



## Variations in the soil micro-food web structure and its relationship with soil C and N mineralization during secondary succession of subalpine forests

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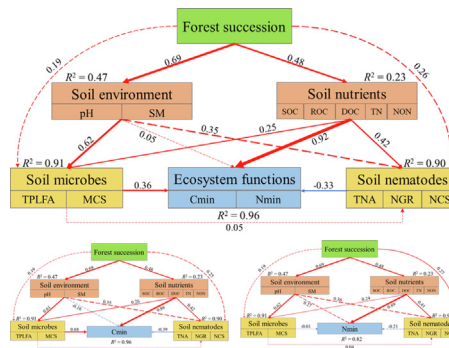
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### HIGHLIGHTS

- Forest secondary succession enriched and stabilized the soil micro-food web.
- Soil C and N mineralization rates were lowest in the grassland and highest in the coniferous forest.
- Soil C and N mineralization was positively correlated with soil micro-food web abundance and diversity.
- Soil nutrients and micro-food web jointly determined the ecosystem functions.

### GRAPHICAL ABSTRACT



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### ABSTRACT

The soil micro-food web is an important network of belowground trophic relationships and it participates directly and indirectly in soil ecological processes. In recent decades, the roles of the soil micro-food web in regulating ecosystem functions in grasslands and agroecosystems have received much attention. However, the variations in the soil micro-food web structure and its relationship with ecosystem functions during forest secondary succession remain unclear. In this study, we investigated how forest secondary succession affected the soil micro-food web (including soil microbes and nematodes) and soil carbon and nitrogen mineralization across a successional sequence of “grasslands – shrublands – broadleaf forests – coniferous forests” in a subalpine region of southwestern China. With forest successional development, the total soil microbial biomass and the biomass of each microbial group generally increased. The significant influences of forest succession on soil nematodes were mainly reflected in several trophic groups with high colonizer-persister values (particularly bacterivore3, herbivore5 and omnivore-predator5) that are sensitive to environmental disturbance. The increases in the connectance and nematode genus richness, diversity, and maturity index indicated an increasingly stable and complex soil micro-food web with forest succession, which was closely related to soil nutrients, particularly the soil carbon contents. Additionally, we found that the soil carbon and nitrogen mineralization rates also exhibited generally increasing trends during forest succession, which had significant positive correlations with the soil micro-food web composition and structure. The path analysis results indicated that the variances in ecosystem functions induced by forest succession were significantly determined by soil nutrients and soil microbial and nematode communities. Overall, these results suggested that forest succession enriched and stabilized the soil micro-food web and promoted ecosystem functions via the increase in soil nutrients, and the soil micro-food web played an important role in regulating ecosystem functions during forest succession.

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## 1. Introduction

In the global context of continuous forest loss and degradation, forest restoration is widely recognized as imperative and has been put into practice in recent decades (Crouzeilles et al., 2017; Erbaugh et al., 2020). Secondary forests, formed by the natural generation of degraded vegetation, can be highly efficient and low-cost in increasing forest cover, enhancing biodiversity conservation and improving ecosystem functions (Chazdon, 2008; Teixeira et al., 2020). However, previous studies have focused largely on the aboveground factors that regulate forest secondary succession (Lohbeck et al., 2015; Lai et al., 2017; Bretfeld et al., 2018). Given the growing awareness of the importance of belowground ecosystems, a pressing need arises to understand the changes in soil biota communities with forest successional development and to further explore their influences on ecosystem functions and aboveground community dynamics (Bardgett and van der Putten, 2014). The soil micro-food web, which consists of soil microbial and nematode communities and plays important roles in belowground ecological processes, has received much attention in the last decade (Kardol et al., 2016; Ranoarisoa et al., 2020; Guan et al., 2021). However, studies on the structure and functions of the soil micro-food web during forest succession are still lacking.

As a component of the soil detrital food web, the soil micro-food web includes diverse biotic communities from multiple trophic levels and functional guilds, such as soil microbes (e.g., bacteria and fungi), soil microbivores (e.g., bacterivorous and fungivorous nematodes) at low trophic levels, and soil micropredators (e.g., omnivore-predator nematodes) at high trophic levels (Richter et al., 2019; Wang et al., 2020). Previous studies have shown that the plant community exhibits strong effects on the soil food web structure (Bezemer et al., 2010; Eisenhauer et al., 2013). Plant root and leaf litter, which provide essential nutrient resources for soil micro-food web organisms, are reported to regulate the soil micro-food web in various ways (Sauvadet et al., 2016; Fu et al., 2017). Thus, vegetation regeneration during forest succession may also result in changes in the soil micro-food web. As components of the soil micro-food web, soil microbes and nematodes have shown significant responses to forest succession (Qiang et al., 2021; Liu et al., 2022b; Zhang et al., 2022). However, these studies focused only on a single soil biota, which might limit our systematic knowledge of the responses of the belowground communities during forest succession. Given the intense trophic relationships between soil microbes and nematodes, an integrated study on the soil micro-food web during forest succession could facilitate a comprehensive understanding of the impacts of forest succession on belowground ecosystems.

Forest succession from logged lands to secondary forests has exhibited high efficiency and advantages in recovering the supply of ecosystem services (Teixeira et al., 2020; Cortés-Calderón et al., 2021). This is largely ascribed to the important roles of secondary forests in maintaining and conserving biodiversity (Balvanera et al., 2006; Maestre et al., 2012), not only for aboveground but also for belowground communities (Kardol et al., 2016). Microbial and nematode communities in belowground environments can be directly or indirectly involved in multiple soil ecological processes, such as organic matter decomposition and soil carbon (C) and nitrogen (N) mineralization (Wang et al., 2017; Ranoarisoa et al., 2018; Wu et al., 2019). Soil C and N mineralization, which serve as important ecosystem functions, greatly affect soil carbon emissions and provide essential nutrients for plant growth (Manzoni and Porporato, 2009; Wang et al., 2020). Thus, the structure and characteristics of the soil micro-food web, such as the abundances of functional guilds, the metabolic footprints of soil nematodes and the connectance of the soil micro-food web, are often used to analyse and evaluate the variations in ecological functions this micro-food web provides (Zhang et al., 2017a; Guan et al., 2018; Wu et al., 2021). However, empirical evidence of the relationship between the soil micro-food web structure and ecosystem functions during forest succession remains insufficient. The incomplete understanding of the quantitative evaluations of ecosystem functions and the effects of the soil micro-food web structure on ecosystem functions during forest succession limits our ability to explore the potential belowground mechanisms regulating forest ecosystem functions across secondary succession.

In this study, we examined how forest secondary succession affected the soil micro-food web (including microbes and nematodes) and its associated ecosystem functions (soil C and N mineralization) in the subalpine region of southwestern China. These subalpine secondary forests, located on the eastern part of the Qinghai-Tibet Plateau, gradually formed on the logged lands after severe deforestation from the 1910s to the 1990s (Liu, 2002). The secondary successional sequence of “grasslands – shrublands – broadleaf forests – coniferous forests” that formed in this area therefore provides a natural platform for our study (Liu et al., 2022b; Zhang et al., 2022). Hence, our aim was to determine the variations in the soil micro-food web structure and functions and to explore the relationships between the soil micro-food web structure and ecosystem functions during forest succession on logged lands. Along the forest successional sequence, we hypothesized that 1) the composition of the soil micro-food web is increasingly abundant and diverse and the structure is increasingly complex because of the close linking between aboveground and belowground communities and 2) ecosystem functions are positively related to the soil micro-food web abundance and diversity because abundant soil microbial and nematode communities will promote the mineralization of nutrients.

## 2. Materials and methods

### 2.1. Study site

This study was conducted in the Miyaluo forest of Lixian County in Sichuan, China (31°43'N; 102°44'E; 3000 m above sea level). This area belongs to the subalpine valley area that transitions from the Tibetan Plateau to the Sichuan Basin, which is characterized by a montane monsoon climate (Cao et al., 2020). The annual mean temperature is approximately 8.7 °C (ranging from 0.6 °C in January to 16.4 °C in July), and the annual precipitation fluctuates between 600 and 1100 mm (Xu et al., 2021). The soils are classified as a mountain brown soil series in Chinese soil taxonomy (Cao et al., 2020). In this area, the northeastern slope of a mountain, where secondary succession has taken place on this previously logged land, was selected as a representative study site. To investigate the soil micro-food web and its relationship with soil C and N mineralization during secondary succession of subalpine forests, we selected a successional sequence consisting of grassland (approximately 20 years), shrubland (30 to 40 years), broadleaf and coniferous forests (>50 years) at this site. The grassland is dominated by *Rumex nepalensis*, *Anemone rivularis* and multiple genera of Poaceae; the shrubland is dominated by *Malus kansuensis*, *Quercus semecarpifolia*, *Berberis sichuanica* and *Rosa sweginzowii*; the broadleaf forest is dominated by *Betula platyphylla* and *Betula albosinensis*; and the coniferous forest is dominated by *Picea asperata* and *Abies squamata*.

### 2.2. Soil sampling

Soil sampling was conducted in July 2021. For each successional stage, including grassland, shrubland, broadleaf forest and coniferous forest, five randomly established plots were spaced >20 m apart. The plots at different stages were spaced about 100 m apart. The sizes of these plots distributed in random blocks were 2 × 2 m<sup>2</sup> in the grassland, 5 × 5 m<sup>2</sup> in the shrubland and 10 × 10 m<sup>2</sup> in the forest. Before soil sampling, the surface plant litter was removed. Then, three soil cores (diameter 5 cm, depth 20 cm) were drilled and collected. Soil samples within each plot were homogenized into one sample. After roots and stones were removed, the soil sample was passed through a 2 mm sieve and separated into two parts. One part was kept fresh to determine the soil moisture, soil microbial and nematode communities and soil C and N mineralization rates, and the other part was air-dried to determine the soil physicochemical properties (except soil moisture).

### 2.3. Soil physicochemical properties

Soil pH was measured in a 1:2.5 (w:v) soil:deionized water suspension using a digital pH meter (FE28-Standard, Mettler-Toledo, Switzerland).

The soil moisture (SM) was measured by oven-drying fresh soil samples at 105 °C for 24 h. Soil organic carbon (SOC), dissolved organic carbon (DOC) and readily oxidizable carbon (ROC) were determined using the potassium dichromate heating method, a TOC/TN analyser (Multi N/C 2100, Analytik, Jena, Germany) and the  $\text{KMnO}_4$  (333 mM) oxidation procedure, respectively. Soil total nitrogen (TN) was determined by the Kjeldahl digestion method. Ammonium N ( $\text{NH}_4^+$ -N) and nitrate N ( $\text{NO}_3^-$ -N) were extracted from soils using a 2 M KCl solution and were measured by the indophenol blue and phenol disulfonic acid methods, respectively, with a microplate reader (Varioskan LUX, Thermo Scientific, America).

#### 2.4. Soil microbial community composition

The composition of the soil microbial communities was determined by phospholipid fatty acid (PLFA) analysis as described by Guan et al. (2021). We extracted fatty acids from approximately 3 g of freeze-dried soil and analysed them using an Agilent 490 gas chromatograph (Agilent Technologies) equipped with flame ionization detection (FID). Quantification was performed using an external standard of 19:0 methyl ester, and the abundance of each individual fatty acid was expressed as PLFAs  $\text{nmol g}^{-1}$  dry soil. The following biomarkers were used: bacteria (i14:0, a15:0, i15:0, a16:0, i16:0, 16:1 $\omega$ 7c, 16:1 $\omega$ 9c, a17:0, i17:0, 17:1 $\omega$ 8c, cy17:0, 18:1 $\omega$ 5c, 18:1 $\omega$ 7c), fungi (18:1 $\omega$ 9c, 18:2 $\omega$ 6,9c, 18:3 $\omega$ 6c), actinomycetes (Act) (10Me16:0, 10Me17:0, 10Me18:0) and arbuscular mycorrhizal fungi (AMF) (16:1 $\omega$ 5c) (Hu et al., 2017; Wang et al., 2020; Guan et al., 2021). The PLFAs were converted into microbial biomass carbon using the following formulas: bacterial biomass, 363.6  $\text{nmol PLFA} = 1 \text{ mg carbon}$ ; fungal biomass, 11.8  $\text{nmol PLFA} = 1 \text{ mg carbon}$ ; and AMF biomass, 1.047 neutral lipid fatty acid = 1  $\mu\text{g carbon}$  (Guan et al., 2018; Wu et al., 2021).

#### 2.5. Soil nematode community composition

Soil nematodes were extracted from 100 g of fresh soil sample using a modification of the shallow dish method (Liu et al., 2022a). The collected nematodes were killed and fixed in 4 % formalin solution, and the nematode suspensions were stored in 10 ml centrifuge tubes and then counted with a dissecting microscope (BXT-1304, Bingyu, China). After the total number of nematodes was counted, at least 100 randomly chosen individuals from each sample were identified to the genus level with an inverted biological microscope (BMC500, Phenix, China) at 400 $\times$  magnification. All nematodes were identified when there were <100 individuals in a sample. The identified nematodes were assigned to four trophic groups, i.e., bacterivores (Ba), fungivores (Fu), herbivores (He) and omnivore-predators (Op), according to Yeates et al. (1993). The abundance of total nematodes and trophic groups was expressed as the number of individuals per 100 g dry soil.

The Shannon–Wiener index,  $H' = -\sum P_i(\ln P_i)$ , was used as an indicator of soil nematode diversity, where  $P_i$  is the proportion of the  $i$ th genus in a sample (Freckman and Ettema, 1993). The maturity index (MI) and plant parasitic index (PPI) were calculated using the same formula:  $\text{MI (PPI)} = \sum V_i \times P_i$ , where  $V_i$  is the colonizer-persister (c-p) value of genus  $i$  (Bongers, 1990; Domínguez-Begines et al., 2019). Each nematode had a corresponding c-p value ranging from enrichment colonizers (c-p 1) and disturbance colonizers (c-p 2) to persisters (c-p 5) based on its genus classification (Bongers, 1990; Shaw et al., 2019), which was used to calculate the MI and PPI. Moreover, the MI was calculated using the abundance of free-living nematodes, while the PPI was calculated using the abundance of herbivores. Additionally, the fresh weight of the soil nematodes was obtained from the database of the Nematode Ecophysiological Parameter (<http://nemaplex.ucdavis.edu>) (Li et al., 2020). It is generally assumed that 20 % of the fresh weight of the soil nematodes was the dry weight and 52 % of their dry weight was the carbon content. Thus, the carbon content in nematode biomass ( $\text{NeC} = 52 \% \times 20 \% \text{ Wt}/100 (\mu\text{g/g})$ , where Wt indicates the fresh weight of soil nematodes (Ferris, 2010; Guan et al., 2018). The

connectance (C) of a food web is an indicator to quantify the trophic interactions within the food web channel and is believed to reflect its stability (Sánchez-Moreno et al., 2011; Ferris et al., 2012). After assuming that all predators in the soil sample are free to hunt, the realized connectance ( $S = ab$ , where  $a$  and  $b$  are the numbers of occurrences of any predator and any prey in each sampling plot of one successional stage, respectively; the potential connectance ( $F = AB$ , where  $A$  and  $B$  are the numbers of occurrences of predator and prey groups in all sampling plots of one successional stage; thus, connection ( $C = S/F$ ) (Guan et al., 2018; Wu et al., 2021).

#### 2.6. Soil C and N mineralization rates

Soil C and N mineralization rates were measured by aerobic incubation as described by Wang et al. (2020). Briefly, for each soil sample, 20 g of fresh soil was incubated in a 100 ml specimen cup. The cup was covered with a small, perforated plastic cap to allow gas exchange and reduce water evaporation. Then, the specimen cups were placed in a constant temperature (25 °C) incubator in the dark for 21 days. The specimen cups were weighed and watered periodically (every two to three days) to keep the soil samples at the original moisture. After 1, 2, 3, 7, 11, 15 and 21 days of incubation, soil  $\text{CO}_2$  efflux was measured using a Li-Cor 830  $\text{CO}_2$  gas analyser. The  $\text{CO}_2$  release rate was calculated as the slope of the regression line of the  $\text{CO}_2$  efflux in each measurement. Thus, the soil C mineralization rate ( $\mu\text{g/g/d}$ ) was calculated using the average  $\text{CO}_2$  release rate over the incubation period, the volume of the specimen cup and the dry weight of the soil sample. At the end of the incubation, the  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N concentrations of the soil samples in the specimen cups were determined using the same method as described above. Given the original concentrations of soil  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N we measured, the soil N mineralization rate ( $\mu\text{g/g/d}$ ) was calculated as the change in the total inorganic nitrogen content before and after incubation.

#### 2.7. Statistical analysis

One-way analysis of variance (ANOVA) was performed to examine the influences of forest succession on soil microbial PLFA biomass (total PLFAs, bacteria, fungi, actinomycetes, AMF), soil nematode abundance (total nematodes and functional guilds), soil properties (SM, pH, SOC, ROC, DOC, TN,  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N) and ecosystem functions (soil C and N mineralization rates). The least significant difference (LSD) post hoc test was applied to check for significant differences among different forest successional stages at the  $P < 0.05$  level. The composition of soil microbial and nematode communities in relation to forest successional stages was explored by principal component analysis (PCA) based on the relative abundances (hellinger transformed) of microbial PLFAs and nematode genera, respectively. The variations in soil microbial and nematode community composition were tested by permutational analysis of variance (PERMANOVA). The first principal component (PC1) was used to indicate the community structure, i.e., microbial community structure (MCS) and nematode community structure (NCS). Stepwise multiple regression (SMR) was performed to determine significant factors that affected the soil microbial and nematode communities. Linear regression analysis was applied to examine the relationships between the soil micro-food web structure and ecosystem functions. Partial least squares path modelling (PLS-PM) was applied to reveal the relationships among forest succession, soil environment (indicated by SM and pH), soil nutrients (indicated by SOC, ROC, DOC, TN,  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N), soil microbes (indicated by total microbial biomass and MCS), soil nematodes (indicated by total nematode abundance, nematode genus richness and NCS) and ecosystem functions (indicated by soil C and N mineralization rates) using the function 'inner plot' in the R package 'plsmpm' (Sanchez et al., 2015). The direction and strength of the relationships between these latent variables are represented by the arrows and the path coefficients on them. The degree of explanation ( $R^2$ ) of each variable was also provided in the PLS-PM, and the models were

assessed using the goodness of fit (GOF) statistic. The carbon flow channels in the soil micro-food web during forest succession were also analysed by PLS-PM. Except for the PCA conducted using the Microbiome Database (<http://egcloud.cib.cn/index-cn.html>), all statistical analyses were performed using R-4.0.3 software (R Core Team, 2020).

### 3. Results

#### 3.1. Soil micro-food web composition changes with forest succession

Soil microbial PLFA biomass was significantly affected by forest succession ( $P < 0.05$ , Fig. 1). The PLFA biomasses of total microbes, bacteria, fungi, Act and AMF exhibited similar trends of coniferous forest > shrubland > broadleaf forest > grassland. In total, 37 nematode genera were identified in the soils at the four successional stages (Table S1). Although no significant differences were found for the abundance of total nematodes among successional stages, the nematode trophic group abundances were significantly affected by forest succession, particularly those of Ba3, He5 and Op5 ( $P < 0.05$ , Table 1). Forest succession also had marginally significant effects on the abundances of total bacterivores, Ba2, Ba4 and He3. Compared with soils in the grassland, the soils in the shrubland, broadleaf forest and coniferous forest had higher abundances of microbivores and omnivore-predators. In contrast, herbivore abundance showed a decreasing trend with forest succession. Additionally, Fu3 (i.e., *Diphtherophora*) and He5 (i.e., *Xiphinema*) were specific nematode guilds in the shrubland and grassland, respectively. Principal component analysis also showed significant differences in the soil microbial and nematode community compositions among the forest successional stages (Fig. 2). The genus richness, Shannon's diversity index and maturity index of the nematode community significantly increased during forest succession, while the plant parasitic index significantly decreased (Fig. S1). The connectance value of the soil micro-food web gradually increased, but not significantly, with forest successional development (Fig. S2).

#### 3.2. Relationships between ecosystem functions and the soil micro-food web

The highest soil C and N mineralization rates were found in the coniferous forest and shrubland, followed by the broadleaf forest and grassland (Fig. 3A and C). Compared with the grassland, the soil C mineralization rate increased approximately fivefold in the broadleaf forest and ten times in the shrubland and coniferous forest, while the soil N mineralization rate increased approximately threefold in the broadleaf forest and sixfold in the shrubland and coniferous forest. In addition, both soil C and N mineralization rates were remarkably and positively related to total nematode abundance, nematode diversity, nematode genus richness and total microbial biomass (Fig. 3B and D). Moreover, the soil C and N

mineralization rates were positively related to the PLFA biomass of all microbial communities and the abundances of most nematode trophic groups (Figs. S3 and S4). In contrast, no significant relationships were noted between the soil C and N mineralization rates and herbivore abundance (Fig. S4H).

#### 3.3. Relative effects of forest succession, soil properties and the soil micro-food web on ecosystem functions

PLS-PM was used to reveal the relationships among forest succession, soil environment, soil nutrients, soil microbial communities, soil nematode communities and ecosystem functions (Fig. 4). The PLS-PM analysis indicated that forest succession directly impacted the soil environment and soil nutrients and thus altered the soil micro-food web and further regulated the ecosystem functions (Fig. 4A). The variances in the soil microbial community were determined by both the soil environment and soil nutrients (path coefficients: 0.62 and 0.25, respectively, Fig. 4A). In contrast, the variances in the soil nematode community were directly determined by soil nutrients (0.42, Fig. 4A). The variances in ecosystem functions were mainly determined by soil nutrients (0.92) and were partly determined by soil microbial and nematode communities (0.36 and  $-0.33$ , respectively, Fig. 4A). Soil nutrients exhibited the strongest total effects on ecosystem functions, followed by forest succession and the soil micro-food web (Fig. 4B). Additionally, soil nutrients together with the soil micro-food web directly determined soil C mineralization, while soil N mineralization was mainly determined by soil nutrients without significant effects of the soil micro-food web (Fig. S5).

In the carbon flow channels through the soil micro-food web during forest succession explored by the PLS-PM, the path modelling explained 66 % of the variances in both soil bacterial and fungal biomass carbon and 34–72 % of the variances in the biomass carbon of the soil nematode trophic groups (Fig. 5A). Among the possible carbon flow paths from the carbon substrate (i.e., SOC, ROC and DOC) to the biomass carbon of the omnivore-predators at the highest trophic level of the soil micro-food web, we found that soil bacteria and bacterivores may have played dominant roles.

## 4. Discussion

#### 4.1. Effects of forest succession on the soil micro-food web

The soil micro-food web structure differed significantly during forest secondary succession, which was characterized by increasing microbial biomasses and nematode abundances and diversity. In line with previous studies showing greater soil microbial biomass after afforestation (Zhang et al., 2019; Zheng et al., 2019; Hu et al., 2020), our results showed that the soil

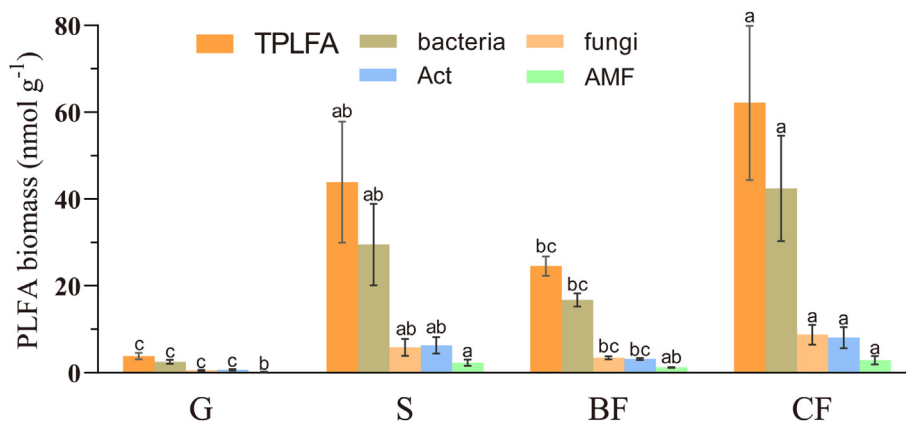


Fig. 1. Microbial PLFA biomass ( $\text{nmol g}^{-1}$ ) at different successional stages. TPLFA, total microbial biomass; Act, actinomycetes; AMF, arbuscular mycorrhizal fungi; G, grasslands; S, shrublands; BF, broadleaf forests; CF, coniferous forests. Different lowercase letters indicate significant differences among different successional stages for each microbial PLFAs ( $P < 0.05$ ).



**Table 1**  
Abundances of total and different nematode trophic groups (ind. 100 g<sup>-1</sup> dry soil) at different successional stages.

Stage	TNA	Bacterivores				Fungivores				Herbivores			Omnivore-predators						
		Total	Ba1	Ba2	Ba3	Ba4	Total	Fu2	Fu3	Fu4	Total	He2	He3	He5	Total	Op3	Op4	Op5	
G	141.5	26.9 b	–	26.5 b	0.4 b	–	35.1	33.6	–	1.5	67.7	0.6 b	55.8 a	11.3	11.8 b	–	11.8	–	
S	592.7	207.9 a	18.8	148.6 a	32.9 a	7.5 ab	52.8	40.4	1.7	10.8	67.5	17.2 ab	50.2 a	–	264.5 a	15.2	215.7	33.7 ab	
BF	354.6	88.9 ab	31.0	49.4 b	8.2 b	0.3 b	118.4	95.8	–	22.6	47.9	13.0 ab	35.0 ab	–	99.4 ab	6.2	82.0	11.3 b	
CF	445.1	140.8 ab	16.0	93.0 ab	15.9 ab	15.8 a	94.9	67.4	–	27.6	32.8	31.8 a	1.0 b	–	176.6 ab	23.2	106.4	46.9 a	
ANOVA																			
F	1.44	3.11	0.78	2.71	4.09	2.80	0.89	0.79	2.45	0.97	1.02	1.91	3.01	57.6	2.01	1.53	1.72	3.40	
P	0.269	0.056	0.524	0.080	0.025	0.073	0.469	0.519	0.101	0.432	0.409	0.169	0.061	0.000	0.154	0.244	0.204	0.044	

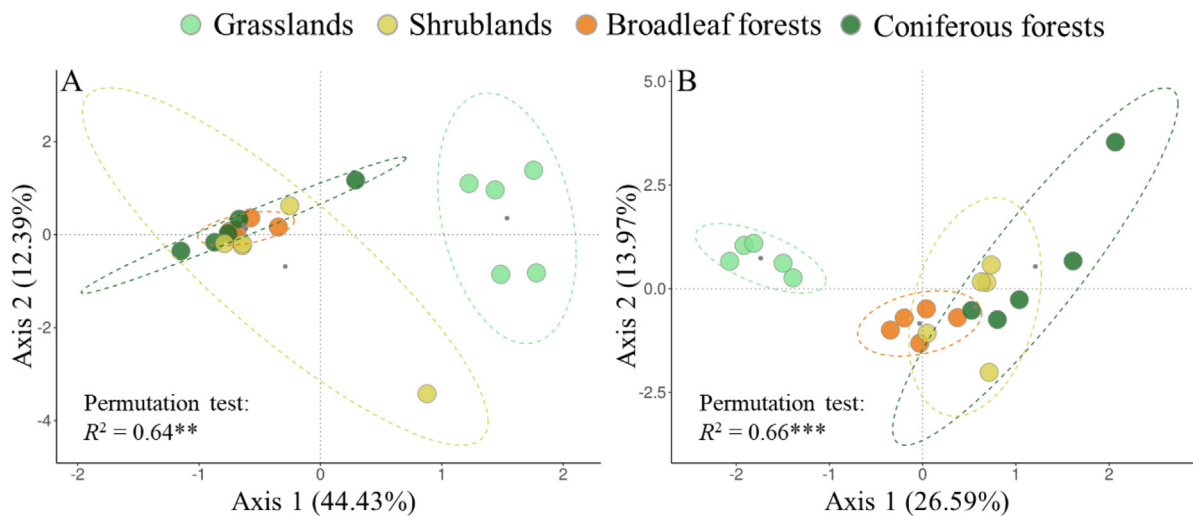
G, grasslands; S, shrublands; BF, broadleaf forests; CF, coniferous forests; TNA, total nematode abundance; Ba, bacterivores; Fu, fungivores; He, herbivores; Op, omnivore-predators. The numbers following the trophic groups represent the c-p values. Different lowercase letters indicate significant differences among different successional stages for each nematode trophic group ( $P < 0.05$ ).

microbial biomass exhibited a general increasing trend from the early-successional grassland to the late-successional coniferous forest in our study. However, an unexpected result deviating from our first hypothesis was that shrubland soil had the second highest microbial biomass, which was higher than that of broadleaf forest soil. Likewise, Yan et al. (2020) found that shrubs had stronger effects than trees and herbs on the accumulation of microbial biomass in shallow karst fissures, which was ascribed to the dense lateral roots of shrubs that effectively reduced runoff infiltration. Together with these studies, our results implied that plant communities at a certain successional stage could have important effects beyond expectations on soil microbial biomass, such as the mid-successional shrubland dominated by various plant species (*Malus kansuensis*, *Quercus semecarpifolia*, *Berberis sichuanica* and *Rosa sweginzowii*) at our study sites. In addition, consistent with the findings of previous studies conducted in another subalpine region (Yoshitake et al., 2013; Xiong et al., 2021), our results showed that compared with fungal biomass, bacterial biomass contributed more to the increase in total microbial biomass during subalpine forest succession. This might be because bacteria have a considerably shorter turnover time than fungi (Kuz'yakov, 2010; Ren et al., 2021), so bacterial biomass could be more strongly promoted by elevated nutrient availability than fungal biomass during forest succession.

In contrast to our results, a previous study in this subalpine area reported that the abundances of total nematodes and microbivores significantly increased from grassland and shrubland to forest (Liu et al., 2022b). This difference in the variation trend in nematode abundances might be associated with the environmental conditions of the sampling sites, as the sampling site in this study was located at a lower altitude

(3000 m above sea level) than that of Liu et al. (2022b) (3680 m above sea level). Moreover, compared with the findings reported by Liu et al. (2022b), the relatively higher species richness and biomass in the shrubland in this study could have contributed to higher nutrient inputs into the soils, which improved the nematode abundance. In contrast, Mondino et al. (2011) showed that the total nematode abundance in ridge forest soils was greater than that in herbaceous floodplain soils, which was greater than that in thicket soils. These results indicated that the variations in nematode abundance during forest succession were highly heterogeneous, depending on the environmental conditions and vegetation characteristics (including plant communities and soil properties). In addition, we found that the abundances of the nematodes with high c-p values in each trophic group (e.g., Ba3, He5 and Op5), rather than the abundances of those with low c-p values (e.g., Ba1, He2, Op3 and Op4), were significantly influenced by forest succession, probably because the nematodes with high c-p values are more sensitive to environmental disturbance than those with low c-p values (Bongers and Bongers, 1998; Shaw et al., 2019).

In this study, the principal component analysis results agree with previous researches suggesting that the change in plant species during succession could have significant effects on soil microbial and nematode communities (Cesarz et al., 2013; Qiang et al., 2021; Liu et al., 2022b). Confirming our first hypothesis, significantly increased genus richness, Shannon–Wiener index and maturity index of soil nematodes were observed during successional development, indicating a more diverse soil micro-food web as the environmental disturbance gradually stabilized (Li et al., 2015; Sun et al., 2019). The increasing, although not significant, connectance of the soil micro-food web also suggested that the soil micro-food web was becoming



**Fig. 2.** Principal components analysis (PCA) of soil microbial and nematode communities during forest succession, based on the relative abundances of each phospholipid fatty acids (PLFA) (A) and nematode genera (B). Percentage explained variance of each axis is also presented. The lower left corner of each PCA figure shows the permutation test result; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

