

Effects of zokor mounds on the stability of soil micro-foodweb in lightly disturbed alpine meadows on the Qinghai-Tibet Plateau

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Research Article

Keywords: Qinghai-Tibet Plateau, Zokor mound, Soil micro-foodweb, Nematode metabolic footprint, Diversity index, Decomposition pathway

Posted Date: December 6th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-3495424/v1>

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Abstract

The plateau zokor (*Myospalax baileyi*) is a subterranean rodent endemic to the alpine meadow of the Qinghai-Tibetan Plateau. Zokor mound building changes plant communities and soil conditions. In our study, the soil microorganisms and nematode communities, and soil physicochemical properties in the zokor mounds with dominant plant groups *Potentilla anserina* (PM), *Leontopodium leontopodioides* (LM) and naked new zokor mound (NM) were investigated in the eastern Qinghai-Tibet Plateau. The Chao1 index and Shannon diversity index of bacteria and nematodes in PM were higher than those in other treatments. At the same time, the Chao1 index of fungi in PM was also higher. However, fungal Shannon diversity index, total nematode metabolic footprint, bacterivorous nematode metabolic footprint and omnivorous predatory nematode metabolic footprint were higher in NM. The metabolic footprint of plant-parasitic nematodes in LM was higher than that of other treatments. The functional metabolic footprint, enrichment index and structural index of soil nematodes all showed the order of NM > CK > PM > LM, indicating that the soil micro-foodweb structure of NM is relatively stable. Energy flow analysis showed that bacterial and fungal energy flow channels were dominant in PM, while plant energy flow channels had the highest proportion in LM, indicating that soil food web energy conversion and utilization efficiency in PM was higher. In addition, we found that the NM soil micro-foodweb was dominated by fungal decomposition, while PM was dominated by bacterial decomposition. Furthermore, bottom-up effects of nutrients in zokor mounds of different vegetation types determine the structure and activity of these pathways.

1. Introduction

The alpine meadow on the Qinghai-Tibet Plateau is a highly complex and persistent ecosystem, providing essential food sources and habitats for herbivores and serving as an ideal site for studying the relationship between herbivore disturbance, plant community structure, and ecosystem function. Herbivores directly impact grassland vegetation through activities such as foraging, excretion, and trampling, which, in turn, affect the characteristics of biological communities and material cycling processes. In the past few decades, severe rodent disasters have occurred in alpine meadows due to climate change and human disturbance. Grassland degradation is a significant cause of rodent infestation, and rodent infestation is the primary driving force behind accelerated grassland degradation, serving as a key indicator of the degree of grassland degradation (Akiyama and Kawamura 2007, Li et al. 2009b). The plateau zokor (*Myospalax baileyi*) is the predominant herbivorous subterranean rodent in alpine meadows. Its unique underground habitat habits have led to the creation of a complex underground tunnel system, which plays a crucial role in maintaining biodiversity, energy flow, and material circulation within alpine grassland ecosystems. As a result, the plateau zokor is often referred to as the "ecosystem engineer" of the alpine grassland (Zhang 2007, Kang et al. 2022, Zhang and Liu 2003). Plateau zokors mainly feed on forage rhizomes, and their excavation activities bring fresh soil from underground to the surface of the grassland. This process results in the formation of mounds of varying sizes and triggers different secondary succession processes, leading to changes in both vertical and horizontal soil structures. Consequently, these changes affect the material cycle rate of the ecosystem

and the characteristics of the biological community. Each plateau zokor can move thousands of times its body weight in soil to the surface every year, and their digging behavior directly results in bare grassland patches and vegetation death (Naichang and Quanye 2011).

Soil microorganisms and nematodes are the two most abundant types of organisms in the soil, occupying key positions in the food web, participating in important soil ecological processes such as organic matter decomposition and nutrient transformation, and are the most active parts of the soil ecosystem (Wall and Moore 1999, Yeates 1979). Zokor activities, including excavation and excretion, alter soil's physical and chemical properties and vegetation distribution. Consequently, these activities significantly influence the soil microorganisms and nematode communities due to their vulnerability to soil factors like moisture (Bakonyi et al. 2007), nutrient content (Bjørnlund et al. 2012), pH regulation (Kitagami, Tanikawa and Matsuda 2020). At present, the primary focus of research on alpine meadows in the Qinghai-Tibet Plateau centers around soil physical and chemical properties, as well as vegetation community structure (Tang et al. 2015, Li et al. 2023, Yang et al. 2023). However, literature pertaining to soil nematodes is relatively scarce, with most known studies relying on traditional research methods-morphological identification (Chu et al. 2020, Li et al. 2009a). Additionally, there exists a significant gap in comprehensive research concerning the interaction between soil nematodes and microbial communities in alpine meadows, thus limiting our understanding of the changes within the soil micro-foodweb in this ecosystem. The soil micro-foodweb, as a component of the detrital food web, plays a crucial role in soil ecological processes by participating directly or indirectly in nutrient cycling through resource substrate consumption, consequently influencing the functions of terrestrial ecosystems (Chu et al. 2020). Comprising soil microorganisms, small soil animals, and their predators, changes in the structure of the soil micro-foodweb can indicate alterations in the energy channels of organic matter, while also influencing the growth of aboveground plants through certain feedback mechanisms (de Sosa et al. 2018). Consequently, an in-depth investigation of the structure, composition, and role of the soil micro-foodweb in the soil ecosystem bears significant scientific importance, contributing to a better understanding of underground ecological processes involving soil organisms, and unveiling insights into soil health, quality, and sustainability.

Some studies have shown that zokor disturbance may increase the abundance of soil microorganisms and nematodes (Ye et al. 2023, Su et al. 2020); other studies have shown that zokor disturbance may reduce the abundance of soil microorganisms and nematodes (Hu et al. 2017, Zhang et al. 2023). The impact of zokor disturbance on soil microbial and nematode communities can vary significantly depending on soil type, climatic conditions, and vegetation type (Renčo, Gömöryová and Čerevková 2020). Yang et al. (Yang et al. 2017) observed a typical plant community structure during the vegetation succession process of zokor mounds, with dominant species like *Elsholtzia densa*, *Potentilla anserina*, and *Leontopodium leontopodioides*. Furthermore, due to variations in the formation time of zokor mounds, even those within the same space-time range undergo different stages of plant community succession, resulting in diverse plant community structures and dominant populations. This study hypothesizes that typical vegetation types on zokor mounds in an alpine meadow have significant effects on soil microbial and nematode diversity, community structure, and metabolic footprint. To verify this

hypothesis, zokor mounds with different vegetation types after zokor disturbance were selected as the research objects, and the response of soil nematodes to zokor disturbance was explored. Additionally, the characteristics of the soil microbial community were considered in analyzing the soil quality of the alpine meadow on the Qinghai-Tibet Plateau from the perspective of the soil micro-foodweb. This study introduces new biological indicators for evaluating the ecological effects of alpine meadows on the Qinghai-Tibet Plateau and offers a theoretical reference for the sustainable utilization of these meadows.

2. Materials and methods

2.1. Experimental design

The experimental plots were located in an alpine meadow ecosystem in Zuoni County, southern Gansu Province, Northwest China (103°03'32.68" E, 34°51'42.87" N) (Fig. 1). The local climate is characterized as continental alpine, with cold and wet conditions. The mean annual temperature is 2.3°C, and the average annual precipitation is approximately 640mm, with 80% occurring from May to August. The annual mean sunshine time and frost-free season last for around 1800–2600 hours and approximately 105 days, respectively. The soil type is meadow soil, and the main vegetation type is alpine meadow dominated by *Kobresia pygmaea*. The main associated species include *Elymus nutans*, *Poa pratensis*, *Anemone rivularis*, *Potentilla anserina*, *Potentilla fragarioides*, *Saussurea japonica*, etc.

In August 2022, we conducted a random selection of alpine meadows with relatively flat and consistent terrain that experienced fencing in warm seasons and grazing in cold seasons as the survey area. Within this survey area, we identified three types of zokor mounds based on the dominant plant groups: *Potentilla anserina* (PM), *Leontopodium leontopodioides* (LM) and naked new zokor mound (NM). We selected four zokor mounds from each type to form four repetitions. Additionally, following the principle of proximity, we selected a control sample circle (CK) in undisturbed grassland within the same area where zokor activity was absent. All selected zokor mound colonies were of similar size to CK (approximately 50 cm in diameter).

2.2. soil sampling

In August 2022, five 0–20 cm soil cores (2 cm in diameter) were collected and completely mixed into one composite sample at each selected zokor mounds and control. The total number of soil samples collected was 4 treatments × 4 replicate sites = 16. The mixed soils were then sieved through 2 mm and any visible plant materials were removed. The sieved soil samples were then divided into three parts. One part stored at 4°C for determination of soil water content (SW), microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN). The second part stored at -80°C for DNA extraction. The remaining soil was used for physicochemical analyses.

2.3. Soil properties

Soil water content (SW) was measured by drying method (105°C, 24h). Soil pH was measured in a 1:2.5 (v/v) soil:water suspension with a digital pH meter (Mettler Toledo, Switzerland). DDSJ-308F conductivity

meter is used to measure soil conductivity (soil:water is 1:5). SOC was determined using the dichromate oxidation method (Yeomans and Bremner 1988). The contents of soil microbial biomass carbon (MBC) (Vance, Brookes and Jenkinson 1987) and microbial biomass nitrogen (MBN) (Brookes et al. 1985) were determined by chloroform fumigation extraction method, and the concentrations of MBC and MBN in soil extracts were analyzed using a TOC total organic carbon/total nitrogen analyzer (Multi C/N 2100S, Analytik, Jena, Germany). Soil total nitrogen (TN) was analyzed by the Kjeldahl method (Bremner 1960). Soil total P (TP) was determined by the H₂SO₄–HClO₄ fusion method (Bender and Wood 2000). Available phosphorus (AP) was extracted with 0.5 M sodium bicarbonate and measured by molybdenum blue colorimetry (Olsen 1954). Available potassium (AK) was extracted by 1M ammonium acetate and determined by flame emission spectrophotometry (Eckert and Watson 1996).

2.4. DNA extraction, PCR amplification and sequencing

Total soil microbial DNA was extracted from 0.5 g soil by using an Ultraclean™ Soil DNA Isolation Kit (Mobile Biometry, USA). DNA quality was measured with a Nanodrop® ND-2000 UV-Vis spectrophotometer (Thermo Fisher Scientific, Schwerte, Germany). The V4-V5 region of the bacterial 16S rRNA gene was amplified with the primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') (Zeng and An 2021). The ITS region of the fungi was amplified with the primers TS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3') (Martin and Rygielwicz 2005). For nematodes, PCR reactions used the primers NF1 (5'-GGTGGTGCATGGCCGTTCTTAGTT-3') and 18S r2bR (5'-TACAAAGGGCAGGGACGTAAT-3') to amplify the V4 region of 18S rRNA gene (Kenmotsu et al. 2021). Polymerase Chain Reaction (PCR) analysis was performed in triplicate according to the following thermal program: pre-denaturation at 95°C for 5 min, denaturation at 95°C for 30 s, annealing at 50°C for 30 s, extension at 72°C for 40 s, and final extension at 72°C for 7 min, 25 cycles. PCR products were subjected to electrophoresis on 1.8% agarose gels, further purified using a Monarch DNA Gel Extraction Kit and quantified using NanoDrop Spectrophotometer. The purified amplicons were paired-end sequenced with Illumina Novaseq6000 at the Biomarker Company (Beijing, China).

2.5. Data analysis

2.5.1 Soil nematode community analysis

The c-p scale is assigned based on the r and K characteristics of soil nematodes, which are categorized into four trophic groups: plant parasites (PP), bacterivores (BF), fungivores (FF), and omnivores-predators (OP). Nematodes with low nutrient levels have a c-p value of 1–2, while those with high nutrient levels have a c-p value of 3–5 (Ferris 2010). The nematode metabolic footprint (NMF) is calculated using the biomass (fresh weight, W) of each nematode group listed in the "Nematode-Plant Expert Information System" (<http://plpnemweb.ucdavis.edu/nemaplex>). The NMF is determined by the equation: $NMF = \sum (N_t \times (0.1 \times (W_t \div m_t) + 0.273(W_t^{0.75})))$, where N_t represents the abundance of t-type nematode groups, while W_t and m_t represent soil nematode weight and c-p values, respectively. The NMF is further

categorized into bacterivores metabolic footprint (BFMF), fungivores metabolic footprint (FFMF), plant parasites metabolic footprint (PPMF), and omnivores-predators metabolic footprints (OPMF). These footprints represent the carbon and energy input into the food web from bacteria, fungi, and plants, respectively. The OPMF reflects the input of carbon and energy by omnivorous predatory nematodes at higher trophic levels. To measure the overall metabolic footprint of a nematode population, the total nematode metabolic footprint (TNMF) is calculated. The NMF is also divided into enrichment footprints and structural footprints based on the nematode's life history strategy. The enrichment footprint (Fe) indicates the metabolic response of nematodes to resource enrichment, while the structure footprint (Fs) represents the metabolic contribution of nematode taxa at higher trophic levels. The functional metabolic footprint (FMF) is expressed as the area occupied by the enrichment footprint and the structural footprint, given by the formula $(Fs \times Fe)/2$. The FMF assesses the performance of the food web in regulating and maintaining its metabolic equilibrium (Ferris 2010).

The energy flow analysis of the soil food web follows the method of Ferris et al (Ferris 2010). The vertex of the triangle is located at coordinate point (50, 86.6), while the lower left corner is at (0, 0), and the lower right corner is at (100, 0). Utilizing geometric knowledge, we calculated the coordinate points determining the center of the triangle and the midpoints of its three sides. For each treatment, seven coordinate points were derived from the relative metabolic footprints of bacterivorous, fungivorous, and plant-parasitic nematodes.

2.5.2 Statistical analysis

We conducted a one-way analysis of variance (ANOVA) to assess the impact of zokor mounds with different vegetation types on soil physicochemical properties, microorganism, and nematode communities. If significant differences were found, we used the least significant difference (LSD) test ($p < 0.05$) to compare the means of all soil parameters. Soil properties, microorganism, and nematode community charts and figures were generated using SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA) and Origin 2021, respectively. Canoco 5.0 software was used to determine the interactions between soil physicochemical properties, microorganism and nematode communities. For relevant ecological network and structural equation modeling (SEM) analyses, we utilized the "igraph" package and "piecewise SEM" package in R software, respectively. All data were processed using Excel 2010.

3. Results

3.1 Environmental factors

The soil physical and chemical properties exhibited varying degrees of variation among the treatments, including NM, PM, LM, and CK (Table 1). Specifically, the PM treatment showed significantly lower levels of SOC, TP, TN, and AP compared to the other treatments ($P < 0.05$). The NM treatment exhibited significantly lower soil water content (SW) than the other treatments ($P < 0.05$), while the LM treatment had significantly lower soil pH, AP, MBC, and MBN compared to the other treatments ($P < 0.05$).

Table 1
Physicochemical properties of the grassland soils used in this study

	SW%	pH	EC	SOC g kg ⁻¹	TP g kg ⁻¹	TN g kg ⁻¹	AP mg kg ⁻¹	AK mg kg ⁻¹	MBC mg kg ⁻¹	MBN mg kg ⁻²
CK	24.60 ± 0.27a	6.31 ± 0.05ab	55.03 ± 1.88b	47.97 ± 0.91a	0.93 ± 0.03a	1.57 ± 0.01a	13.96 ± 0.38a	13.96 ± 0.38b	814.03 ± 33.46a	73.09 ± 0.59c
NM	16.90 ± 0.53c	6.37 ± 0.03a	65.40 ± 2.51a	43.08 ± 1.20b	0.50 ± 0.17bc	1.53 ± 0.02a	14.30 ± 1.03a	15.52 ± 0.50a	651.11 ± 8.73b	94.85 ± 2.42a
PM	19.77 ± 1.08b	6.27 ± 0.01bc	46.65 ± 1.79c	36.68 ± 1.51c	0.42 ± 0.02c	1.44 ± 0.02b	11.13 ± 0.26b	15.34 ± 0.43a	538.00 ± 15.05c	80.13 ± 3.57b
LM	18.54 ± 0.24bc	6.22 ± 0.01c	47.28 ± 1.48c	42.85 ± 0.96b	0.74 ± 0.03ab	1.52 ± 0.03a	12.03 ± 0.39b	11.62 ± 0.45b	513.53 ± 25.31c	64.50 ± 1.21d

Notes: Values are means ± SE for quadruplicate replicates. Different lowercase letters indicate significant differences with a P value < 0.05 based on the analysis of variance. SW: soil water content; SOC: soil organic carbon; TP: total phosphorus; TN: total nitrogen; AP: available phosphorus; AK: available potassium; MBC: microbial biomass carbon; MBN: microbial biomass nitrogen.

