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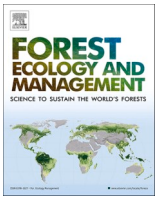

Nutrient enrichment shapes litter micro-food webs in a subtropical plantation

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ABSTRACT

Tropical and subtropical forests are important for terrestrial gross primary production. These forests are limited by nutrient availability and are vulnerable to nutrient enrichment under global change. However, little is known about how and why belowground biodiversity responds to nutrient enrichment during litter decomposition in these forests – the fundamental process fuelling nutrients to the soil system while supporting carbon sequestration. We conducted a 6-year field microcosm experiment and used a linear mixed effect to investigate the effects of nutrient enrichment on micro-food webs (i.e., microbes and nematodes) of leaf and root litters in a subtropical plantation. We found strong effects of nutrient enrichment on diversity and structure of microbes and nematodes during litter decomposition. For instance, fertilization (nitrogen+phosphorus; N + P) significantly decreased fungal richness of diversity (OTUs richness) throughout the decomposition process, and shifted the litter biota toward lower bacterial evenness of diversity (OTUs evenness), with higher relative abundances of fungi and herbivores at the humus-near stage. Nutrient enrichment also modulated leaf and root litter micro-food webs in different ways. NP addition had stronger positive effects on leaf litter bacterial oligotrophs:copiotrophs at the early stage, and stronger positive effects on root litter fungi:bacteria, but stronger negative effects on leaf litter fungal oligotrophs:copiotrophs at the humus-near stage. Overall, our results indicate that nutrient enrichment significantly alters microbes and microfauna associated with litter decomposition in subtropical forests, with important consequences for nutrient replenishment and soil organic carbon formation.

1. Introduction

Tropical and subtropical forests account for approximately one-third of the Earth's terrestrial gross primary production (Lewis et al., 2015), occupying a critical position in the functioning of terrestrial ecosystems. Native tropical ecosystems are often highly productive and geologically old environments with limited nutrient availability due to nutrient depletion during intensive heavy rainfall events (Du et al., 2020). Notably, tropical and subtropical regions are the major sinks for atmospheric nitrogen (N) and phosphorus (P) deposition (Galloway et al., 2004). It would be expected that human-induced NP enrichment alleviates NP limitations in these forests; however, recent meta-analyses and experimental studies suggest that NP enrichment has moderate to weak effects on plant growth and exerts no effect at the community level in

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tropical forests (Wright et al., 2018; Wright, 2019; Manu et al., 2022). The potential reasons may be that most of the deposited P is fixed by soils and that local plants are adapted to low-nutrient environments (Wright et al., 2018). However, the underlying mechanisms of this unexpected response of tropical forests to NP enrichment remain largely elusive (Wright et al., 2018), which hinders our understanding and predictions of current and future tropical and subtropical forest dynamics.

Belowground micro-food webs, complex size-structured networks formed by trophic groups of organisms (i.e., microbes and nematodes), are closely related to plant performance (Baldrian et al., 2023; Li et al., 2023a). These organisms are especially important in litter decomposition, which both fuel the soil system with nutrients and regulate soil organic carbon (SOC) formation (Liang et al., 2017, 2019; Sokol and Bradford, 2019; Craig et al., 2022). Greater microbial evenness allows to mitigate C loss and N leaching from substrates, while greater soil fauna richness is associated with higher substrate C and N mineralization (de Vries et al., 2012; Liu et al., 2023). Depositing organism necromass accounts for a considerable proportion of SOC (Ludwig et al., 2015; Liang et al., 2017, 2019; Wang et al., 2021). The organic carbon in necromass is bound to mineral particles and stabilized as mineral-associated organic matter (Angst et al., 2024). Compared with fungal-based decomposition pathway, bacterial-based (especially r-strategy community with a higher prevalence of copiotrophs) decomposition pathway is more efficient in microbial necromass production (Fierer et al., 2007; Glassman et al., 2018; Shao et al., 2021) and inhibits the turnover of soil stable C pools (Su et al., 2022).

Previous studies have provided important insights into shifts in bulk soil biota under NP enrichment in tropical and subtropical forests by removing litter decomposition residues. These studies have showed that NP enrichment suppresses nematodes, decreases fungal richness, and alters the decomposition pathways (e.g. fungal-based or bacterial-based) (Zhao et al., 2014; Fu et al., 2017; Ma et al., 2022). Indeed, organisms dwelling in litter and bulk soil are intricately linked through energy and nutrient exchanges (Bunn et al., 2019; Chen et al., 2020; Lang et al., 2021). However, examining bulk soil diversity without considering litter habitats misses a significant portion of belowground diversity in tropical and subtropical forests, approximately 66 % of nematode diversity and 20 % of bacterial diversity (Powers et al., 2009; Mao et al., 2022). The unique organism assemblages in distinct habitats (Fierer, 2017; Kitagami et al., 2020; Potapov, 2022), together with divergent responses of different organism assemblages to global change scenarios (Glassman et al., 2018; Chen et al., 2022a), suggest that litter and bulk soil biota may exhibit divergent trajectories under global change. However, knowledge of the response dynamics of litter food webs to NP enrichment is lacking in tropical and subtropical forests, despite studies on leaf litter microbial communities (Kerekes et al., 2013; Krashevska et al., 2014); this is particularly true for the food webs in root litter residues. This knowledge gap hinders our understanding of the effects of NP enrichment on belowground biota and, consequently, on ecosystem functions in tropical and subtropical forests.

Chinese fir ranks first in terms of forest plantation area in China and occupies 36 % of global subtropical plantations (Payn et al., 2015; Qu et al., 2022). Therefore, we conducted a 6-year litter decomposition experiment in a Chinese fir plantation to investigate the effects of NP addition on litter micro-food webs (including bacteria, fungi, and nematodes). We placed 250 g of leaf litter on the mineral soil surface, and 50 g of root litter at a depth of 5 cm in the mineral soil. The large initial litter mass allowed us to trace the litter micro-food webs at both the early and humus-near decomposition stages. We tested the following hypotheses: 1) NP addition has stronger negative effects on the bacterial-based decomposition pathway because NP addition would decrease litter pH (Gao et al., 2024), diversity and relative abundance of fast-growing bacteria are more adversely affected by acidification (Li et al., 2023b), and bacterivore nematodes will correlate with bacteria (Cesarz et al., 2013; