SOIL ECOLOGY LETTERS

SEL RESEARCH ARTICLE
Volume 6 / Issue 4 / 240241 / 2024
https://doi.org/10.1007/s42832-024-0241-7

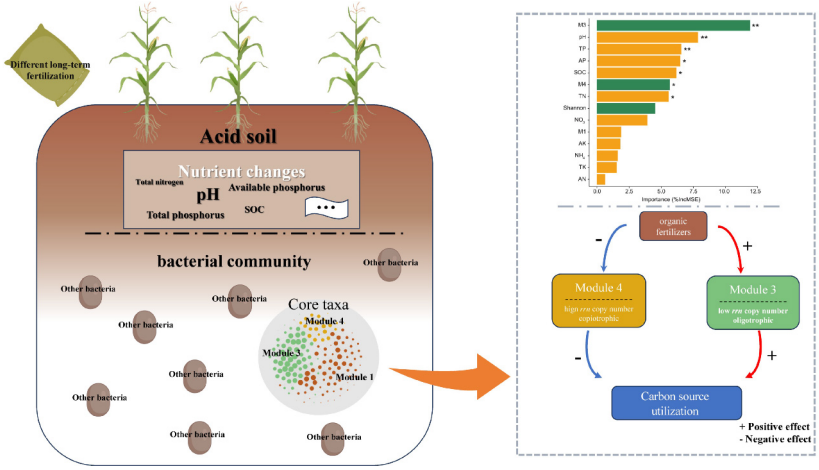
Effects of core soil microbial taxa on soil carbon source utilization under different long-term fertilization treatments in Ultisol

Haoran Mao[1,2], Guilong Li[1], Ke Leng[1], Luyuan Sun[1], Kailou Liu[3], Yongxin Lin[4], Jia Liu[1,*], Xingjia Xiang[2,*]

1 Soil and Fertilizer & Resources and Environment Institute, Jiangxi Academy of Agricultural Sciences, Nanchang 330200, China
2 Anhui Province Key Laboratory of Wetland Ecosystem Protection and Restoration, School of Resources and Environmental Engineering, Anhui University, Hefei 230601, China
3 Jiangxi Institute of Red Soil and Germplasm Resources, Nanchang 331717, China
4 Key Laboratory for Humid Subtropical Eco-geographical Processes of the Ministry of Education, School of Geographical Sciences, Fujian Normal University, Fuzhou 350007, China
* Corresponding authors. E-mail: liujia@jxaas.cn (J. Liu); xjxiang@ahu.edu.cn (X. Xiang)
Received September 7, 2023; Revised January 23, 2024; Accepted February 1, 2024

ABSTRACT

• Core taxa play an important role in regulating soil carbon metabolism.
• Ecological cluster with oligotrophic made key contributions to soil carbon metabolism.
• Microbial cluster characteristics link microorganisms to carbon metabolism.



Characterizing the ecological roles of core soil microbial species in soil carbon metabolism is critically important for enhancing carbon sequestration in agricultural systems; however, no studies to date have determined the effects of core soil microbial taxa on carbon metabolism under various long-term fertilization practices. Here, we collected soil samples from field plots that had been subjected to different fertilization practices for nearly 30 years and examined the long-term effects of fertilization on the preferences of core soil bacterial taxa for different carbon sources. We also examined the relative contribution of core soil bacterial taxa in utilization of different carbon source types in Biolog Eco microplates. Long-term fertilization treatment had a significant effect on soil properties and bacterial community structure. The core taxa were closely related to soil carbon source utilization. The co-occurrence network showed that the major ecological clusters containing core taxa made key contributions to soil carbon source utilization. The organic fertilization increased the abundance of a core cluster with a low weighted average *rrn* copy number. This ecological cluster was the most important factor affecting soil carbon source utilization even among soil physicochemical factors considered. Our findings indicate that core taxa characterized by oligotrophic bacteria have a major effect on carbon source utilization in Ultisols.

Keywords core taxa, long-term fertilization, life-history strategy, agricultural ecosystems, sequencing

1 Introduction

Soil carbon (C) pools are crucial components of the global C cycle, ensuring the sustainability of agroecosystems and serving as a key indicators of soil fertility (Lal, 2004; Stockmann et al., 2013). Concurrently, soil microorganisms are essential contributors to the complex processes of soil C metabolism (Schimel and Schaeffer, 2012; Liang et al., 2017). Research on soil microbial communities can provide valuable insights to enhance soil C sequestration and agricultural production. However, soil microbes are sensitive to various environmental variables, and several factors influence their C metabolism activity (Hartmann and Six, 2023). For example, fertilization, which is commonly used to increase the yields of crops, has a major effect on soil

microbial communities (Leff et al., 2015). Fertilization affects the ecosystem functions performed by microorganisms and their metabolic activities in various ways. The effects of fertilizer application on the properties of soil organic matter, nutrients, and pH vary depending on the type of fertilizer applied, which can have implications for microbial C metabolism in arable systems (Xiao et al., 2021; Wang et al., 2022a). Previous studies have shown that manure fertilization increases the abundance of bacterial taxa related to C metabolism, as well as net soil C gain (Abdalla et al., 2022); manure fertilization can also have more impact on soil C source metabolic activity than chemical fertilizers (Hu et al., 2022). Comparing the effects of organic and chemical fertilizers on the soil microbiome can provide useful information for optimizing soil C sequestration and cropland quality. Studying the role of fertilization in microbiota-mediated C cycling is essential for developing accurate estimates of soil C stocks and changes in nutrient dynamics in agricultural ecosystems.

The abundance and diversity of soil bacteria poses a major challenge for studies aimed at identifying the key factors driving ecosystem function (Fierer, 2017). However, an increasing number of studies have demonstrated that some specific microbial taxa have greater effects on ecosystem function than the entire community (Wertz et al., 2007; Louca et al., 2018). The composition of abundant and ubiquitous core taxa, which are key components of ecosystems, greatly contributes to shaping community structure and environmental functions (Shade and Handelsman, 2012; Delgado-Baquerizo et al., 2018). Previous studies have indicated that core taxa are strongly linked to enhanced habitat resistance, ecological versatility, and nutrient cycling (Jiao et al., 2019, 2022a, 2022b). Being part of a narrowed "most wanted" list of the entire microbial taxa is essential for understanding the relative contributions of microbial communities to ecosystem functioning (Delgado-Baquerizo et al., 2018). Recent studies have shown that microbial interactions and organize into well-established ecological clusters that contain microbes with similar ecological preferences, and these ecological clusters have a major effect on ecosystem functioning (Wang et al., 2022b; Yang et al., 2022; Fan et al., 2023). Ecological clusters comprise the core microbial taxa, which are essential for maintaining functional stability, and they explain most of the observed changes in soil organic C under a ridge-furrow with plastic mulching system (Du et al., 2023; Li et al., 2023). Thus, identification of the core microbial clusters can enhance our understanding of the roles of microbes in soil C cycling in agroecosystems and provide insights with implications for soil ecosystem conservation and management.

Ultisol is a major soil type in southern China. However, Ultisol is fragile and barren due to its strong leaching and weathering properties, which severely constrain crop productivity (Xiang et al., 2021). Thus, long-term fertilization is necessary to maintain crop yields in Ultisol areas (Zhang et al., 2014). Ultisol comprises 21% of the soil area in China, and 40% of the food produced for the Chinese population is derived from areas with Ultisols (Xiang et al., 2021). Characterizing the effects of different fertilization treatments on core taxa involved in C source utilization in Ultisols is essential for optimizing fertilizer allocation; the results of such studies can also provide insights that could be used to increase crop yields under acidic conditions and promote the sustainability of agroecosystems. However, few studies have investigated the effects of microbial taxa on soil C source utilization in acidic soils. Here, we conducted a long-term field experiment in which plots of maize grown on Ultisol were subjected to different fertilization treatments. Specifically, we evaluated (1) the importance of core bacterial taxa in regulating soil C source utilization and (2) the C source preferentially utilized by different ecological clusters. We identified core bacterial taxa from Ultisol soil samples using high-throughput sequencing technology. The utilization profiles of various C substrates on Biolog Eco microplates serves as an indicator for evaluating microbial C sources utilization. The aim of this study was to elucidate the links between bacterial communities and soil C source utilization under long-term fertilization conditions.

2 Materials and methods

2.1 Experimental site and soil sampling

The long-term fertilization experiment was initiated in 1986 at the Jiangxi Institute of Red Soil in Jinxian County, Nanchang, Jiangxi Province, China (28°37′ N, 116°26′ E). The average annual precipitation and temperature of this experimental area are 1,549 mm and 18 °C, respectively. The region experiences a typical subtropical climate. The soils in the field experiment were derived from Quaternary red clay and identified as Ultisol. The cropping system consisted of a spring and autumn maize (cv. Yedan 13) –winter fallow rotation. Spring maize was planted in early April and harvested in late July, and summer corn was planted at the end of July and harvested at the beginning of November; the winter fallow period was from the beginning of November to the beginning of April. Plots (22.22 m^2; 5.555 m × 4 m) were arranged in a completely randomized block design with three replications.

We collected eight soil samples from all the treatments, and these were divided into the following three groups based on their chemical substrates: (1) non-fertilizers (NF, i.e., CK); (2) chemical fertilizers (CF), including mineral nitrogen (N) fertilizer (N), mineral N and phosphorus (P) fertilizer (NP), mineral N and potassium (K) fertilizer (NK), mineral N,

P, and K fertilizer (NPK), and dual mineral N, P, and K fertilizer (HNPK); and (3) organic fertilizers (OF), including pig manure alone (M) and mineral N, P, and K fertilizer plus pig manure (NPKM). Urea was used for the N fertilizer, calcium-magnesium phosphate was used for the P fertilizer, and potassium chloride was used for the K fertilizer. P fertilizer, K fertilizer, and pig manure were applied as base fertilizers. Two-thirds of the N fertilizer dose was used as the base fertilizer, and one-third was used as a top dressing.

Soil samples were collected in May 2015. Four soil cores were collected from the four corners of each sample plot (0–10 cm depth) and mixed thoroughly as a composite sample. The basic chemical properties of the original soil are shown in Table 1. All soil samples were sealed and immediately transported to the laboratory on dry ice. Each soil sample was completely mixed and sieved through a 2 mm sieve. For each sample, one part was stored at −80 °C for DNA extraction, and one part was stored at 4 °C for soil physicochemical analysis.

2.2 Soil chemical properties

Soil pH was measured using a pH meter (FE20, Mettler Toledo, Switzerland) in a 1:2.5 mass/volume soil-water suspension for 30 min. Automated segmented flow analysis was used to measure the concentration of ammonium (NH_4^+) and nitrate (NO_3^-) (Bran + Luebbe AAIII, Germany). The Kjeldahl digestion-distillation procedure was used to determine the total N (TN) concentration, and the molybdenum blue colorimetric method and flame photometry were used to measure the total P (TP) and total K (TK) concentration, respectively. In addition, alkali hydrolysis was used to determine the available N (AN) concentration, the available P (AP) concentration was determined using Bray's method, and the available K (AK) concentration was determined using flame photometry. The SOC concentration was determined using the potassium dichromate oxidation method.

2.3 DNA extraction and MiSeq sequencing

DNA was extracted from fresh soil samples (0.5 g) using a FastDNA® SPIN Kit (MP Biomedicals, Santa Ana, CA). The DNA was then dissolved in 60 μL of TE buffer, and a NanoDrop ND-1 000 spectrophotometer (Thermo Scientific, USA) was used to measure the DNA concentration. The DNA was stored at –20 °C.

The F515/R907 primer was used as a template for amplifying the V4–V5 highly variable region of the bacterial 16S rRNA gene fragment. The Illumina MiSeq platform (300 paired-end sequencing) at Majorbio (Shanghai, China) was used to sequence the amplicon libraries. The original fastq files were merged using FLASH software. QIIME v.1.9.0 software was used to remove low-quality sequences, and UCLUST software was used to classify the remaining sequences into operational taxonomic units (OTUs) with 97% sequence similarity (Caporaso et al., 2010). The sequences were annotated via comparison against the SILVA v.132 database. The SILVA (available at the website arb-silva.de) database was used to assign taxonomic designations to bacterial OTU. To standardize the sequencing effort, the OTU matrix of each sample was extracted to 29 107 sequences.

2.4 Utilization of different carbon source types by soil microbial in Biolog Eco microplates

The capacity of soil microbial communities to utilize various C sources was evaluated using Biolog Eco Plates™ (Biolog Inc., USA). The Biolog Eco Plates™ contained 31 different C sources, and these could be divided into six substrate groups depending on their properties, including carbohydrates, amino acids, carboxylic acids, phenolic acids, polymers, and amines. First, fresh soil samples (5 g) were mixed with 45 mL of pre-sterilized 0.85% (w/v) sodium chloride solution, shaken vigorously at 200 rpm for 30 min, and allowed to settle for 10 min. The supernatant was diluted 10-fold and inoculated into the Biolog Eco microplates with a spiking volume of 150 μL per well. The plates were then incubated at 25°C for 10 d, and the absorbance was recorded every 24 h at 590 nm and 750 nm. The average well color developed (AWCD) was calculated by the difference between the absorbance at 590 nm and the absorbance at 750 nm. Finally, the absorbance at 120 h was used.

$$\mathrm{AWCD} = \sum (C - R)/31$$

where C is the absorbance value of each well based on the subtraction at two wavelengths, and R is the absorbance value of the control wells based on the subtraction at two wavelengths.

2.5 Statistical analyses

All statistical analyses were conducted in R Studio (version 4.2.1), and the "*ggplot2*" package was used to make figures to visualize the results. The "*picante*" package was used to calculate the α-diversity of the bacterial community. To clarify differences in bacterial β-diversity and C source utilization under different treatments, we conducted principal coordinate analysis (PCoA) and principal component analysis, respectively, based on the Bray−Curtis distance using the "*vegan*" package. ANOVA and Tukey's HSD test were used to determine the significance of differences in several variables, including soil chemistry, α-diversity, *rrn* copy number, and relative abundance, among fertilization treatments; these tests were carried out using the "TukeyHSD" function in the

Table 1 Soil chemical properties under different fertilization treatments.

Soil properties	Soil pH	SOC (g kg^{-1})	TN (g kg^{-1})	TP (g kg^{-1})	TK (g kg^{-1})	NO_3^--N (mg kg^{-1})	NH_4^+ -N (mg kg^{-1})	AN (mg kg^{-1})	AP (mg kg^{-1})	AK (mg kg^{-1})
CK	5.24 ± 0.07e	6.81 ± 0.09e	0.94 ± 0.03bc	0.60 ± 0.08ef	13.67 ± 0.17cd	4.03 ± 0.33d	0.57 ± 0.15c	71.05 ± 5.61d	15.59 ± 2.83e	96.67 ± 7.22e
N	4.45 ± 0.06i	7.59 ± 0.14d	0.94 ± 0.06bc	0.59 ± 0.01f	13.35 ± 0.18d	5.37 ± 0.69bcd	1.35 ± 0.31a	74.73 ± 2.12cd	14.93 ± 2.13e	86.67 ± 7.22e
NP	5.03 ± 0.08f	8.17 ± 0.49cd	0.90 ± 0.09bc	0.85 ± 0.11cd	14.15 ± 0.25bc	5.38 ± 0.79bcd	0.80 ± 0.14bc	86.98 ± 7.65bc	37.09 ± 7.17d	83.33 ± 18.93e
NK	4.69 ± 0.08h	8.84 ± 0.62bc	1.00 ± 0.05b	0.76 ± 0.06de	15.01 ± 0.14a	8.33 ± 0.49a	1.40 ± 0.12a	96.78 ± 13.91ab	27.53 ± 6.88de	205.00 ± 38.97c
NPK	4.89 ± 0.11fg	8.74 ± 0.27bc	0.95 ± 0.05bc	0.77 ± 0.12de	14.98 ± 0.12a	5.37 ± 1.79bcd	0.81 ± 0.04bc	85.75 ± 11.81bc	28.10 ± 9.93de	190.83 ± 8.04c
HNPK	4.82 ± 0.05gh	9.03 ± 0.39b	1.02 ± 0.07b	0.98 ± 0.03c	14.86 ± 0.33ab	4.89 ± 1.67cd	1.01 ± 0.34b	89.43 ± 9.25b	52.89 ± 4.42c	283.33 ± 26.02a
M	6.52 ± 0.06a	10.54 ± 0.44a	1.24 ± 0.06a	1.78 ± 0.12b	13.24 ± 0.37d	6.52 ± 0.37abc	0.62 ± 0.18c	99.23 ± 7.35ab	225.81 ± 11.88b	155.83 ± 9.46d
NPKM	6.12 ± 0.05b	11.19 ± 0.34a	1.22 ± 0.09a	1.94 ± 0.13a	14.92 ± 0.30a	6.92 ± 1.04ab	0.72 ± 0.04bc	107.80 ± 5.61a	253.94 ± 5.46a	250.00 ± 0.00b

The values in brackets indicate the standard deviation of the mean. Letters following brackets indicate significant differences according to one-way ANOVA followed by Tukey's HSD test ($p < 0.05$). SOC: Soil organic carbon; TN: total nitrogen; TP: total phosphorus; TK: total potassium; AN: available nitrogen; AP: available phosphorus; AK: available potassium. The values represent mean ± standard error. Different letters indicate significant differences according to one-way ANOVA followed by Tukey's HSD test ($p < 0.05$).

"*stats*" package.

To identify core taxa, we selected the most abundant taxa (>10% relative abundance) as well as taxa that were prevalent in all soil samples (Jiao et al., 2022a). The *rrn* copy number in OTUs was determined via comparison of the *rrn* sequences against the DB database (Stoddard et al., 2015). Following previous studies, the known rRNA copy number starting at the lowest level of classification was multiplied by the relative abundance of each OTU to calculate the weighted average *rrn* copy number. Due to microbial growth characteristics, copiotrophs often possess multiple *rrn* copy number to sustain rapid growth and resource acquisition, whereas oligotrophic bacteria on the contrary (Dai et al., 2022; Lin et al., 2022).

Random forests were predicted using decision trees based on bootstrap samples of the dataset. The bacterial taxa with relative abundance >0.001% as predictors of C sources utilization. Three-quarters of the entire dataset was randomly selected as the training set, and the rest of the data was used as the test set. After 999 iterations, the algorithm calculates the increase in mean squared error (%IncMSE), which is used to determine the importance of the variable; This was determined using the "*rfPermute*" package. This variable indicates the importance of the model predictors, with larger values indicating higher variable importance. We used 24 soil samples to construct a co-occurrence network of core taxa according to Spearman correlation coefficients between different OTUs using the "*igraph*" package. *p*-values were corrected using the false discovery rate. Finally, the Gephi tool was used to visualize the network using the following criteria: Spearman's $r > 0.75$ and $p_{adj} < 0.05$. The number of co-occurring nodes and modules (ecological clusters) was calculated using the Gephi tool.

3 Results

3.1 Effects of fertilization treatments on soil physicochemical properties and utilization of different types of carbon sources by microbial

Fertilization treatment had a major effect on soil physicochemical properties. Soil pH was significantly higher in the OF treatment than in the other treatments. The concentration of SOC, TP, AP, and TN was also significantly higher in the OF treatment than in the other treatments (Table 1). The C source utilization of the soil bacterial community was characterized using the community-level physiological profile method. Our results revealed that long-term fertilization had a major effect on C source utilization (Fig. S1). C source utilization in soil microorganisms was altered to a greater degree in the fertilizer application treatments than in the NF treatment, and the largest difference was observed in the OF treatment. The AWCD value was significantly higher in the OF treatment than in the NF and CF treatments ($p < 0.05$) (Fig. 1A). No significant differences in the AWCD value were observed among the NF and CF treatments. Furthermore, the degradation of the six categories of C sources among the treatments was the same as the pattern of variation in AWCD among treatments, with the highest AWCD value in OF treatment and without differences of AWCD values between the NF and CF treatments ($p < 0.05$) (Fig. 1B).

3.2 Effects of fertilization treatments on bacterial diversity and community composition

No significant differences in the bacterial richness index were observed among the different fertilization treatments; however, the bacterial Shannon index was significantly higher in the OF treatment than in the other treatments ($p < 0.05$) (Fig. 2A; Table S1). In addition, the bacterial Shannon index was significantly lower in the N treatment than in the other treatments. The PCoA revealed significant changes in the composition of the soil bacterial community following long-term fertilization (Fig. 2B). And we observed the relative abundance of bacterial communities in each treatment exhibit differences at the phylum level (Fig. 2C). The microbial communities are mainly composed of *Acidobacteria* (27.26%), *Proteobacteria* (23.68%), *Chloroflexi* (13.61%), *Bacteroidetes* (8.70%). Further analysis was performed at the number of OTUs in the phyla level between CF and OF treatments. The number of OTUs in the phyla *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* was higher and the number of OTUs in the phyla *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, and *Planctomycetes* was lower in the OF treatment than in the CF treatment (Fig. S2).

3.3 Relationships between core taxa and microbial carbon source utilization activity

A total of 163 OTUs were identified as the core taxa, and they primarily included *Proteobacteria*, *Acidobacteria*, and *Actinobacteria* (Fig. 3A). We used a random forest model to evaluate the effect of core taxa on soil C source utilization and found that the core taxa made a large contribution to soil C utilization ($p < 0.001$) (Fig. 3B).

We constructed a correlation-based co-occurrence network of the core taxa to clarify potential interactions among them. The network comprised 153 nodes and 2376 edges (Fig. 3C). We identified three major ecological clusters. Analysis of the relationship between the ecological clusters and AWCD using ordinary least squares regression revealed a positive correlation between the relative abundance of Module 3 and AWCD ($R^2 = 0.64$, $p < 0.001$); however, a

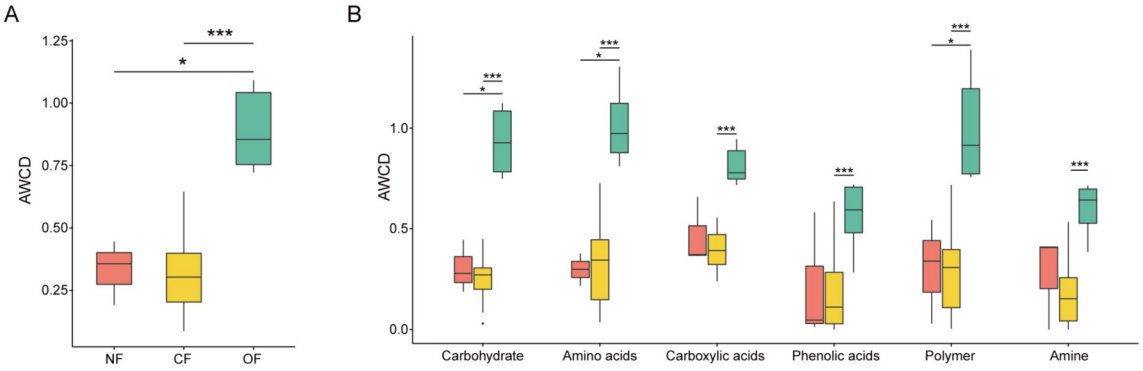


Fig. 1 Utilization of different carbon source types by soil microbial in Biolog Eco microplates (A) reflected by the average well color development (AWCD), and utilization of six categories carbon substrate (B) under different fertilization treatments.

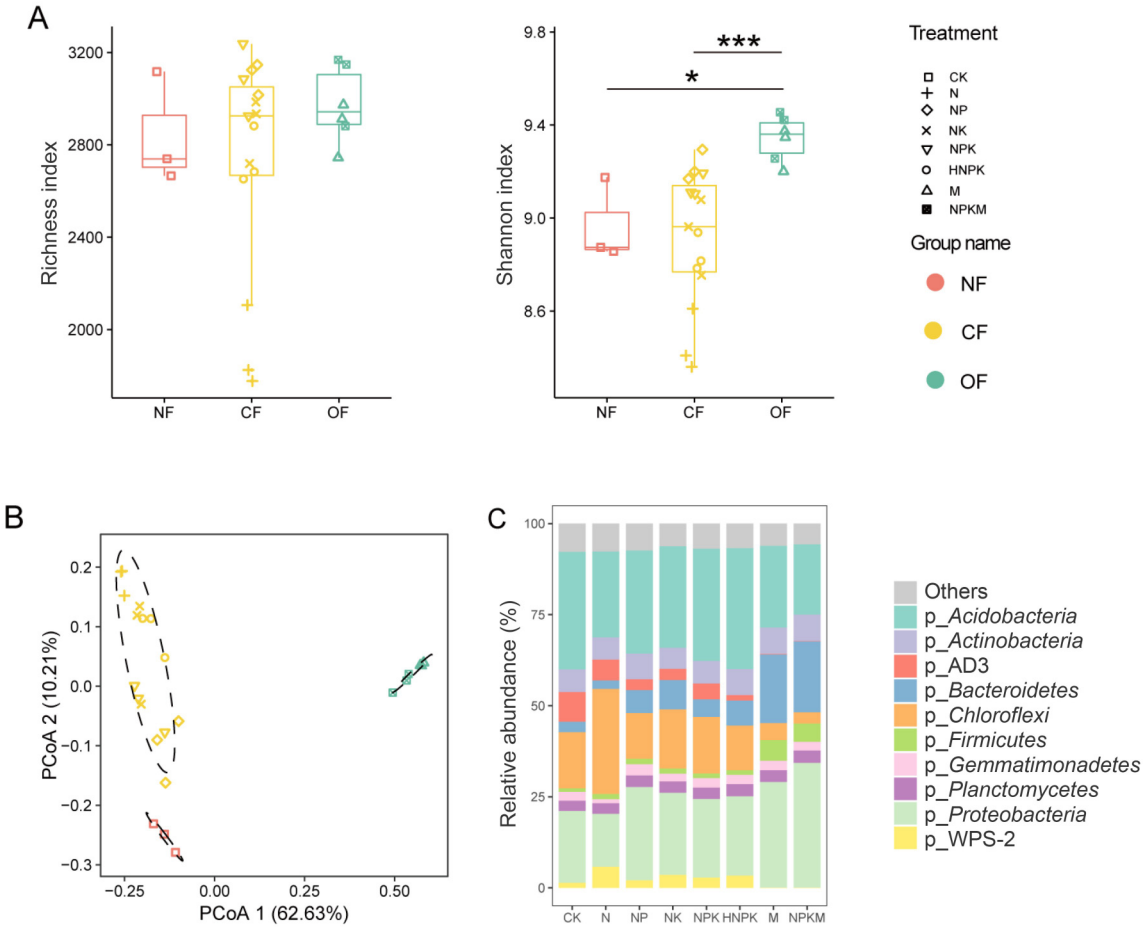


Fig. 2 Effects of fertilization treatments on bacterial community diversity and composition. (A) Effect of different fertilization treatments on the richness index and Shannon index, ***, $p < 0.001$; **, $p < 0.01$; and *, $p < 0.05$. (B) Principal coordinate analysis showing the effects of different fertilization treatments on patterns of microbial carbon source utilization. (C) Relative abundances of major bacterial phyla under different fertilization treatments.

negative correlation was observed between the abundance of Module 4 and AWCD ($R^2 = 0.37$, $p < 0.05$) (Fig. 3D).

3.4 Relationships between ecological clusters and the carbon source utilization activities of microbes

The relative abundance of Module 3 was significantly higher in the OF treatment than in the CF treatment ($p < 0.001$); the relative abundance of Module 4 was significantly lower in the OF treatment than in the CF treatment ($p < 0.001$) (Figs. 5A and 5B). We also analyzed the correlations between network ecological modules and characteristic C sources using Biolog Eco microplates. Module 3 was positively correlated with the metabolism of

phenylethylamine, L-serine, and α-cyclodextrin and negatively correlated with the metabolism of D-glucosaminic acid. The opposite correlations were observed for Module 4 (Fig. 4A and Fig. 4B). A random forest model was used to identify the key factors affecting soil C metabolism. Module 3 was the most important factor affecting AWCD, followed by key chemical factors such as pH and TP (Fig. 3E). In addition, the weighted average *rrn* copy number was significantly higher in Module 4 than in Module 3 (p < 0.001) (Fig. 5C).

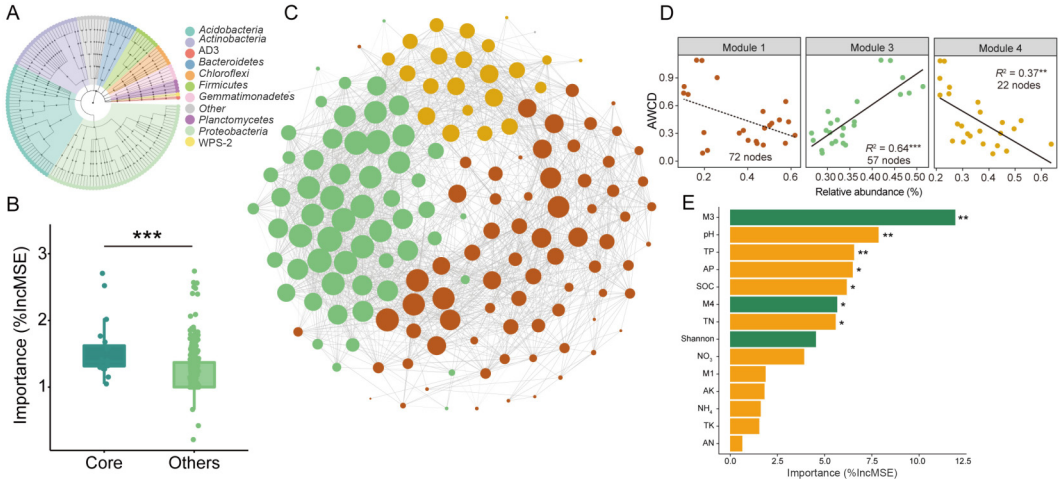


Fig. 3 Linkages between core taxa and the carbon source utilization activities of microbes. (A) Circle tree plot showing the composition of core taxa at the phylum level. (B) Predicted effects of microbial taxa on soil carbon source utilization according to random forest analysis. (C) Co-occurrence networks containing multiple ecological clusters based on the soil samples. Circles of the same color belong to the same ecological cluster, and the size of the circles is positively correlated with the relative abundances of OTUs. (D) Linear relationship between the relative abundance of major ecological clusters and AWCD. The linear relationship was analyzed using the least squares method. (E) Predicted effects of different factors on soil carbon source utilization according to random forest analysis. The increase in mean squared error (%IncMSE) of the variables was used to estimate the significance of these predictors. M3: relative abundance of taxa within ecological cluster 3; M4: relative abundance of taxa within ecological cluster 4; SOC: Soil organic carbon; TN: total nitrogen; TP: total phosphorus; TK: total potassium; AN: available nitrogen; AP: available phosphorus; AK: available potassium; NO_3, nitrate nitrogen; NH_4, ammonium nitrogen. ***, p < 0.001; **, p < 0.01; and *, p < 0.05.

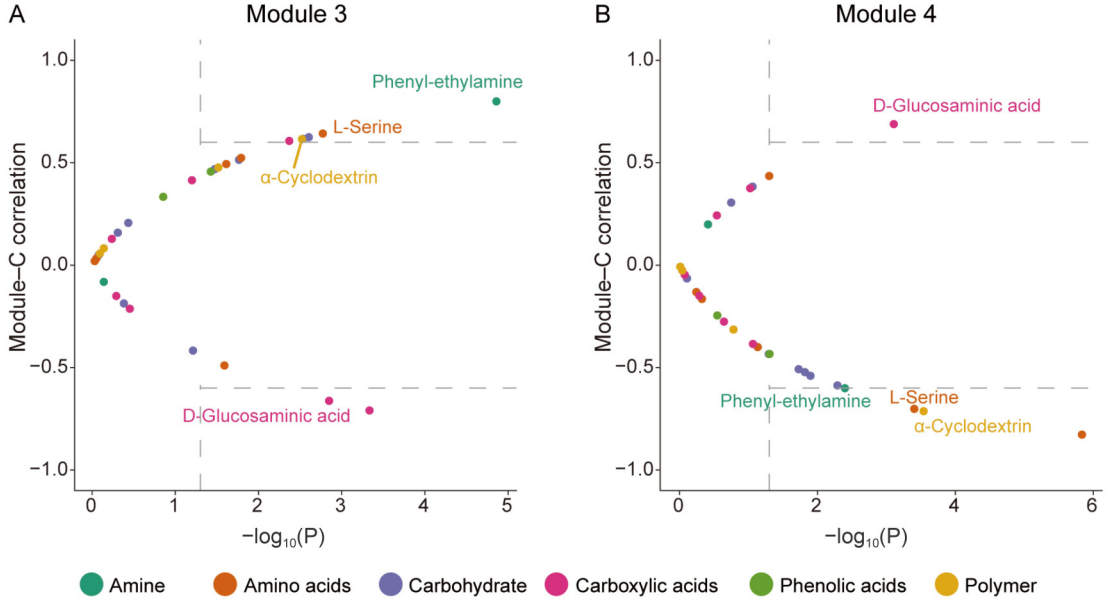


Fig. 4 Correlations between soil carbon sources and ecological clusters M3 (A) and M4 (B). Each point corresponds to a carbon source in the Biolog Eco microplates, the y-axis shows the Spearman's correlation coefficients between the ecological clusters and the characteristic C-source, and the x-axis shows the $-\log_{10}$(adjusted p -value); the significance of the differences increases towards the right-hand side of the plot.

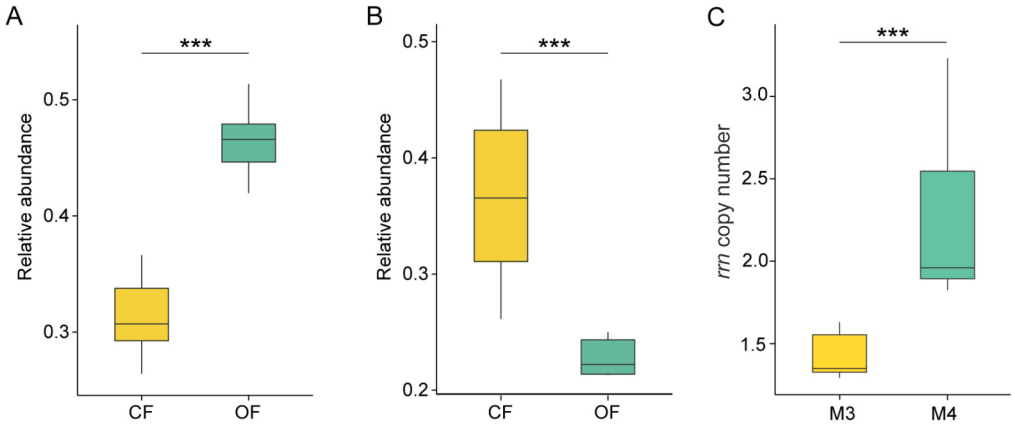


Fig. 5 Linkages between ecological clusters and carbon sources utilization. Boxplots showing the relative abundances of ecological clusters M3 (A) and M4 (B) under organic and chemical fertilizer application. (C) Boxplots indicating the *rrn* copy number of ecological clusters M3 and M4. ***, $p < 0.001$; **, $p < 0.01$; and *, $p < 0.05$.

4 Discussion

The Biolog analysis showed that organic fertilizer significantly increased the AWCD value and had a positive effect on C source utilization activity (Fig. 1). We observed different patterns of C source utilization in different fertilization treatments. Compared with inorganic fertilizers, organic fertilizers have a more complex matrix composition, and extensive research has shown that most of them have an improvement effect on soil environment such as pH (Kumar et al., 2017; Liu et al., 2018; Bian et al., 2022). The application of inorganic fertilizer accelerates the acidification of Ultisol; however, the application of organic fertilizers can increase soil pH, thereby preventing acidification (Francioli et al., 2016; Xiao et al., 2021). The pH is critically important for limiting the decomposition of C sources by microorganisms and affects metabolic activity (Sheng et al., 2016). The entry of inorganic fertilizers, especially N fertilizers, into the soil causes soil acidification (Raza et al., 2020) and can trigger heavy metal toxicity (Wang et al., 2022a), which can restrict the activity of soil microbes. Severe soil acidification can induce strong niche-based filtering and lead to a decrease in α-diversity. In addition, soil acidification reduces the availability of P (Xun et al., 2016), which is often a limiting nutrient in agroecosystems. Previous research has shown that the availability of P in soil affects soil microbial activity (He et al., 2022) and increases the microbial demand for C (Ma et al., 2023). Organic fertilizers application is superior to inorganic fertilizers for promoting increases in nutrients such as TP.

The application of exogenous fertilizers has a major effect on the structure and composition of topsoil microbial communities (Xiang et al., 2020). The results of our study indicate that the application of inorganic and organic fertilizers induces major changes in bacterial community structure (Fig. 2). This can be attributed in large part to variation in the concentration of soil nutrients and the soil environment among fertilization treatments (Cai et al., 2019). Additional investigation of the bacterial community revealed that OTUs in the phyla *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* were more abundant in the application of organic fertilizers (Fig. 2C). Both resource availability and soil pH play key roles in shaping the structure of microbial communities (Rousk et al., 2010; Wei et al., 2017). The application of organic fertilizers can enhance the fertility of soil to a greater degree than inorganic fertilizers application; this can promote a more copiotrophic soil environment, improve soil pH, and create a more favorable habitat. These factors affect the C source utilization activity and diversity of soil microbial communities.

Characterizing the effect of core taxa on C source utilization can enhance our understanding of the roles of microbial communities in soil C sequestration and agricultural development (Toju et al., 2018; Li et al., 2023). We found that core taxa had the greatest effect on soil C source utilization compared with other taxa (Fig. 3B). The abundant and ubiquitous core taxa occupy wide ecological niches and can efficiently utilize resources (Barberán et al., 2014; Jiao et al., 2022a); they also play key roles in the maintenance of multiple ecosystem functions and are closely tied to specific community functions (Philippot et al., 2010; Shade and Handelsman, 2012; Yeoh et al., 2017). Although core taxa were widely present between treatments, their bacterial biomarkers differed slightly under different fertilization treatments (Fig. S3), which may be a key factor contributing to differences in C source utilization. Therefore, we classified the core taxa and found that ecological clusters formed by core taxa with similar ecological preferences were significantly correlated with soil C source utilization. We found that the relative abundance of Module 3 and 4 was significantly affected by different fertilization treatments and that different modules had opposite C source utilization preferences. The relative abundance of Module 3, for example, was signifi-

cantly and positively correlated with the metabolism of the relatively recalcitrant C source α-cyclodextrin. These underline the effects of different fertilization on soil C source utilization, possibly through influencing patterns of community clustering (Li et al., 2023). Some critical soil functions are often performed primarily by specific soil microbials (Zhu et al., 2021). Module 3 serves as the most significant predictor of C sources utilization. The increased abundance of these taxa may affect functions related to utilization soil C sources.

We attempted to use life-history strategy to look for differences between the two ecological modules. Some current studies tend to simply classify life-history strategies based only on the relative abundance of bacterial phyla (Dai et al., 2021). Different functional groups OTUs exist under the same phyla, and a higher level of precision is needed to describe life‐history (Ho et al., 2017; Delgado-Baquerizo et al., 2018; Li et al., 2023). Empirical studies have shown that it is feasible to use *rrn* copy number to predict the growth rate of individual bacteria with higher precision than microbial phylum relative abundance (Roller et al., 2016; Dai et al., 2022). And the results further showed that the *rrn* copy number in Module 3 was lower than the *rrn* copy number in Module 4 (Fig. 5C). This implies that Module 3, the most important predictor of C sources utilization, consists mainly of oligotrophic bacteria. Whereas the presence of a large number of low *rrn* copy number OTUs in Module 3, probably from the phylum *Proteobacteria* and *Actinobacteria*, increased with organic fertilization and was significantly and positively correlated with AWCD (Fig. S3). Organic fertilization increases the Module 3/Module 4 ratio in core taxa, i.e., the Oligo/Copio ratio, to influence the positive impact of soil microorganisms on soil C source utilization. We thus speculate that this could be due to the fact that in the native Ultisol system, which is characterized by soil acidification, nutrient deficiencies, and harsh environments (Xiang et al., 2021), its selection through natural selection has filtered out some of oligotrophic bacteria that are able to utilize C sources efficiently and have well-adapted. Oligotrophic bacteria have a broad substrate utilization spectrum (Chen et al., 2021). With the input of organic fertilizers, which introduced high-quality nutrient sources and improved microbial habitats, Module 3, as part of the core taxa, can still play an active role in copiotrophic environment. In addition, recent studies have shown that the application of pig manure recalcitrant organic C and can reduce the content of labile C (Wu et al., 2020), as well as that the application of organic fertilizers leads to a microbial community in the topsoil that prefers the utilization of recalcitrant C (Liu et al., 2022). This condition could make these oligotrophic bacteria are more effective in degrading these recalcitrant C sources, significantly affecting C utilization in the soil. In contrast chemical fertilization reduces the Module 3/Module 4 ratio, and although there is a risk of soil acidification, the long-term stabilization inputs of exogenous inorganic fertilizers can still lead to a certain dominance of copiotrophic bacteria (Dai et al., 2018; Feng et al., 2022) and may affect the competitiveness of cluster 3. This results maybe in a limited contribution to AWCD increase.

It is also notable that co-occurrence network analysis does have some limitations. Thus, further experimental evidence would be required to support and verify in this study. In addition, although the effects of core bacterial clusters on C source utilization were intensively studied. However, due to limited sample availability, some other biological factors that may contribute to these processes were not included, e.g., the effects of fungi, archaea, protists, and other bioturbations, which need to be further investigated. Future research may focus more on specific microorganisms in these ecological clusters, and use cultivation experiments to provide more reliable evidence to support conclusions. This could help to find “dominant bacteria” that contribute to ecosystem function.

5 Conclusions

We examined the effects of different exogenous fertilizer inputs on soil microbial C source utilization under long-term fertilization and found that organic fertilization promoted soil C source utilization. This was likely caused by a series of changes in environmental variables, such as pH, TP, and SOC, associated with organic fertilizer application. We also found that core microorganisms had a major effect on soil C source utilization and identified microbial ecological clusters related to soil C source utilization. Module 3, in which clusters are characterized as oligotrophic bacteria, is the most important factor influencing C source utilization. Overall, our results enhance our understanding of the effects of core clusters on soil ecosystem functioning and provide new insights that could aid the development of improved fertilization strategies, as well as the sustainable management of Ultisol.

Acknowledgments

This work was supported by the National Key Research and Development Program of China (Grant No. 2021YFD1901201-05), the National Natural Science Foundation of China (Grant No. 41661052), Agricultural Projects Jiangxi Province (Grant No. JXNK202307-01), Special Program for Basic Research and Talent Training of Jiangxi Academy of Agricultural Sciences (Grant No. JXSNKYJCRC202301), the Outstanding Youth Research Project of Anhui Province (Grant No. 2022AH030015).

Data availability

The datasets for this study can be found in SRA of the NCBI under accession number PRJNA1009978.

Electronic supplementary material

Supplementary material is available in the online version of this article at https://doi.org/10.1007/s42832-024-0241-7 and is accessible for authorized users.

References

Abdalla, K., Sun, Y., Zarebanadkouki, M., Gaiser, T., Seidel, S., Pausch, J., 2022. Long-term continuous farmyard manure application increases soil carbon when combined with mineral fertilizers due to lower priming effects. Geoderma 428, 116216.

Barberán, A., Ramirez, K.S., Leff, J.W., Bradford, M.A., Wall, D.H., Fierer, N., 2014. Why are some microbes more ubiquitous than others? Predicting the habitat breadth of soil bacteria. Ecology Letters 17, 794–802.

Bian, Q., Wang, X.Y., Bao, X.G., Zhu, L.Y., Xie, Z.B., Che, Z.X., Sun, B., 2022. Exogenous substrate quality determines the dominant keystone taxa linked to carbon mineralization: evidence from a 30-year experiment. Soil Biology and Biochemistry 169, 108683.

Cai, A.D., Xu, M.G., Wang, B.R., Zhang, W.J., Liang, G.P., Hou, E. Q., Luo, Y.Q., 2019. Manure acts as a better fertilizer for increasing crop yields than synthetic fertilizer does by improving soil fertility. Soil and Tillage Research 189, 168–175.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7, 335–336.

Chen, Y.J., Neilson, J.W., Kushwaha, P., Maier, R.M., Barberán, A., 2021. Life-history strategies of soil microbial communities in an arid ecosystem. The ISME Journal 15, 649–657.

Dai, T.J., Wen, D.H., Bates, C.T., Wu, L.W., Guo, X., Liu, S., Su, Y. F., Lei, J.S., Zhou, J.Z., Yang, Y.F., 2022. Nutrient supply controls the linkage between species abundance and ecological interactions in marine bacterial communities. Nature Communications 13, 175.

Dai, Z.M., Su, W.Q., Chen, H.H., Barberán, A., Zhao, H.C., Yu, M.J., Yu, L., Brookes, P.C., Schadt, C.W., Chang, S.X., Xu, J.M., 2018. Long-term nitrogen fertilization decreases bacterial diversity and favors the growth of *Actinobacteria* and *Proteobacteria* in agroecosystems across the globe. Global Change Biology 24, 3452–3461.

Dai, Z.M., Zang, H.D., Chen, J., Fu, Y.Y., Wang, X.H., Liu, H.T., Shen, C.C., Wang, J.J., Kuzyakov, Y., Becker, J.N., Hemp, A., Barberán, A., Gunina, A., Chen, H.H., Luo, Y., Xu, J.M., 2021. Metagenomic insights into soil microbial communities involved in carbon cycling along an elevation climosequences. Environmental Microbiology 23, 4631–4645.

Delgado-Baquerizo, M., Oliverio, A.M., Brewer, T.E., Benavent-González, A., Eldridge, D.J., Bardgett, R.D., Maestre, F.T., Singh, B.K., Fierer, N., 2018. A global atlas of the dominant bacteria found in soil. Science 359, 320–325.

Du, S., Li, X.Q., Feng, J., Huang, Q.Y., Liu, Y.R., 2023. Soil core microbiota drive community resistance to mercury stress and maintain functional stability. Science of the Total Environment 894, 165056.

Fan, K.K., Chu, H.Y., Eldridge, D.J., Gaitan, J.J., Liu, Y.R., Sokoya, B., Wang, J.T., Hu, H.W., He, J.Z., Sun, W., Cui, H.Y., Alfaro, F. D., Abades, S., Bastida, F., Díaz-López, M., Bamigboye, A.R., Berdugo, M., Blanco-Pastor, J.L., Grebenc, T., Duran, J., Illán, J. G., Makhalanyane, T.P., Mukherjee, A., Nahberger, T.U., Peñaloza-Bojacá, G.F., Plaza, C., Verma, J.P., Rey, A., Rodríguez, A., Siebe, C., Teixido, A.L., Trivedi, P., Wang, L., Wang, J.Y., Yang, T.X., Zhou, X.Q., Zhou, X.B., Zaady, E., Tedersoo, L., Delgado-Baquerizo, M., 2023. Soil biodiversity supports the delivery of multiple ecosystem functions in urban greenspaces. Nature Ecology & Evolution 7, 113–126.

Feng, Y.Z., Delgado-Baquerizo, M., Zhu, Y.G., Han, X.Z., Han, X.R., Xin, X.L., Li, W., Guo, Z.B., Dang, T.H., Li, C.H., Zhu, B., Cai, Z. J., Li, D.M., Zhang, J.B., 2022. Responses of soil bacterial diversity to fertilization are driven by local environmental context across China. Engineering 12, 164–170.

Fierer, N., 2017. Embracing the unknown: disentangling the complexities of the soil microbiome. Nature Reviews Microbiology 15, 579–590.

Francioli, D., Schulz, E., Lentendu, G., Wubet, T., Buscot, F., Reitz, T., 2016. Mineral vs. organic amendments: microbial community structure, activity and abundance of agriculturally relevant microbes are driven by long-term fertilization strategies. Frontiers in Microbiology 7, 1446.

Hartmann, M., Six, J., 2023. Soil structure and microbiome functions in agroecosystems. Nature Reviews Earth & Environment 4, 4–18.

He, W., Ye, W.H., Sun, M.J., Li, Y.P., Chen, M.M., Wei, M., Hu, G. Q., Yang, Q.G., Pan, H., Lou, Y.H., Wang, H., Zhuge, Y.P., 2022. Soil phosphorus availability and stoichiometry determine microbial activity and functional diversity of fluvo-aquic soils under long-term fertilization regimes. Journal of Soils and Sediments 22, 1214–1227.

Ho, A., Di Lonardo, D.P., Bodelier, P.L.E., 2017. Revisiting life strategy concepts in environmental microbial ecology. FEMS Microbiology Ecology 93, fix006.

Hu, X.J., Gu, H.D., Liu, J.J., Wei, D., Zhu, P., Cui, X.A., Zhou, B.K., Chen, X.L., Jin, J., Liu, X.B., Wang, G.H., 2022. Metagenomics reveals divergent functional profiles of soil carbon and nitrogen cycling under long-term addition of chemical and organic fertilizers in the black soil region. Geoderma 418, 115846.

Jiao, S., Chen, W.M., Wei, G.H., 2022a. Core microbiota drive functional stability of soil microbiome in reforestation ecosystems. Global Change Biology 28, 1038–1047.

Jiao, S., Qi, J.J., Jin, C.J., Liu, Y., Wang, Y., Pan, H.B., Chen, S., Liang, C.L., Peng, Z.H., Chen, B.B., Qian, X., Wei, G.H., 2022b. Core phylotypes enhance the resistance of soil microbiome to environmental changes to maintain multifunctionality in agricultural ecosystems. Global Change Biology 28, 6653–6664.

Jiao, S., Xu, Y.Q., Zhang, J., Hao, X., Lu, Y.H., 2019. Core microbiota

in agricultural soils and their potential associations with nutrient cycling. mSystems 4, e00313–18.

Kumar, U., Shahid, M., Tripathi, R., Mohanty, S., Kumar, A., Bhattacharyya, P., Lal, B., Gautam, P., Raja, R., Panda, B.B., Jambhulkar, N.N., Shukla, A.K., Nayak, A.K., 2017. Variation of functional diversity of soil microbial community in sub-humid tropical rice-rice cropping system under long-term organic and inorganic fertilization. Ecological Indicators 73, 536–543.

Lal, R., 2004. Soil carbon sequestration impacts on global climate change and food security. Science 304, 1623–1627.

Leff, J.W., Jones, S.E., Prober, S.M., Barberán, A., Borer, E.T., Firn, J.L., Harpole, W.S., Hobbie, S.E., Hofmockel, K.S., Knops, J.M. H., McCulley, R.L., La Pierre, K., Risch, A.C., Seabloom, E.W., Schütz, M., Steenbock, C., Stevens, C.J., Fierer, N., 2015. Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. Proceedings of the National Academy of Sciences of the United States of America 112, 10967–10972.

Li, Y.Z., Hou, Y.T., Hou, Q.M., Long, M., Yang, Y.L., Wang, Z.T., Liao, Y.C., 2023. Long-term plastic mulching decreases rhizoplane soil carbon sequestration by decreasing microbial anabolism. Science of the Total Environment 868, 161713.

Liang, C., Schimel, J.P., Jastrow, J.D., 2017. The importance of anabolism in microbial control over soil carbon storage. Nature Microbiology 2, 17105.

Lin, Q., De Vrieze, J., Fang, X.Y., Li, L.J., Li, X.Z., 2022. Microbial life strategy with high rRNA operon copy number facilitates the energy and nutrient flux in anaerobic digestion. Water Research 226, 119307.

Liu, J., Liu, M., Wu, M., Jiang, C.Y., Chen, X.F., Cai, Z.J., Wang, B. R., Zhang, J., Zhang, T.L., Li, Z.P., 2018. Soil pH rather than nutrients drive changes in microbial community following long-term fertilization in acidic Ultisols of southern China. Journal of Soils and Sediments 18, 1853–1864.