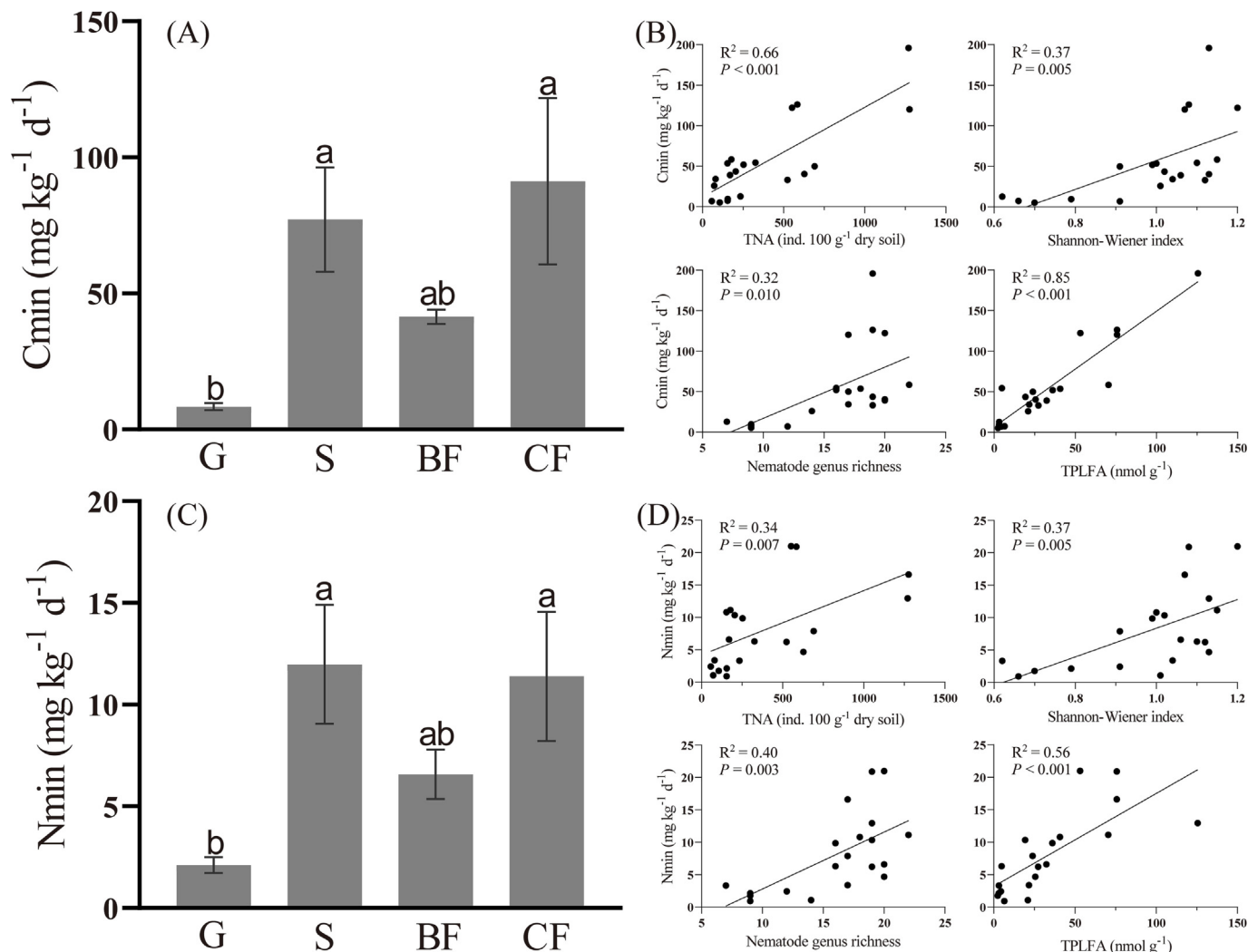
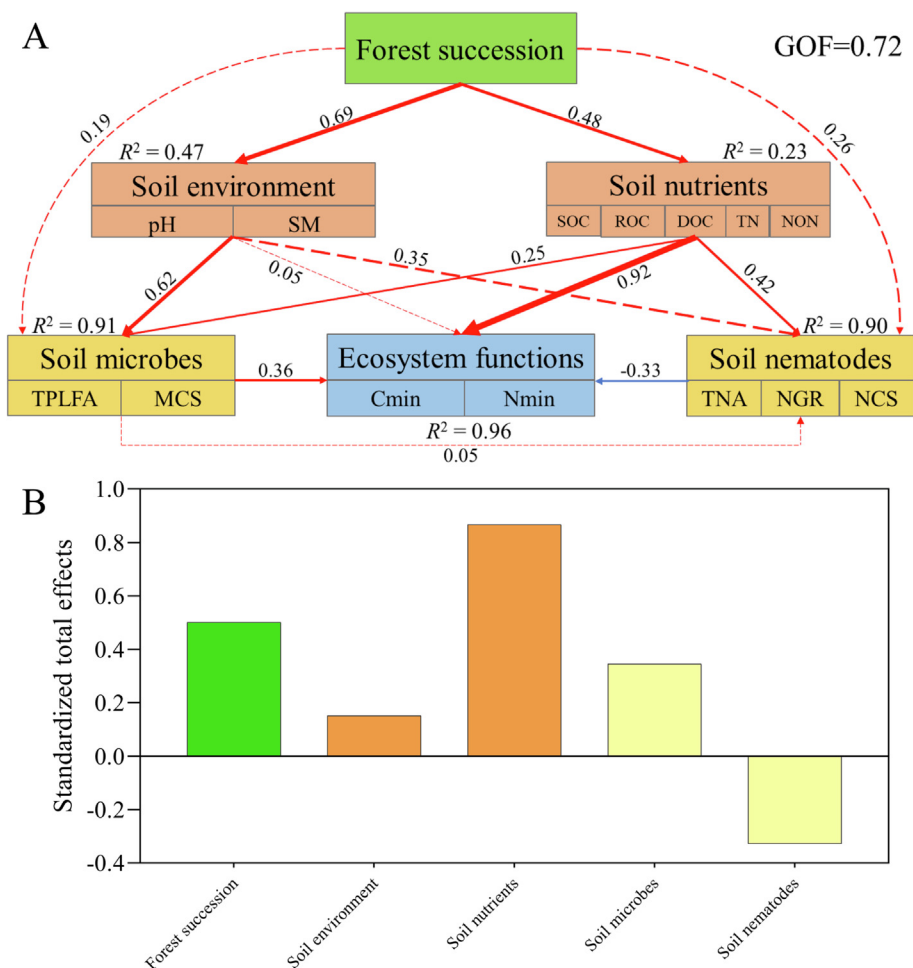


Fig. 3. Soil carbon (A) and nitrogen (C) mineralization rates (mg kg<sup>-1</sup> d<sup>-1</sup>) at different successional stages, and linear regressions between soil carbon (B) or nitrogen (D) mineralization rates and soil nematode or microbial communities. G, grasslands; S, shrublands; BF, broadleaf forests; CF, coniferous forests; Cmin, carbon mineralization rate; Nmin, nitrogen mineralization rate; TNA, total nematode abundance; TPLFA, total microbial biomass. Different lowercase letters indicate significant differences among different successional stages ( $P < 0.05$ ).

more stable during forest succession (Guan et al., 2018). Corroborating previous studies (Susyan et al., 2011; Wu et al., 2021; Liu et al., 2022b), our results further confirmed that forest succession, particularly the restoration of secondary forests, could have remarkably promoting effects on soil biodiversity and micro-food web stability. In addition, by identifying the significant soil variables regulating the soil microbial and nematode communities using the stepwise multiple regression method, we found that soil nutrients, including soil carbon and nitrogen, showed remarkable impacts on the soil microbial and nematode communities (Table 3). Soil carbon and nitrogen serve as important resources that impact the growth and reproduction of soil microbial and nematode communities (Sul et al., 2013; Ng et al., 2014; Pan et al., 2020). Thus, the elevated resource availability (except soil NO<sub>3</sub><sup>-</sup>-N) during forest succession (Table 2) might greatly contribute to the abundance and diversity of the soil micro-food web composition and structure. In contrast, the nematode genus richness was exclusively affected by the soil environment (i.e., soil moisture and pH). The soil moisture content provides essential habitat conditions for soil nematodes, which often have significant promoting effects on nematode genus richness (Song et al., 2016). Likewise, soil pH was generally reported to influence soil nematode communities (Holland et al., 2018; Pan et al., 2022). Overall, our results implied that the bottom-up effects (resource control) mediated by soil nutrients could play an important role in regulating the soil micro-food web during forest succession.

#### 4.2. Effects of forest succession on ecosystem functions

In this study, we found that the soil C and N mineralization rates exhibited general increasing trends during forest succession. Our results confirmed the view that forest secondary succession could have played an important role in promoting soil nutrient cycling and ecosystem functions (Shimamoto et al., 2018; Teixeira et al., 2020). In previous studies, above-ground plant species richness and biodiversity were generally expected to be positively correlated with ecosystem functions (Balvanera et al., 2006; Eisenhauer et al., 2013). However, consistent with our second hypothesis, significant positive relationships were noted between the soil micro-food structure and ecosystem functions. The positive correlations between nematode diversity and the soil C and N mineralization rates suggested that positive biodiversity effects on ecosystem functions also existed in the belowground ecosystem. Additionally, the soil C and N mineralization rates were significantly and positively related to the abundances of most nematode trophic groups (except herbivores) and the biomass of soil microbes in our study. Consistent with our results, previous studies also suggested that soil microbial and nematode communities could promote soil nutrient availability (Gebremikael et al., 2016; Rashid et al., 2016). In contrast, herbivorous nematodes always interact with plants (Wondafraash et al., 2013; Hauri and Szendrei, 2022) and therefore exhibited nonsignificant correlations with the soil C and N mineralization rates in our study.