3.2 Composition of soil microorganisms and nematode communities

In this study, a total of 17 phyla and 40 genera were obtained through high-throughput sequencing of bacterial 16S rRNA genes (Fig. 2a). Bacterial phyla that have been classified and have a relative abundance greater than 10% include *Proteobacteria* (21.65%) and *Acidobacteriota* (20.09%). At the same time, some bacterial phyla with low relative abundance (1%~10%) were also detected, mainly including *Verrucomicrobiota* (4.18%), *Bacteroidota* (4.02%), *Myxococcota* (3.80%), and *Methylomirabilota* (3.77%). There are 8 bacterial genera with a relative abundance greater than 1.0% (Fig. 2c). The dominant bacterial genus is *RB41* (3.05%). The taxa with a relative abundance of 1.0–2.0% include *Sphingomonas* (1.95%), *P3OB_42* (1.83%), and *MND1* (1.34%), *Ellin6067* (1.27%), *Nitrospira* (1.23%), *Haliangium* (1.13%), *Subgroup_10* (1.05%). A total of 8 phyla and 60 genera were obtained through high-throughput sequencing of fungal ITS region (Fig. 2b). Fungal phyla with relatively high relative abundance include *Ascomycota* (51.50%) and *Basidiomycota* (31.63%). There are 12 fungal genera with a relative abundance greater than 1% (Fig. 2d). The dominant genera are *Mortierella* (5.10%), *Pseudaleuria* (4.40%), and *Clavaria* (3.68%). The taxa with a relative abundance of 1–2% include *Cladosporium* (1.93%), *Filobasidium* (1.87%), *Vishniacozyma* (1.29%), and *Tetracladium* (1.03%). A total of 19 genera were obtained through high-throughput sequencing of the nematode 18sRNA gene (Fig. 2e), including 5 genera of bacterivorous nematodes, 2 genera of fungivorous nematodes, 9 genera of plant-parasitic nematodes, and 3 genera of omnivorous predatory nematodes, among which plant-parasitic nematodes accounted for the highest ratio (18.61%~45.56%). The dominant nematode genera in the CK treatment were

Filenchus (17.23%), *Paratylenchus* (13.89%) and *Ditylenchus* (10.43%); the dominant nematode genus in the NM treatment was *Enchodelus* (29.09%); the dominant nematode genus in the PM treatment was *Filenchus* (18.20%), *Paratylenchus* (11.44%) and *Basiria* (14.05%); the dominant nematode genera in the LM treatment were *Filenchus* (14.67%), *Paratylenchus* (12.66%) and *Hemicycliophora* (23.26%)

3.3 Diversity of soil microorganism and nematode communities

We assessed the diversity and richness of soil nematode and microorganism communities under different vegetated zokor mounds by measuring the Shannon and Chao1 indices (Fig. 3). The Chao1 index of soil nematodes in the NM treatment was lower than in other treatments, while the Chao1 index of soil bacteria and fungi in the LM treatment was also lower compared to other treatments. Conversely, the Chao1 index of soil nematodes, bacteria, and fungi in the PM treatment was higher than in other treatments. Regarding the Shannon diversity index, the NM treatment showed lower values for soil nematodes and bacteria compared to other treatments, while the Shannon index for soil fungi in the NM treatment was higher than in other treatments. The LM treatment exhibited a lower Shannon index for soil fungi than the other treatments. On the other hand, the Shannon index for soil nematodes and bacteria in the PM treatment was higher than in other treatments.

We conducted principal component analysis (PCA) of the Bray-Curtis algorithm to analyze structural changes in nematode, bacterial, and fungal communities, aiming to determine the impact of zokor mounds with different vegetation types on their structures (Fig. 4). The results revealed significant effects of different zokor mound vegetation types on the soil nematode, bacterial, and fungal community structures. The nematode communities were divided into two groups: CK, NM, and LM formed one group, while PM constituted the other group. Notably, the nematode community structure in PM exhibited distinct differences from the other treatment groups, indicating significant changes in the soil nematode community structure as vegetation grew on the zokor mounds. For bacterial communities, PCA identified three groups: CK and LM each formed one group, while NM and PM clustered together, differing significantly from CK. Similarly, LM also differed significantly from CK. Concerning fungal communities, PCA divided them into two groups, with LM in one group, and the remaining three treatments forming another group. Overall, our findings demonstrate that zokor mounds with different vegetation types have a substantial impact on the structures of nematode, bacterial, and fungal communities.

3.4 Metabolic footprint and food web energy flow analysis of soil nematodes

The nematode metabolic footprint serves as a valuable indicator of the metabolic activity and functional status of the habitat ecosystem. It effectively reflects the carbon metabolism pathway, energy flow state of the food web, and the overall size of the regional food web function. In the NM treatment, the total nematode metabolic footprint (TNMF), bacterivores metabolic footprint (BFMF), and omnivores-predators metabolic footprints (OPMF) were higher compared to the other treatments. Conversely, the LM treatment exhibited lower TNMF, BFMF, and OPMF compared to the other treatments, while the plant parasites

metabolic footprint (PPMF) was higher (Fig. 5a). The functional metabolic footprint (FMF), represented by the area enclosed by the enrichment footprint and the structural footprint, serves as a measure of the food web's ability to regulate and maintain its metabolic equilibrium. Larger FMF values indicate stronger regulation of the habitat food web by nematode populations. In this study, FMF, enrichment index, and structure index values followed the order: NM > CK > PM > LM (Fig. 5b). PM and LM treatments fell within quadrant C, while CK and NM treatments were in quadrant B. The analysis of food web energy flow (Fig. 5c) demonstrated that the PM treatment had the largest proportion of bacterial and fungal energy flow channels, while plant energy flow channels had the smallest proportion. Conversely, the LM treatment displayed the opposite pattern. The NM treatment fell between the PM and LM treatments, suggesting that the energy conversion and utilization efficiency of the soil food web in the PM treatment were relatively high.

3.4 Soil Micro-foodWeb Relationships

The interactions between soil microbes and nematodes in zokor mound soils with different vegetation types exhibited distinct differences (Fig. 6 and Table 2). The NM treatment had a relatively higher total number of linkages compared to the CK treatment, whereas both the PM and LM treatments showed lower total numbers of linkages than CK. In zokor mound soils with different vegetation types, the NM treatment had a higher number of positive and negative links compared to other treatments, with a relatively lower proportion of positive links. Conversely, the PM and LM treatments had a higher proportion of positive links. Moreover, key species in the network varied among zokor mound soils with different vegetation types. We constructed Structural Equation Modeling (SEM) of different soil microbial and nematode components to calculate the carbon fluxes in the soil food web in the zokor mounds with different vegetation. The study revealed that fungal richness and bacterial diversity had a direct impact on the carbon metabolic footprint of bacterivores. Additionally, fungal and bacterial community structures directly influenced the carbon metabolic footprint of fungivores. Moreover, the carbon metabolic footprint of omnivores-predators was directly affected by fungal richness and fungal and bacterial community structures. Furthermore, bacterivores (BF) and fungivores (FF) had a direct impact on the metabolic carbon footprint of omnivores-predators. Notably, bacterivores (BF), fungivores (FF), and omnivores-predators (OP) played significant roles in contributing to soil organic carbon. The SEM model explained 71.0% of the difference in the carbon metabolic footprint of omnivores-predators, 49.0% of the difference in the carbon metabolic footprint of bacterivores, 7% of the difference in the carbon metabolic footprint of fungivores, and 89.0% of the variation in soil organic carbon (Fig. 7).

Table 2
Topological properties of soil micro-foodweb network in the zokor mounds with different vegetation

Topological properties	CK	NM	PM	LM
Number of nodes	52	48	48	48
Total links	96	120	86	81
Positive links	70	73	72	57
Negative links	26	47	14	25
% Positive links	72.92%	60.83%	83.72%	70.37%

3.4 Correlation analysis between soil microorganism and nematode communities and soil physicochemical properties

In this study, we conducted redundancy analysis (Fig. 8) using soil microorganism and nematode community genus levels as response variables, and SW, pH, SOC, TN, AP, TP, MBC, and MBN as explanatory variables. The total explanatory degrees of soil nematode community, bacterial community, and fungal community were 30.32%, 42.48%, and 30.39%, respectively. Redundancy analysis of soil physicochemical properties and soil nematode communities revealed several positive correlations. *Enchodelus*, *Aphelenchus*, *Bitylenchus*, *Nygolaimus*, and *Panagrolaimus* were positively correlated with AP, TN, EC, pH, and SOC, while *Lelenchus*, *Boleodorus*, *Ditylenchus*, *Prodesmodora*, and *Teratocephalus* were positively correlated with AK, SW, MBC, and MBN (Fig. 7a). Additionally, redundancy analysis indicated that certain bacterial genera, such as *P3OB_42*, *Bryobacter*, *RB41*, and *Bradyrhizobium*, were positively correlated with SW, SOC, TN, AP, and TP; *MND1*, *Reyranella*, *IS_44*, and *Dongia* were positively correlated with AK, SW, MBC, and MBN; and *Sphingomonas*, *Arenimonas*, *Nitrospira*, and *Gaiella* were positively correlated with AP (Fig. 8b). In relation to soil fungal communities, *Pseudaleuria*, *Diversispora*, *Mortierella*, and *Leohumicola* were positively correlated with AP; *Dermoloma* and *Trichoglossum* were positively correlated with TN; and *Hygrocybe*, *Cupophyllus*, *Hemileucoglossum*, and *Clavulinopsis* were positively correlated with SW ($P=0.046$), PH ($P=0.01$), AK ($P=0.006$) and MBN ($P=0.015$) (Fig. 8c). Additionally, Mantel analysis (Fig. 8d) indicated that SW, pH, AK, and MBN significantly affected soil bacterial communities, while SW ($P=0.002$) and AP ($P=0.014$) significantly influenced soil fungal communities. However, the correlation between individual soil physical and chemical factors and soil nematodes did not reach a significant level, suggesting that the soil nematode community is jointly affected by various soil physical and chemical properties.

