10.1038/ismej.2016.65>
  34. Delgado-Baquerizo, Manuel, Fernando T. Maestre, Peter B. Reich, Thomas C. Jeffries, Juan J. Gaitan, Daniel Encinar, Miguel Berdugo, Colin D. Campbell, and Brajesh K. Singh. 2016. "Microbial Diversity Drives Multifunctionality in Terrestrial Ecosystems." *Nature Communications* 7(1): 10541. <https://doi.org/10.1038/ncomms10541>
  35. Delgado-Baquerizo, Manuel, Peter B. Reich, Chanda Trivedi, David J. Eldridge, Sebastián Abades, Fernando D. Alfaro, Felipe Bastida, et al. 2020. "Multiple Elements of Soil Biodiversity Drive Ecosystem Functions Across Biomes." *Nature Ecology & Evolution* 4: 210–20. <https://doi.org/10.1038/s41559-019-1084-y>
  36. Le Bagousse-Pinguet, Yoann, Nicolas Gross, Hugo Saiz, Fernando T. Maestre, Sonia Ruiz, Marina Dacal, Sergio Asensio, et al. 2021. "Functional Rarity and Evenness Are Key Facets of Biodiversity to Boost Multifunctionality." *Proceedings of the National Academy of Sciences* 118: e2019355118. <https://doi.org/10.1073/pnas.2019355118>
  37. Wagg, Cameron, S. Franz Bender, Franco Widmer, and Marcel G. A. van der Heijden. 2014. "Soil Biodiversity and Soil Community Composition Determine Ecosystem Multifunctionality." *Proceedings of the National Academy of Sciences* 111: 5266–70. <https://doi.org/10.1073/pnas.1320054111>
  38. Tilman, David, Clarence L. Lehman, and Kendall T. Thomson. 1997. "Plant Diversity and Ecosystem Productivity: Theoretical Considerations." *Proceedings of the National Academy of Sciences* 94: 1857–61. <https://doi.org/10.1073/pnas.94.5.1857>
  39. Saleem, Muhammad, Jie Hu, and Alexandre Jousset. 2019. "More Than the Sum of Its Parts: Microbiome Biodiversity as a Driver of Plant Growth and Soil Health." *Annual Review of Ecology, Evolution, and Systematics* 50: 145–68. <https://doi.org/10.1146/annurev-ecolsys-110617-062605>
  40. Yachi, Shigeo, and Michel Loreau. 1999. "Biodiversity and Ecosystem Productivity in a Fluctuating Environment: The Insurance Hypothesis." *Proceedings of the National Academy of Sciences* 96: 1463–8. <https://doi.org/10.1073/pnas.96.4.1463>
  41. Banerjee, Samiran, Klaus Schlaeppi, and Marcel G. A. van der Heijden. 2018. "Keystone Taxa as Drivers of Microbiome Structure and Functioning." *Nature Reviews Microbiology* 16: 567–76. <https://doi.org/10.1038/s41579-018-0024-1>
  42. Qiu, Liping, Qian Zhang, Hansong Zhu, Peter B. Reich, Samiran Banerjee, Marcel G. A. van der Heijden, Michael J. Sadowsky, et al. 2021. "Erosion Reduces Soil Microbial Diversity, Network Complexity and Multifunctionality." *The ISME Journal* 15: 2474–89. <https://doi.org/10.1038/s41396-021-00913-1>
  43. Montoya, D., Marian L. Yallopp, and Jane Memmott. 2015. "Functional Group Diversity Increases with Modularity in Complex Food Webs." *Nature Communications* 6(1): 7379. <https://doi.org/10.1038/ncomms8379>
  44. Deng, Qi, Xiaoli Cheng, Dafeng Hui, Qian Zhang, Ming Li, and Quanfa Zhang. 2016. "Soil Microbial Community and Its Interaction With Soil Carbon and Nitrogen Dynamics Following Afforestation in Central China." *Science of the Total Environment* 541: 230–7. <https://doi.org/10.1016/j.scitotenv.2015.09.080>
  45. Clarholm, Marianne. 1985. "Interactions of Bacteria, Protozoa and Plants Leading to Mineralization of Soil Nitrogen." *Soil Biology and Biochemistry* 17: 181–7. [https://doi.org/10.1016/0038-0717\(85\)90113-0](https://doi.org/10.1016/0038-0717(85)90113-0)
  46. Blair, Graeme J., Rod D. B. Lefroy, and Leanne Lisle. 1995. "Soil Carbon Fractions Based on Their Degree of Oxidation, and the Development of a Carbon Management Index for Agricultural Systems." *Australian Journal of Agricultural Research* 46: 1459–66. <https://doi.org/10.1071/ar9951459>
  47. Reysenbach, Anna-Louise, Gene S. Wickham, and Norman R. Pace. 1994. "Phylogenetic Analysis of the Hyperthermophilic Pink Filament Community in Octopus Spring, Yellowstone National Park." *Applied and Environmental Microbiology* 60: 2113–9. <https://doi.org/10.1128/aem.60.6.2113-2119.1994>
  48. White, Thomas J., Thomas Bruns, S. Lee, and John Taylor. 1990. "Amplification and Direct Sequencing of Fungal