**Fig. 4.** Partial least squares path modelling (PLS-PM) showing the effects of forest succession on ecosystem functions via soil environment, soil nutrients, soil microbes and soil nematodes (A), and standardized total effects of these variables on ecosystem functions (B). Numbers adjoining the arrows indicate standardized path coefficients, and the arrow width is proportional to the strength of the association. Red arrows represent positive relationships and blue arrows represent negative relationships. The solid arrows represent significant ( $P < 0.05$ ) relationships and the dashed arrows represent nonsignificant relationships.  $R^2$  values indicate the variance of variables accounted for by the model. SM, soil moisture; SOC, soil organic carbon; ROC, readily oxidizable organic carbon; DOC, dissolved organic carbon; TN, total nitrogen; NON,  $\text{NO}_3^-$ -N; TPLFA, total microbial biomass; MCS, microbial community structure; TNA, total nematode abundance; NCS, nematode community structure; NGR, nematode genus richness; Cmin, carbon mineralization rate; Nmin, nitrogen mineralization rate; GOF, goodness of fit.

Through the PLS-PM that illustrated the regulation paths of forest succession with respect to the ecosystem functions, we found that the variations in the ecosystem functions induced by forest succession were significantly and directly determined by soil nutrients and soil microbial and nematode communities, among which soil nutrients may have had the strongest effects (Fig. 5). Soil nutrients, including soil C and N, served as the main resource substance and could exhibit potential bottom-up effects on nutrient mineralization. The increase in soil nutrients was generally expected to promote soil C and N mineralization (Nave et al., 2009; Watts et al., 2010). Additionally, our results showed that soil nutrients exhibited significant indirect effects on ecosystem functions via soil microbial and nematode communities. A growing body of studies has recognized the important contributions of soil biota to soil nutrient mineralization (Holtkamp et al., 2011; Frouz, 2018) and concluded that it is essential to incorporate soil biota into soil C and N cycling models (de Vries et al., 2013). Consistently, through our path modelling of soil C and N mineralization, we found that the soil micro-food web played a significant role in affecting the ecosystem functions (indicated by soil C and N mineralization) induced by forest succession, confirming that incorporating soil micro-food web data into soil C and N cycling models of forest succession can yield informative results. However, we found that the soil microbial and nematode communities significantly affected soil C mineralization rather than N mineralization

(Fig. S5). This might be associated with our study area being characterized by N limitation (Zhang et al., 2017b). The relatively low N content in soils might impose restrictions on the contribution of the soil micro-food web to N mineralization, implying that the ecological functions of the soil micro-food web might be limited to some extent by the resource level. Collectively, our results suggested that the increase in soil nutrient contents was the main driving force in the recovery of ecosystem functions during forest succession, while the soil micro-food web also played an important role in affecting ecosystem functions.

The carbon flow through the soil food web might be driven by the feeding interactions among soil biotic communities and could be indirectly inferred by the biomass of soil biotic communities in the soil food web (Albers et al., 2006; Lenoir et al., 2007; Zhang et al., 2015). In this study, we found that forest secondary succession significantly regulated the carbon flow through the soil micro-food web via indirect effects on bacterial and fungal channels and direct effects on herbivorous nematodes. These results demonstrated that the energy flow channels of the soil food web during forest secondary succession were dominated by the predator-prey channel, from one trophic level to the next. For herbivorous nematodes, however, the resource input from plant communities instead of predation might be more dominant. More importantly, our results suggested that the bacterial channel showed a remarkable effect on