4. Discussion

4.1 Effects of Zokor Mounds with Different Vegetation Types on Soil Physical and Chemical Properties

Plateau zokors are subterranean rodents that inhabit grasslands year-round and excavate mounds. While feeding on and disturbing underground vegetation roots, they deposit the excavated soil on the surface, covering the original grassland vegetation. This creates voids with heterogeneous soil in the mouse mounds, initiating patch vegetation succession (Pech et al. 2007), providing opportunities for opportunistic species establishment and spread (Wesche, Nadrowski and Retzer 2007). Over time, species with weaker competitive abilities are excluded in the later stages of succession due to their inability to adapt to the environment, resulting in changes to soil physical, chemical, and biological characteristics (Odum 1969). In this study, NM, PM, and LM zokor mounds represent typical vegetation community structures in the early, middle, and late stages of secondary succession, respectively. The soil physical and chemical properties of zokor mounds with different vegetation structures significantly differ. Zokor disturbance increased the soil AP content in the mound patches but reduced soil water content, TP, AK, MBC, MBN, and SOC contents. The TN and pH contents of the new zokor mound increased, consistent with Li et al.'s research (Li et al. 2009b). The lower soil water content in the new zokor mounds results from zokors bringing deeper soil to the surface during mound formation, leading to increased evaporation and reduced soil water content. As vegetation establishes in the patches and livestock trampling occurs during winter grazing, the vegetation coverage and species diversity of PM and LM zokor mounds increase. (Li et al. 2009b). The increase in soil AP content may be due to: the extensive contact of zokor feeding detritus and plant residues with the soil, facilitating thorough decomposition by soil microorganisms; the mixture of zokor feces and urine with the soil, contributing to higher AP content in new zokor mound patches. Regarding soil SOC, TP, MBC, and MBN, zokor disturbance pushes poor soil from lower layers to the surface, creating bare patches, and reduces vegetation coverage, thereby decreasing the organic matter returned to the soil and impacting total nutrient content and microbial biomass carbon and nitrogen. The soil pH change exhibited two trends: the surface soil pH of the new zokor mound was higher than that of the undisturbed control area; over time, as vegetation recovered in the zokor mound, the soil pH decreased due to increased organic acid production from SOC accumulation (Spencer et al. 1985).

4.2 Effects of different vegetation types on the microbial and nematode communities of zokor mounds

The results revealed significant impacts of zokor mounds with different vegetation types on the richness, diversity, and community composition of soil nematodes, bacteria, and fungi. Zokor disturbance induces changes in soil physical and chemical properties, and various nematode, bacterial, and fungal communities exhibit species selectivity towards the environment, resulting in substantial differences in soil nematode and microbial community compositions, thereby validating the study's hypothesis. Soil microbial diversity and abundance are influenced comprehensively by soil water, temperature, nutrients, and gas environment (Díaz-Raviña, Acea and Carballas 1995). Soil aeration conditions, especially

increased oxygen content, promote bacterial richness and diversity. Notably, zokor mounds exhibited higher bacterial richness and diversity than CK, especially in NM and PM, while LM had lower bacterial richness and diversity, likely due to the varying degrees of zokor interference. When disturbance is low, dominant vegetation species are *Potentilla velvet* and *Polygonum viburnum*, whereas high disturbance leads to *edelweiss* and *Polygonum siberica* dominance (Hu et al. 2015). Enhanced soil aeration likely contributes to the increased bacterial richness and diversity in zokor mounds. PM showed the highest fungal richness, LM the lowest, and NM fungal richness and diversity fell in between. This pattern might be associated with the resistance of plants like *Potentilla velvet*, *edelweiss*, and *blue stone grass* to trampling by plateau zokors. These plants contain numerous secondary compounds that effectively resist overgrazing by zokors, leading to their proliferation and continual release of greenhouse gases, which in turn increases atmospheric greenhouse gas concentration (Lerdau et al. 1995). NM had lower nematode richness and diversity than other treatments, potentially due to soil water content and pH, as nematodes are hydrophilic and sensitive to soil pH (Bongers and Ferris 1999). Additionally, zokor interference might cause different habitat preferences, feeding habits, and trophic levels, leading to varying ecological niches for soil nematodes.

Nematode metabolic footprints serve as indicators of carbon metabolic pathways and energy flow states within soil food webs (Ferris 2010). In this study, the metabolic footprint of plant-parasitic nematodes was found to be larger than that of microbiophagous nematodes, implying that most nutrients enter the soil through plant channels in the food web. Furthermore, the analysis of soil nematode flora and food web energy flow revealed that CK and NM were situated in the B quadrant, while PM and LM were in the C quadrant. NM exhibited the largest soil nematode enrichment index and structure index (diamond center position), indicating its ability to enrich soil nutrients and improve the soil environment. This, in turn, reduced the degree of soil disturbance and contributed to a more mature and stable food web structure. The functional footprint of soil nematodes followed the order of NM > CK > PM > LM, with PM having the highest proportion of bacteria and fungi energy flow channels and the lowest proportion of plant energy flow channels. Conversely, LM was positioned between PM and CK, suggesting that the PM soil nematode community displayed a higher carbon utilization rate and possessed the strongest ability to regulate the food web and maintain the metabolic balance between prey and predator populations in the habitat, likely due to its higher abundance of omnivorous-predatory nematodes.

4.3 Effects of different vegetation types of zokor mounds on the decomposition pathways of soil micro-foodweb

Structural equation modeling revealed positive correlations between fungi, bacterivores (BF), and omnivores-predators (OP) under different vegetation types, as well as positive correlations between bacteria, fungivores (FF), and OP. Both bacteria and fungi play crucial roles in decomposition pathways within the soil food web, influencing the rate of soil carbon loss. Notably, the bacterial decomposition pathway facilitates rapid nutrient turnover but is less conducive to soil carbon and nitrogen sequestration compared to the fungal decomposition pathway (Vries et al. 2012). Zokors significantly contribute to the nutrient-rich environment through their excretion of feces and urine during digging (Heth et al. 1996, Zuri,

Gazit and Terkel 1997). These nutrient qualities impact the transformation of soil food web bacterial and fungal decomposition pathways, thus influencing carbon turnover (Fabian et al. 2016). In our study, zokor mounds provided abundant readily available carbon resources, favoring the dominance of bacterial decomposition pathways within the soil food web. However, as the decomposition process progressed, the substrate became refractory, possibly more suitable for fungi decomposition (Bossuyt et al. 2001). Consequently, zokor mounds with different vegetation types may promote the successional shift in the community from bacteria to fungi. We also observed that the fungal decomposition pathway played a more substantial role in increasing soil organic carbon. Previous studies have indicated that fungi are better at protecting carbon pools and retaining a larger proportion of carbon, enhancing carbon stability and microbial source organic matter accumulation (Don et al. 2017, Frey, Thiet and Batten 2006).