- Ribosomal RNA Genes for Phylogenetics." *PCR Protocols: A Guide to Methods and Applications* 18: 315–22. <https://doi.org/10.1016/B978-0-12-372180-8.50042-1>
49. Mori, Hiroshi, Fumito Maruyama, Hiromi Kato, Atsushi Toyoda, Ayumi Dozono, Yoshiyuki Ohtsubo, Yuji Nagata, Asao Fujiyama, Masataka Tsuda, and Ken Kurokawa. 2014. "Design and Experimental Application of a Novel Non-degenerate Universal Primer Set That Amplifies Prokaryotic 16S rRNA Genes With a Low Possibility to Amplify Eukaryotic rRNA Genes." *DNA Research* 21: 217–27. <https://doi.org/10.1093/dnares/dst052>
  50. Bolyen, Evan, Jai Ram Rideout, Matthew R. Dillon, Nicholas A. Bokulich, Christian C. Abnet, Gabriel A. Al-Ghalith, Harriet Alexander, et al. 2019. "Reproducible, Interactive, Scalable and Extensible Microbiome Data Science Using QIIME 2." *Nature Biotechnology* 37: 852–7. <https://doi.org/10.1038/s41587-019-0209-9>
  51. Callahan, Benjamin J., Paul J. McMurdie, Michael J. Rosen, Andrew W. Han, Amy Jo A. Johnson, and Susan P. Holmes. 2016. "DADA2: High-Resolution Sample Inference From Illumina Amplicon Data." *Nature Methods* 13: 581–3. <https://doi.org/10.1038/nmeth.3869>
  52. Bokulich, Nicholas A., Benjamin D. Kaehler, Jai Ram Rideout, Matthew Dillon, Evan Bolyen, Rob Knight, Gavin A. Huttley, and J. Gregory Caporaso. 2018. "Optimizing Taxonomic Classification of Marker-gene Amplicon Sequences With QIIME 2's q2-Feature-Classifer Plugin." *Microbiome* 6: 90. <https://doi.org/10.1186/s40168-018-0470-z>
  53. Quast, Christian, Elmar Pruesse, Pelin Yilmaz, Jan Gerken, Timmy Schweer, Pablo Yarza, Jörg Peplies, and Frank Oliver Glöckner. 2012. "The SILVA Ribosomal RNA Gene Database Project: Improved Data Processing and Web-Based Tools." *Nucleic Acids Research* 41: D590–6. <https://doi.org/10.1093/nar/gks1219>
  54. Kõljalg, Urmas, R. Henrik Nilsson, Kessy Abarenkov, Leho Tedersoo, Andy F. S. Taylor, Mohammad Bahram, Scott T. Bates, et al. 2013. "Towards a Unified Paradigm for Sequence-Based Identification of Fungi." *Molecular Ecology* 22: 5271–7. <https://doi.org/10.1111/mec.12481>
  55. Pielou, Evelyn C. 1966. "The Measurement of Diversity in Different Types of Biological Collections." *Journal of Theoretical Biology* 13: 131–44. [https://doi.org/10.1016/0022-5193\(66\)90013-0](https://doi.org/10.1016/0022-5193(66)90013-0)
  56. Shannon, C. E. 1948. "A Mathematical Theory of Communication." *The Bell System Technical Journal* 27: 379–423. <https://doi.org/10.1002/j.1538-7305.1948.tb01338.x>
  57. Finlay, Bland J., Helaina I. J. Black, Susan Brown, Ken J. Clarke, Genoveva F. Esteban, Ruth M. Hindle, José L. Olmo, Alison Rollett, and Keith Vickerman. 2000. "Estimating the Growth Potential of the Soil Protozoan Community." *Protist* 151: 69–80. <https://doi.org/10.1078/1434-4610-00008>
  58. Stoeck, Thorsten, David Bass, Markus Nebel, Richard Christen, Meredith D. M. Jones, Hans-Werner Breiner, and Thomas A. Richards. 2010. "Multiple Marker Parallel Tag Environmental DNA Sequencing Reveals a Highly Complex Eukaryotic Community in Marine Anoxic Water." *Molecular Ecology* 19: 21–31. <https://doi.org/10.1111/j.1365-294X.2009.04480.x>
  59. Guillou, Laure, Dipankar Bachar, Stéphane Audic, David Bass, Cédric Berney, Lucie Bittner, Christophe Boutte, et al. 2012. "The Protist Ribosomal Reference Database (PR2): A Catalog of Unicellular Eukaryote Small Sub-unit rRNA Sequences With Curated Taxonomy." *Nucleic Acids Research* 41: D597–604. <https://doi.org/10.1093/nar/gks1160>