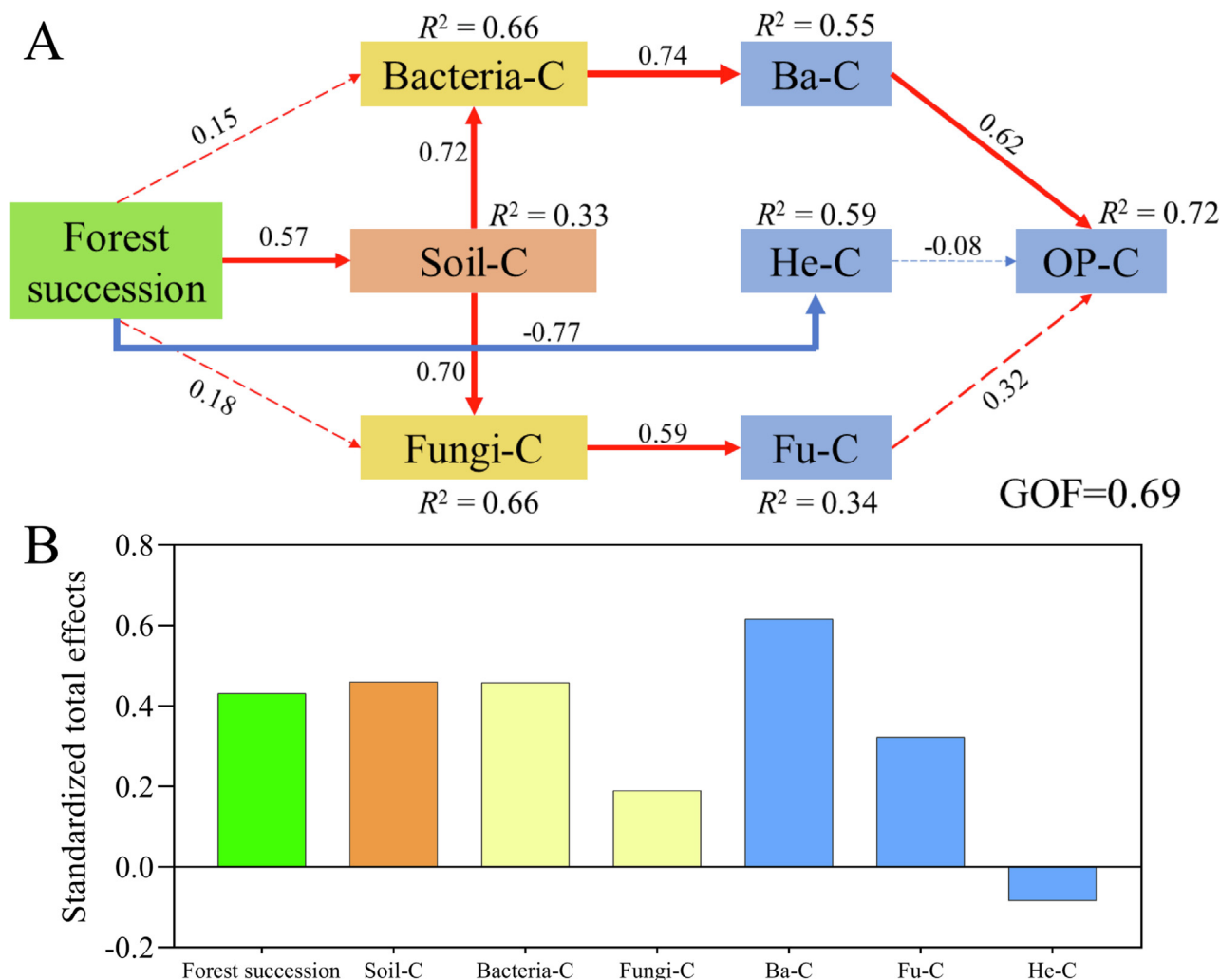


Fig. 5. Carbon flow in the soil micro-food web in response to forest succession explored by partial least squares path modelling (PLS-PM) (A), and standardized total effects of these variables on OP-C (B). Numbers adjoining the arrows indicate standardized path coefficients, and the arrow width is proportional to the strength of the association. Red arrows represent positive relationships and blue arrows represent negative relationships. The solid arrows represent significant ( $P < 0.05$ ) relationships, and the dashed arrows represent nonsignificant relationships.  $R^2$  values indicate the variance of variables accounted for by the model. Soil-C is indicated by SOC (soil organic carbon), ROC (readily oxidizable organic carbon) and DOC (dissolved organic carbon). Bacteria-C, bacterial biomass carbon; Fungi-C, fungal biomass carbon; Ba-C, bacterivore biomass carbon; He-C, herbivore biomass carbon; Fu-C, fungivore biomass carbon; OP-C, omnivore-predator biomass carbon; GOF, goodness of fit.

omnivore-predator biomass carbon, whereas the fungal channel did not, implying an important role of bacteria and bacterivores in the carbon flow through the soil micro-food web during subalpine forest succession, which aligns with the findings of previous studies (Hu et al., 2016; Wu et al., 2021). In our study, the remarkable effects of the bacterial channel on omnivore-predator biomass carbon might contribute greatly to the

accumulation of omnivore-predators at the higher trophic level, given the high abundances of bacteria and bacterivores. These results implied that the bacterial channel of the carbon flow through the soil micro-food web might, to some extent, play an important role in improving the complexity and stability of the soil micro-food web during forest secondary succession.

**Table 3**  
Model summary for the stepwise multiple regression (SMR) of soil nematode and microbial communities on the soil properties.

Soil fauna	Community parameter	Adj. $R^2$ of full model	Contribution of the individual soil variable (%)						
			SOC	ROC	DOC	TN	NHN	SM	pH
Nematodes	TNA	0.563	100.0						
	NCS	0.759			63.7			20.5	15.8
	NGR	0.584						67.8	32.2
	H'	0.619		71.0					29.0
Microbes	TPLFA	0.882		67.0		22.9	10.1		
	MCS	0.880	38.2			11.5		14.4	35.9

TNA, total nematode abundance; NCS, nematode community structure; NGR, nematode genus richness; H', Shannon-Wiener index; TPLFA, total microbial biomass; MCS, microbial community structure; SOC, soil organic carbon; ROC, readily oxidizable organic carbon; DOC, dissolved organic carbon; TN, total nitrogen; NHN,  $\text{NH}_4^+\text{-N}$ ; SM, soil moisture.



**Table 2**

Soil properties (mean ± SE) at different successional stages.

Soil properties	Successional stages				ANOVA	
	Grasslands	Shrublands	Broadleaf forests	Coniferous Forests	F	P
pH	5.97 ± 0.05 b	6.27 ± 0.11 a	6.43 ± 0.04 a	6.31 ± 0.07 a	7.45	0.002
SM (%)	14.78 ± 0.43 c	35.86 ± 3.68 a	24.76 ± 1.01 b	34.95 ± 5.01 a	9.87	<0.001
SOC (g kg <sup>-1</sup> )	19.88 ± 1.42 b	104.77 ± 15.09 a	53.67 ± 5.05 b	106.65 ± 26.59 a	7.38	0.003
ROC (g kg <sup>-1</sup> )	4.36 ± 0.56 c	38.65 ± 6.87 ab	17.77 ± 1.57 bc	40.05 ± 12.49 a	5.77	0.007
DOC (mg kg <sup>-1</sup> )	109.83 ± 2.95 b	148.51 ± 8.07 b	149.66 ± 7.00 b	244.88 ± 43.28 a	6.62	0.004
TN (g kg <sup>-1</sup> )	1.21 ± 0.07 b	5.45 ± 0.84 a	2.19 ± 0.20 b	4.87 ± 1.47 a	5.78	0.007
NHN (mg kg <sup>-1</sup> )	5.60 ± 0.28 b	2.63 ± 0.30 b	13.62 ± 2.87 a	16.83 ± 2.45 a	12.32	<0.001
NON (mg kg <sup>-1</sup> )	40.92 ± 3.00 b	310.50 ± 78.48 a	50.95 ± 14.41 b	90.06 ± 34.72 b	8.47	0.001

SM, soil moisture; SOC, soil organic carbon; ROC, readily oxidizable organic carbon; DOC, dissolved organic carbon; TN, total nitrogen; NHN, NH<sub>4</sub><sup>+</sup>-N; NON, NO<sub>3</sub><sup>-</sup>-N. Different lowercase letters indicate significant differences among different successional stages for each soil property ( $P < 0.05$ ).

## 5. Conclusion

Overall, our study demonstrated that forest succession exhibited significant effects on the composition and structure of the soil micro-food web, through which ecosystem functions were further regulated. The increasingly complex and stable soil micro-food web with forest successional development implied that the belowground biomes exhibited a generally synchronized trend with the aboveground biomes. Additionally, the obviously strong positive correlations between the soil C and N mineralization rates and the soil micro-food web composition and structure further confirmed the positive belowground biodiversity-ecosystem functioning relationships in the field study. Moreover, our PLS-PM results suggested that the soil micro-food web was significantly involved in affecting soil nutrient mineralization, highlighting the important contributions of the soil food web to ecosystem functions during forest succession. However, it must be noted that the present results regarding the soil micro-food web and ecosystem functions during forest succession are essentially context-specific and the extrapolation of these results should be considered with caution. Given the potential effects of environmental conditions and global climate change on the relationships between plant communities and the soil food web, larger-scale investigations across broad ranges of ecosystems and further in-depth explorations of the soil food web (including mesofauna) are still required in future studies.

## CRediT authorship contribution statement

**Jia Liu:** Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Funding acquisition, Writing – original draft, Writing – review & editing. **Kai Fang:** Formal analysis, Visualization, Writing – review & editing. **Yongping Kou:** Data curation, Writing – review & editing. **Ruixue Xia:** Data curation, Investigation. **Heliang He:** Data curation, Investigation. **Wenqiang Zhao:** Funding acquisition, Methodology, Writing – review & editing. **Qing Liu:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

## Data availability

Data will be made available on request.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.163257>.

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