Mineralization of carbon and nitrogen tends to be accelerated due to zokor disturbance (Zhang, Zhang and Liu 2003). Similarly, our findings suggest that the decomposition pathways in this study are influenced by bottom-up effects, where bacteria and fungi exert significant control on microbiophagous nematodes, and changes in bacterivorous (BF) and fungivorous (FF) nematodes are driven by resource input and microbial community characteristics, depending on their life history traits (Ferris and Matute 2003). We observed strong positive correlations between FF, fungi, and omnivores-predators (OP) in the undisturbed control (CK) treatment, and between bacteria and OP in the moderate disturbance (NM) treatment (Fig. 5). These results strongly support the notion that CK and NM enhance the decomposition pathways of fungi and bacteria, respectively. It is worth noting that the decomposition pathways of bacteria and fungi in the soil food web are not mutually exclusive but rather operate simultaneously, involving different carbon transfer and utilization processes (Ruess and Ferris 2004). Different vegetation types in zokor mounds regulate the microbial and nematode community composition and their decomposition pathways in response to varying amounts of organic carbon input. However, zokor disturbance not only accelerates the mineralization of soil carbon but also leads to reduced soil water content, vegetation height, and coverage, resulting in soil drought, fertility decline, and soil salinization. Nevertheless, our study demonstrates that zokor mounds with different vegetation types play a crucial role in enhancing soil biological activity and soil organic carbon accumulation. Moreover, varying carbon-nitrogen ratios have been shown to have distinct effects on the structure and function of soil food webs (DuPont, Ferris and Van Horn 2009).

5. Conclusion

In conclusion, this study significantly advances our comprehension of the decomposition pathways within the micro-foodweb of zokor mound soils, which vary with different vegetation types. The input of organic matter plays a pivotal role in promoting the conversion between bacteria and fungi, consequently influencing the interplay between bacterivorous and fungivorous nematodes. Specifically, the NM treatment enhances the bacterial decomposition pathway, while the CK treatment promotes the fungal decomposition pathway. In the CK treatment, the higher fungal biomass potentially contributes to increased organic carbon sequestration. The nutrient interactions between soil microbial and nematode communities act as key drivers of carbon flow in these decomposition pathways. Overall, the regulatory

effect of NM on the soil food web's decomposition pathway surpasses that of other treatments, thus significantly influencing the carbon metabolic processes within the soil food web.

Declarations

Data availability statement

The microbial and nematode DNA sequences of the 16 soil samples were deposited in the SRA of the NCBI database under Accession nos. NCBI: PRJNA988927 and SRA: SUB13582092.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Author contribution

LLN completed the soil sampling collection from the field. LLN, LLL, XNZ, XYL, and ZBN finished the primary analysis of soil physicochemical properties.>NNL, XYL, YYW, and QQZ analyzed the data and drafted the manuscript. XLL and YBY designed the experiment and improved the structure and logic of the manuscript.

Funding

This research was financially supported by The Youth Science and Technology Fund of Gansu Province (20JR5RA014); Research on the Coordinated Relationship between Land Urbanization and Population Cities (GSAU-ZL-2015-046) and The Scientific Research Start-up Funds for Openly Recruited Doctors of Gansu Agriculture University (2017RCZX32).