  60. Adl, M. S., and V. V. S. R. Gupta. 2006. "Protists in Soil Ecology and Forest Nutrient Cycling." *Canadian Journal of Forest Research* 36: 1805–17. <https://doi.org/10.1139/x06-056>
  61. Yin, Wenying. 2000. *Pictorial Keys to Soil Animals of China*. Beijing: Science Press.
  62. Singer, David, Christophe V. W. Seppey, Guillaume Lentendu, Micah Dunthorn, David Bass, Lassâad Belbahri, Quentin Blandenier, et al. 2021. "Protist Taxonomic and Functional Diversity in Soil, Freshwater and Marine Ecosystems." *Environment International* 146: 106262. <https://doi.org/10.1016/j.envint.2020.106262>
  63. Mao, Xiaofang, Huixin Li, Xiaoyun Chen, and Feng Hu. 2004. "Extraction Efficiency of Soil Nematodes by Different Methods." *Chinese Journal of Ecology* 23: 149–51. <https://doi.org/10.13292/j.100-4890.2004.0101>
  64. Maestre, Fernando T., José L. Quero, Nicholas J. Gotelli, Adrián Escudero, Victoria Ochoa, Manuel Delgado-Baquerizo, Miguel García-Gómez, et al. 2012. "Plant Species Richness and Ecosystem Multifunctionality in Global Drylands." *Science* 335: 214–8. <https://doi.org/10.1126/science.1215442>
  65. Montesinos-Navarro, Alicia, Fernando Hiraldo, José L. Tella, and Guillermo Blanco. 2017. "Network Structure Embracing Mutualism–Antagonism Continuums Increases Community Robustness." *Nature Ecology & Evolution* 1: 1661–9. <https://doi.org/10.1038/s41559-017-0320-6>
  66. Yang, Teng, Leho Tedersoo, Xu Liu, Gui-Feng Gao, Ke Dong, Jonathan M. Adams, and Haiyan Chu. 2022. "Fungi Stabilize Multi-kingdom Community in a High Elevation Timberline Ecosystem." *iMeta* 1: e49. <https://doi.org/10.1002/imt2.49>
  67. Bell, Colin W., Barbara E. Fricks, Jennifer D. Rocca, Jessica M. Steinweg, Shawna K. McMahon, and Matthew D. Wallenstein. 2013. "High-throughput Fluorometric Measurement of Potential Soil Extracellular Enzyme Activities." *Journal of Visualized Experiments* 81: e50961. <https://doi.org/10.3791/50961-v>

## SUPPORTING INFORMATION

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

**Figure S1.** Relative abundances of top 10 taxa of (A) bacteria, (B) fungi, (C) protozoa, and (D) nematode community under treatments of arable systems and restored natural areas in two seasons.

**Figure S2.** Richness (A) and evenness (B) of soil microbiota community (i.e., bacteria, fungi, protozoa, nematode, and the whole microbiota community) in arable system versus restored natural area.

**Figure S3.** The relative standardized activities of eight exoenzymes in arable system versus restored natural area in two seasons.

**Figure S4.** Correlations between the characteristics of bacterial and fungal communities with the characteristics of substrates and microfauna community after the arable system converted to a natural system.

**Table S1.** Network complexity and stability indices (means  $\pm$  SD) of soil microbiota community in arable system and restored natural area.

**Table S2.** The number of positive and negative links, keystone taxa and modules of bacteria, fungal, protozoa, nematode, and the whole microbiota community networks in an arable system versus a restored natural area.

**Table S3.** Network complexity and stability indices (means  $\pm$  SD) based on eight selected exoenzymes involved in carbon and nitrogen cycling under arable system and restored natural area in two seasons.

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