Liu, X.P., Wang, H.T., Wu, Y.J., Bi, Q.F., Ding, K., Lin, X.Y., 2022. Manure application effects on subsoils: abundant taxa initiate the diversity reduction of rare bacteria and community functional alterations. Soil Biology and Biochemistry 174, 108816.

Louca, S., Polz, M.F., Mazel, F., Albright, M.B.N., Huber, J.A., O' Connor, M.I., Ackermann, M., Hahn, A.S., Srivastava, D.S., Crowe, S.A., Doebeli, M., Parfrey, L.W., 2018. Function and functional redundancy in microbial systems. Nature Ecology & Evolution 2, 936–943.

Ma, T., Gao, W.J., Shi, B.W., Yang, Z.Y., Li, Y.F., Zhu, J.X., He, J. S., 2023. Effects of short- and long-term nutrient addition on microbial carbon use efficiency and carbon accumulation efficiency in the Tibetan alpine grassland. Soil and Tillage Research 229, 105657.

Philippot, L., Andersson, S.G.E., Battin, T.J., Prosser, J.I., Schimel, J.P., Whitman, W.B., Hallin, S., 2010. The ecological coherence of high bacterial taxonomic ranks. Nature Reviews Microbiology 8, 523–529.

Raza, S., Miao, N., Wang, P.Z., Ju, X.T., Chen, Z.J., Zhou, J.B., Kuzyakov, Y., 2020. Dramatic loss of inorganic carbon by nitrogen-induced soil acidification in Chinese croplands. Global Change Biology 26, 3738–3751.

Roller, B.R.K., Stoddard, S.F., Schmidt, T.M., 2016. Exploiting rRNA operon copy number to investigate bacterial reproductive strategies. Nature Microbiology 1, 16160.

Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R., Fierer, N., 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. The ISME Journal 4, 1340–1351.

Schimel, J.P., Schaeffer, S.M., 2012. Microbial control over carbon cycling in soil. Frontiers in Microbiology 3, 348.

Shade, A., Handelsman, J., 2012. Beyond the Venn diagram: the hunt for a core microbiome. Environmental Microbiology 14, 4–12.

Sheng, Y.Q., Zhan, Y., Zhu, L.Z., 2016. Reduced carbon sequestration potential of biochar in acidic soil. Science of the Total Environment 572, 129–137.

Stockmann, U., Adams, M.A., Crawford, J.W., Field, D.J., Henakaarchchi, N., Jenkins, M., Minasny, B., McBratney, A.B., de Remy de Courcelles, V., Singh, K., Wheeler, I., Abbott, L., Angers, D.A., Baldock, J., Bird, M., Brookes, P.C., Chenu, C., Jastrow, J.D., Lal, R., Lehmann, J., O'Donnell, A.G., Parton, W. J., Whitehead, D., Zimmermann, M., 2013. The knowns, known unknowns and unknowns of sequestration of soil organic carbon. Agriculture, Ecosystems & Environment 164, 80–99.

Stoddard, S.F., Smith, B.J., Hein, R., Roller, B.R.K., Schmidt, T.M., 2015. *rrn*DB: improved tools for interpreting rRNA gene abundance in bacteria and archaea and a new foundation for future development. Nucleic Acids Research 43, D593–D598.

Toju, H., Peay, K.G., Yamamichi, M., Narisawa, K., Hiruma, K., Naito, K., Fukuda, S., Ushio, M., Nakaoka, S., Onoda, Y., Yoshida, K., Schlaeppi, K., Bai, Y., Sugiura, R., Ichihashi, Y., Minamisawa, K., Kiers, E.T., 2018. Core microbiomes for sustainable agroecosystems. Nature Plants 4, 247–257.

Wang, J.L., Liu, K.L., Zhao, X.Q., Gao, G.F., Wu, Y.H., Shen, R.F., 2022a. Microbial keystone taxa drive crop productivity through shifting aboveground-belowground mineral element flows. Science of the Total Environment 811, 152342.

Wang, Y.F., Chen, P., Wang, F.H., Han, W.X., Qiao, M., Dong, W.X., Hu, C.S., Zhu, D., Chu, H.Y., Zhu, Y.G., 2022b. The ecological clusters of soil organisms drive the ecosystem multifunctionality under long-term fertilization. Environment International 161, 107133.

Wei, M., Hu, G.Q., Wang, H., Bai, E., Lou, Y.H., Zhang, A.J., Zhuge, Y.P., 2017. 35 years of manure and chemical fertilizer application alters soil microbial community composition in a Fluvo-aquic soil in Northern China. European Journal of Soil Biology 82, 27–34.

Wertz, S., Degrange, V., Prosser, J.I., Poly, F., Commeaux, C., Guillaumaud, N., Le Roux, X., 2007. Decline of soil microbial diversity does not influence the resistance and resilience of key soil microbial functional groups following a model disturbance. Environmental Microbiology 9, 2211–2219.

Wu, Z.X., Li, H.H., Liu, Q.L., Ye, C.Y., Yu, F.X., 2020. Application of bio-organic fertilizer, not biochar, in degraded red soil improves soil nutrients and plant growth. Rhizosphere 16, 100264.

Xiang, X.J., Adams, J.M., Qiu, C.F., Qin, W.J., Chen, J.R., Jin, L.L., Xu, C.X., Liu, J., 2021. Nutrient improvement and soil acidification inducing contrary effects on bacterial community structure follow-

ing application of hairy vetch (*Vicia villosa* Roth L.) in Ultisol. Agriculture, Ecosystems & Environment 312, 107348.

Xiang, X.J., Liu, J., Zhang, J., Li, D.M., Xu, C.X., Kuzyakov, Y., 2020. Divergence in fungal abundance and community structure between soils under long-term mineral and organic fertilization. Soil and Tillage Research 196, 104491.

Xiao, Q., Huang, Y.P., Wu, L., Tian, Y.F., Wang, Q.Q., Wang, B.R., Xu, M.G., Zhang, W.J., 2021. Long-term manuring increases microbial carbon use efficiency and mitigates priming effect via alleviated soil acidification and resource limitation. Biology and Fertility of Soils 57, 925–934.

Xun, W.B., Zhao, J., Xue, C., Zhang, G.S., Ran, W., Wang, B.R., Shen, Q.R., Zhang, R.F., 2016. Significant alteration of soil bacterial communities and organic carbon decomposition by different long-term fertilization management conditions of extremely low-productivity arable soil in South China. Environmental Microbiology 18, 1907–1917.

Yang, G.W., Ryo, M., Roy, J., Lammel, D.R., Ballhausen, M.B., Jing, X., Zhu, X.F., Rillig, M.C., 2022. Multiple anthropogenic pressures eliminate the effects of soil microbial diversity on ecosystem functions in experimental microcosms. Nature Communications 13, 4260.

Yeoh, Y.K., Dennis, P.G., Paungfoo-Lonhienne, C., Weber, L., Brackin, R., Ragan, M.A., Schmidt, S., Hugenholtz, P., 2017. Evolutionary conservation of a core root microbiome across plant phyla along a tropical soil chronosequence. Nature Communications 8, 215.

Zhang, X.B., Wu, L.H., Sun, N., Ding, X.S., Li, J.W., Wang, B.R., Li, D.C., 2014. Soil CO_2 and N_2O emissions in maize growing season under different fertilizer regimes in an upland red soil region of South China. Journal of Integrative Agriculture 13, 604–614.

Zhu, D., Delgado-Baquerizo, M., Su, J.Q., Ding, J., Li, H., Gillings, M.R., Penuelas, J., Zhu, Y.G., 2021. Deciphering potential roles of earthworms in mitigation of antibiotic resistance in the soils from diverse ecosystems. Environmental Science & Technology 55, 7445–7455.