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

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Figures

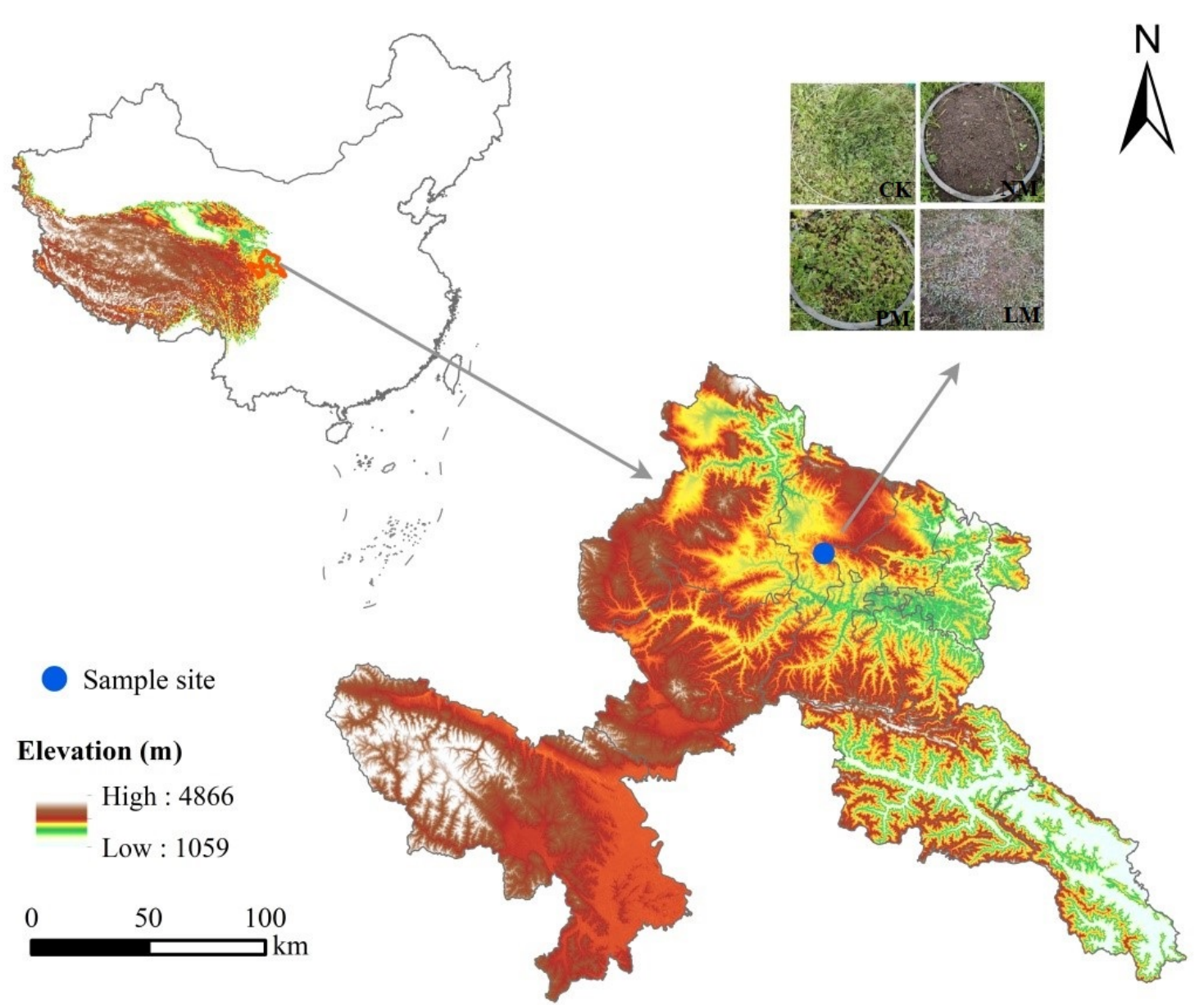


Figure 1

Location of the study site

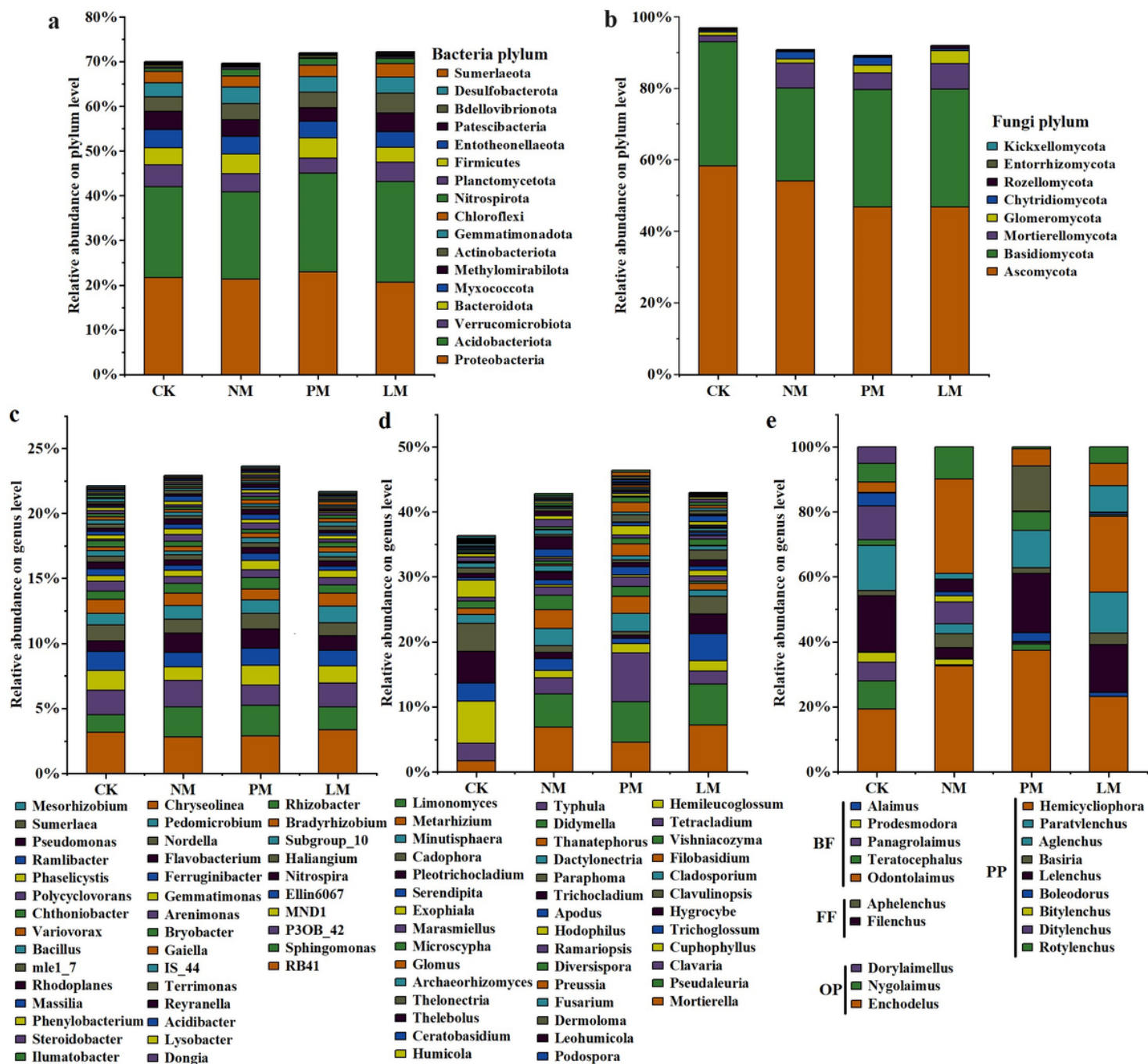


Figure 2

Relative abundance of microbial phyla and genera as well as nematode genera in zokor mound soil under different vegetation types. a: Relative abundance at the bacterial phylum level; b: Relative abundance at the fungal phylum level; c: Relative abundance at the bacterial genus level; d: Relative abundance at the fungal genus level; e: Relative abundance at the nematode genus level; control (CK), bare new zokor mound (NM), zokor mound covered with *Potentilla anserina* (PM), zokor mound covered with *Leontopodium leontopodioides* (LM). BF: bacterivorous nematodes; FF: fungivorous nematodes; PP: plant-parasitic nematodes; OP: omnivorous predatory nematodes.

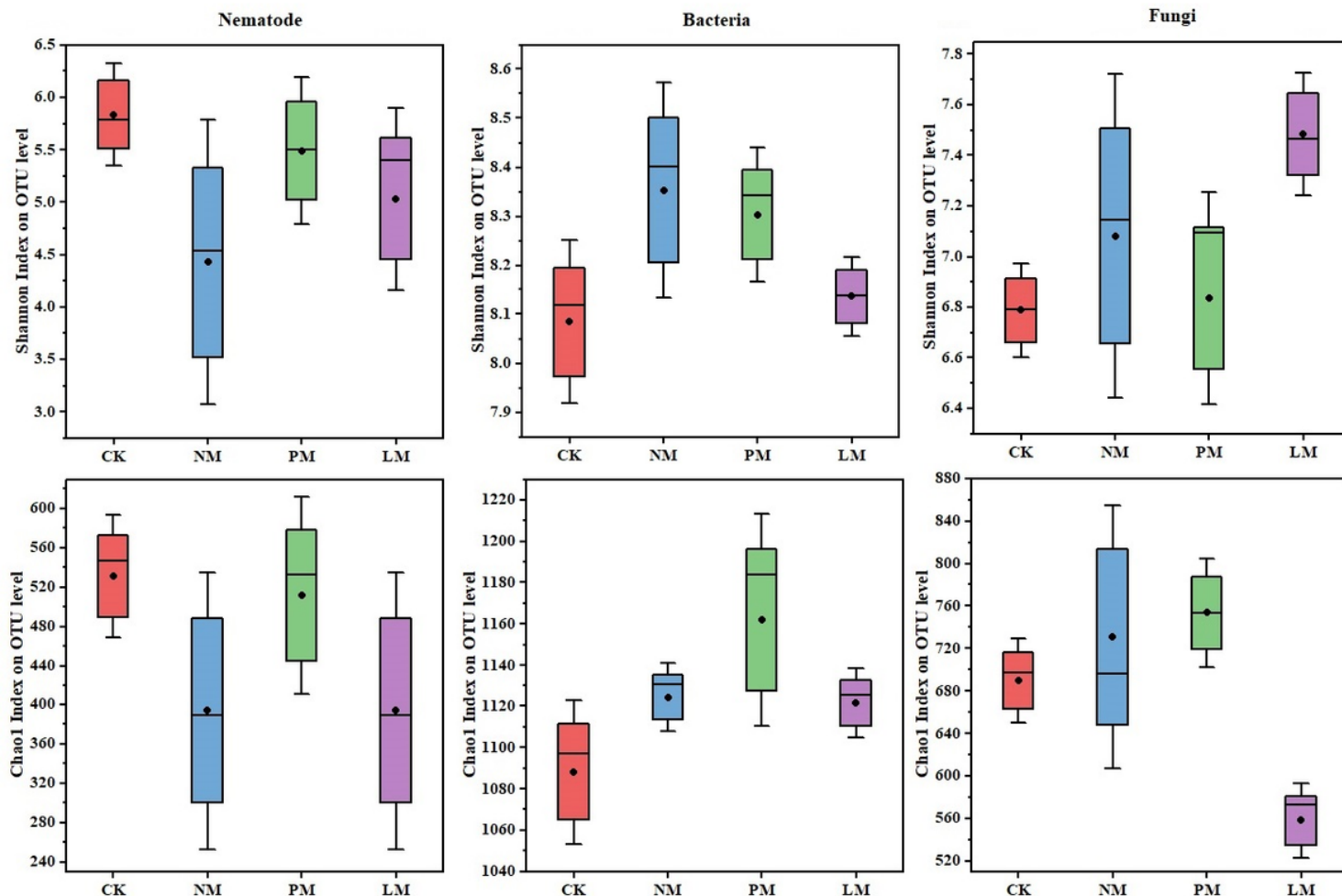


Figure 3

Diversity of soil nematodes, bacteria and fungi in zokor mounds of different vegetation types. control (CK), bare new zokor mound (NM), zokor mound covered with *Potentilla anserina* (PM), zokor mound covered with *Leontopodium leontopodioides* (LM). The size of the violin box depends on the interquartile range of the data. A large box means that the data distribution is discrete, and the data fluctuates greatly. A small box means that the data is concentrated. The upper side of the box is the 75% quantile, and the lower side is the 25% quantile. The upper tentacle of the box is the maximum value of the data, the lower tentacle is the minimum value of the data, and the white dot on the violin plot represents the average value.

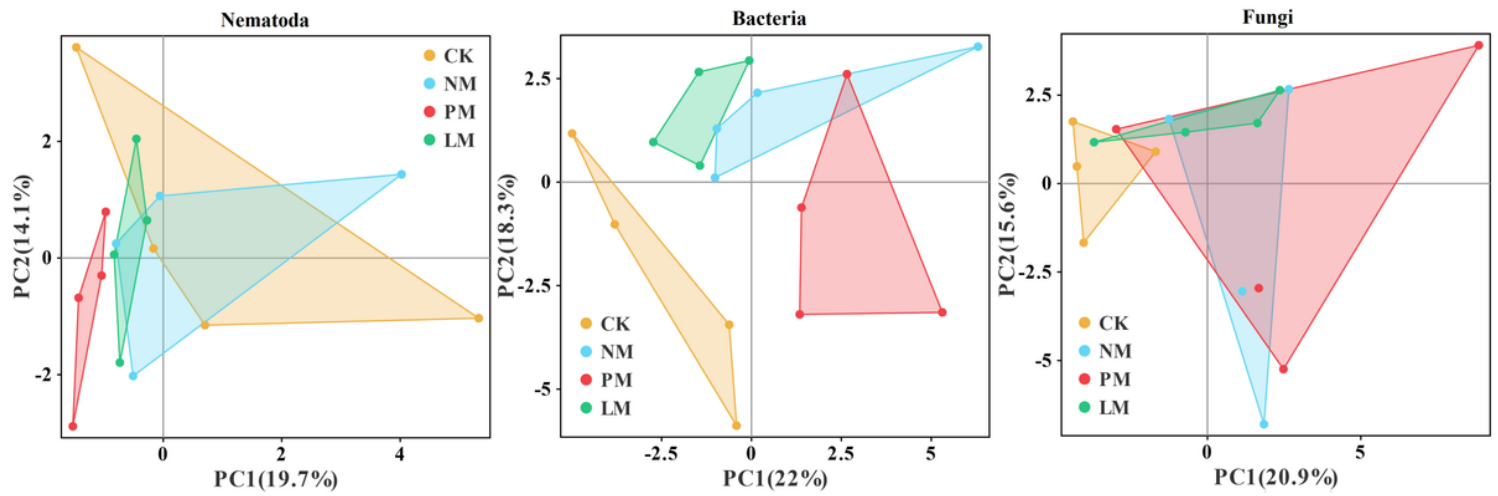


Figure 4

Principal component analysis (PCA) of nematode, bacterial and fungal communities based on Bray-Curtis distances. control (CK), bare new zokor mound (NM), zokor mound covered with *Potentilla anserina* (PM), zokor mound covered with *Leontopodium leontopodioides* (LM).

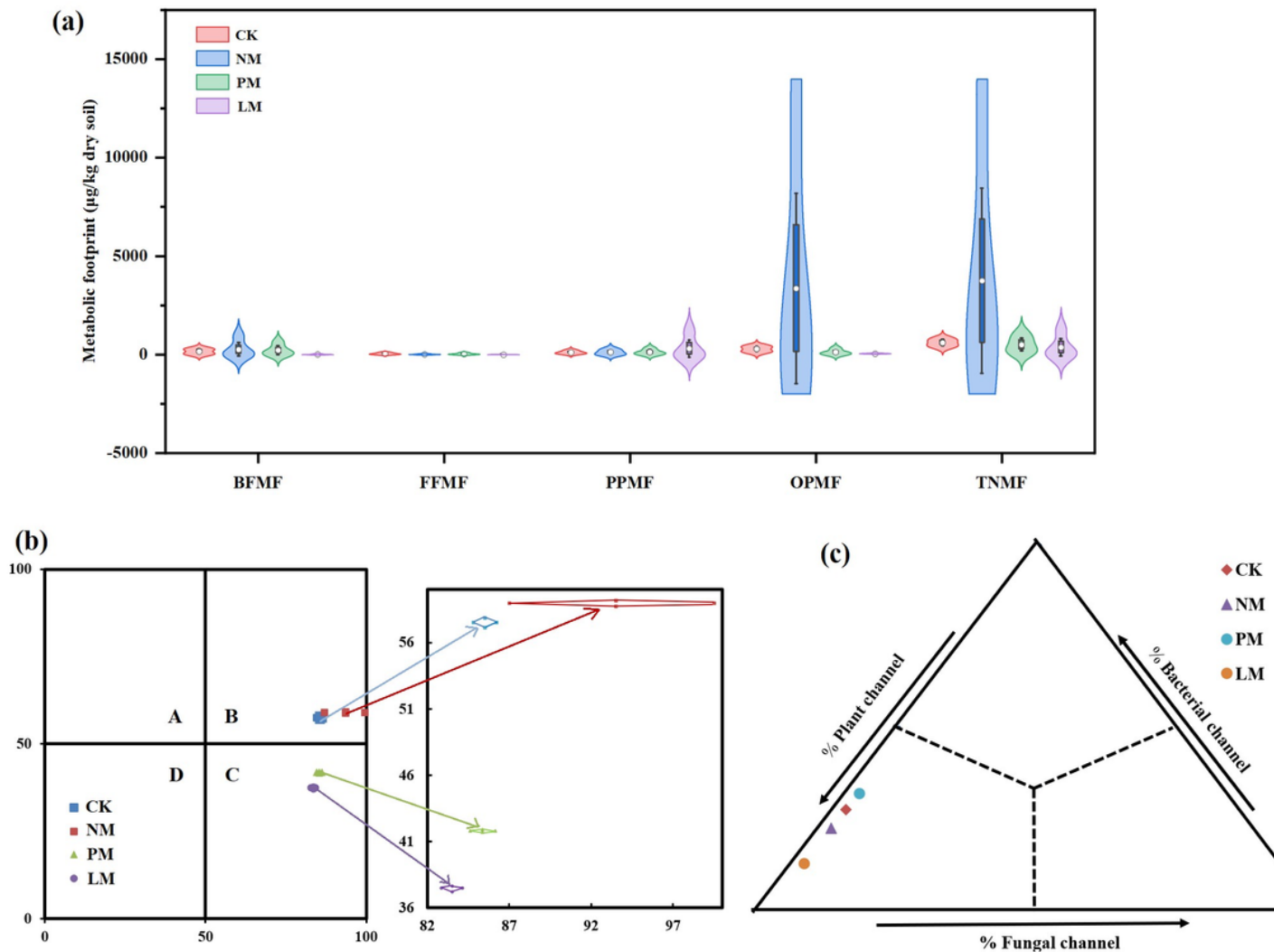


Figure 5

Soil nematode metabolic footprint and food web energy flow analysis in zokor mound soils of different vegetation types. (a) metabolic footprint of trophic taxa; control (CK), bare new zokor mound (NM), zokor mound covered with *Potentilla anserina* (PM), zokor mound covered with *Leontopodium leontopodioides* (LM); BFMF: bacterivores metabolic footprint; FFMF: fungivores metabolic footprint; PPMF: plant parasites metabolic footprint; OPMF: omnivores-predators metabolic footprints; TNMF: Total nematode metabolic footprint. (b) functional metabolic footprint; the functional metabolic footprint is depicted by the sequentially joining points: $(SI-0.5Fs/k, EI)$; $(SI+0.5Fs/k, EI)$; $(SI, EI-0.5Fe/k)$; and $(SI, EI+0.5Fe/k)$. The adjusted k value is 3. (c) food web energy flow analysis;

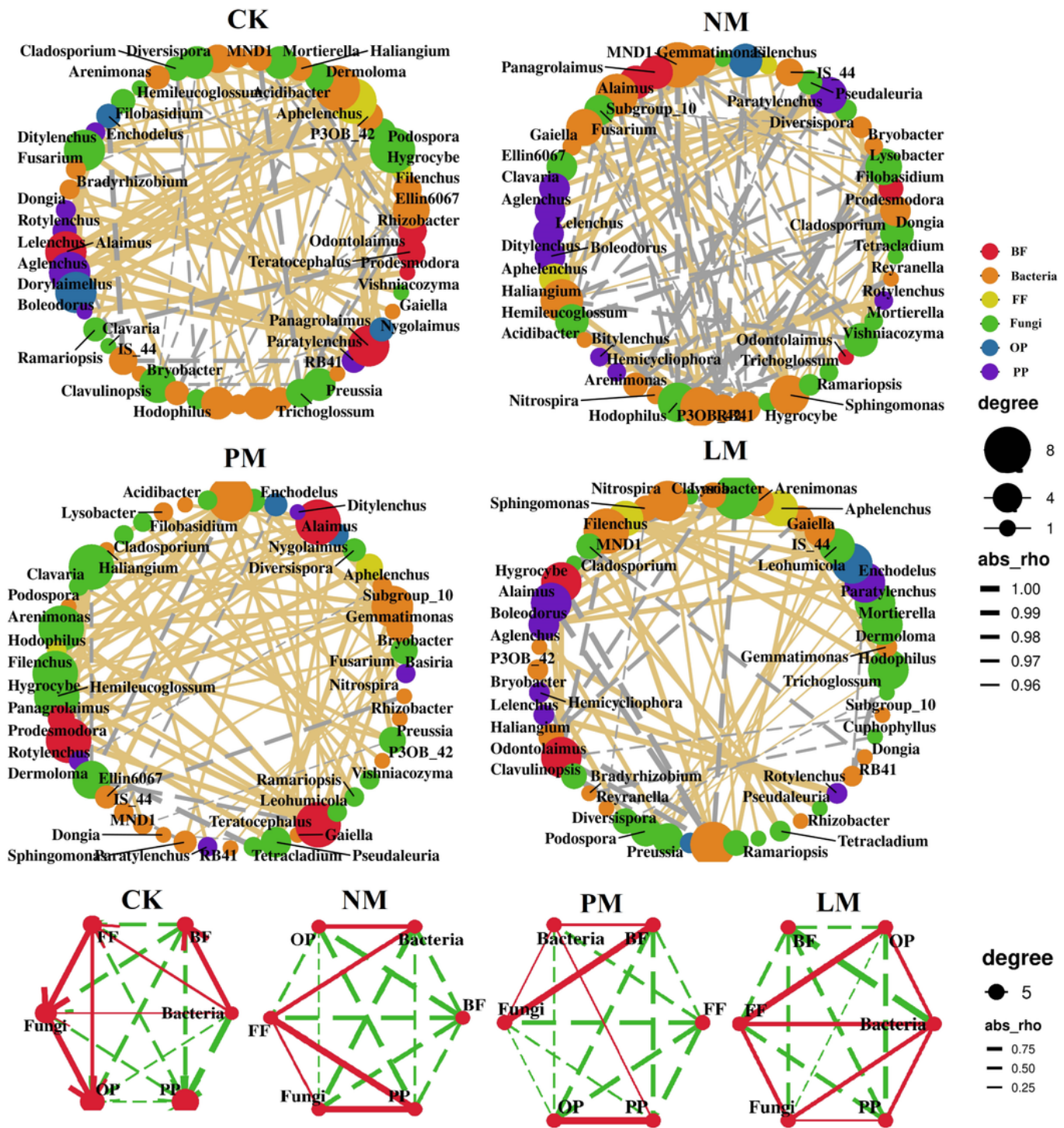


Figure 6

Network visualization of soil micro-foodweb interaction strength. The size of each node is proportional to the centrality score, and genera with larger centrality represent the key species of each network. Lines between each pair of nodes indicate a strong positive (yellow) or negative (dashed gray) interaction, and the thickness of the line indicates the strength of the association.

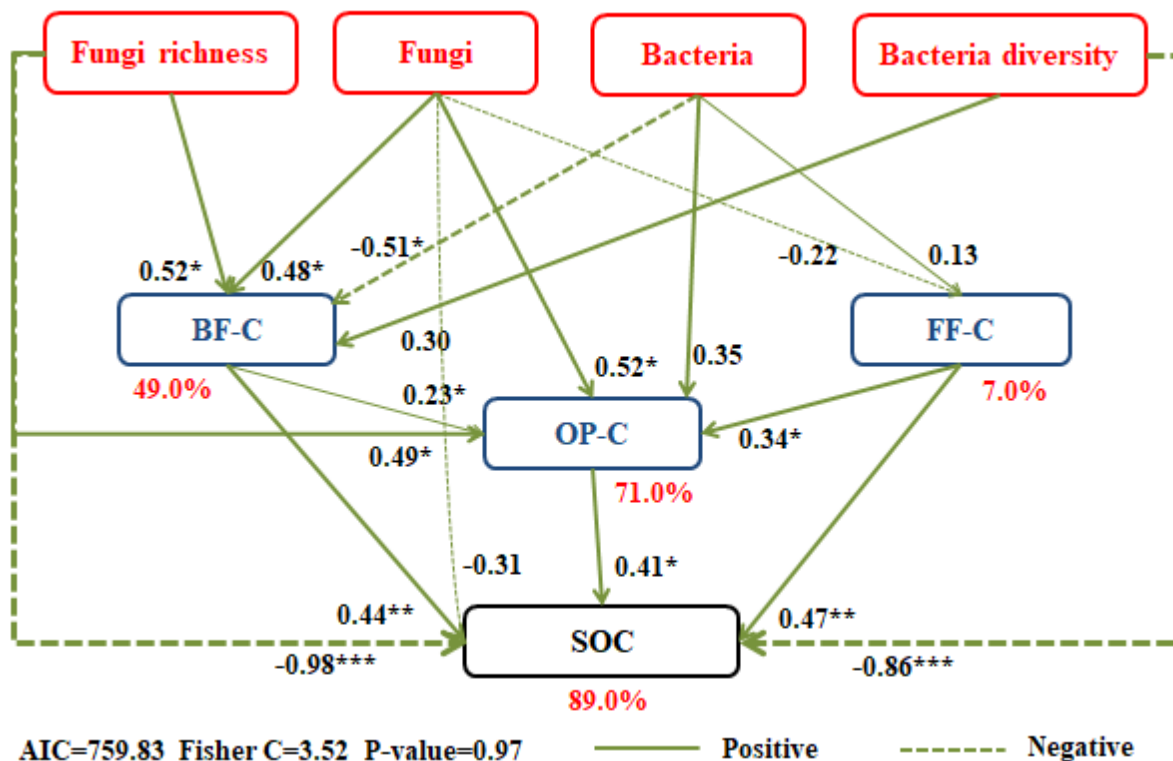


Figure 7

Structural equation modeling of soil micro-foodweb decomposition pathways. The width of the arrows is proportional to the strength of the path coefficient. The continuous red and broken blue arrows represent positive and negative relationships, respectively. BF-C: carbon metabolic footprint of bacterivores; FF-C: carbon metabolic footprint of fungivores; OP-C: carbon metabolic footprint of omnivores-predators; SOC: soil organic carbon.

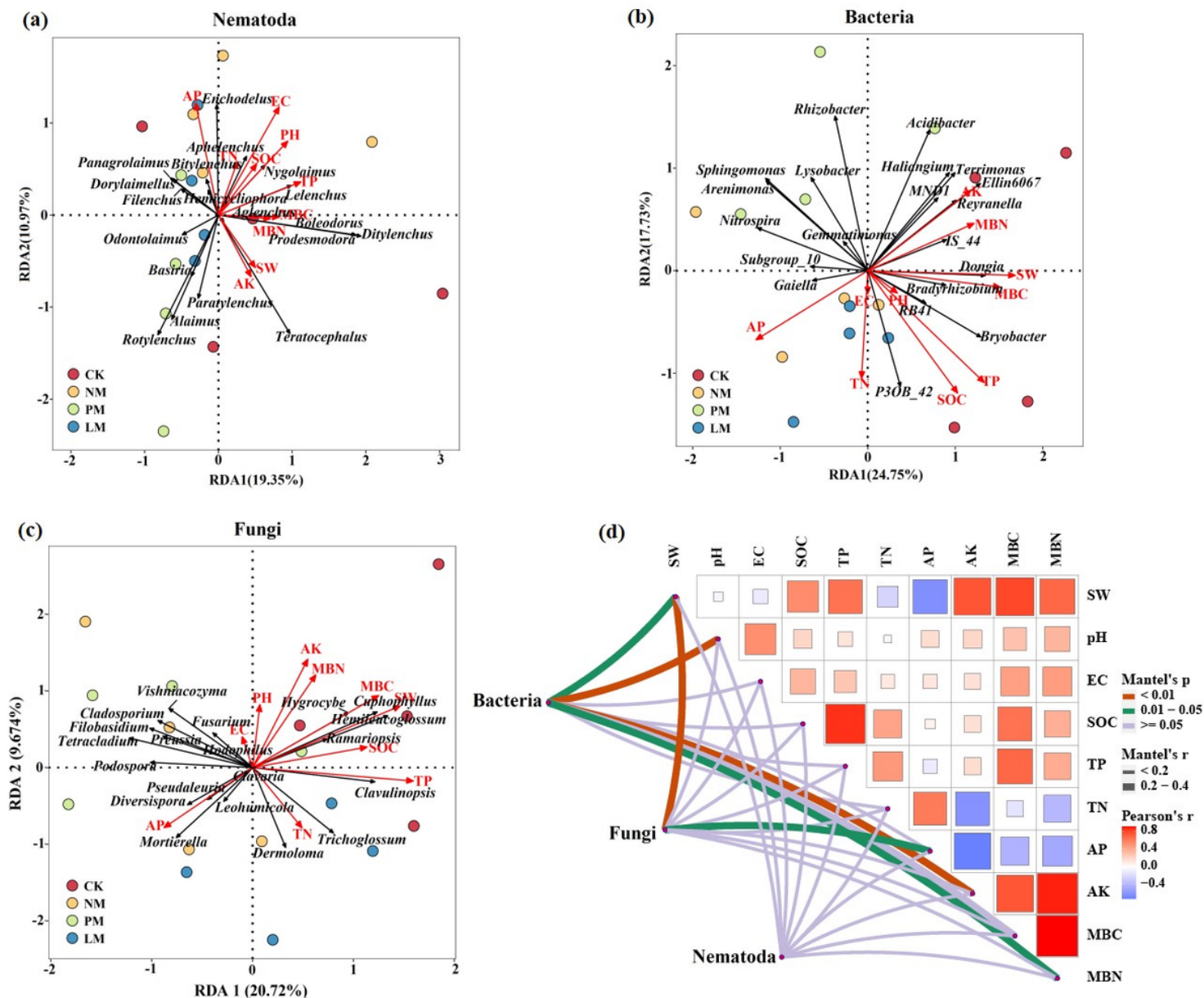


Figure 8

Correlation analysis between soil microorganism and nematode communities and soil physicochemical factors. (a): Redundancy analysis of soil nematode community and environmental factors. (b): Redundancy analysis of soil bacterial community and environmental factors. (c) Redundancy analysis of soil fungal community and environmental factors. (d): Bacteria, fungi and nematoda were related to each environmental factor by Mantel tests. Edge width corresponds to the Mantel's r statistic for the corresponding distance correlations, and edge colour denotes the statistical significance. The blue and red colours show negative and positive relationships, respectively, between the two variables. The stronger the colour, the stronger the relationships.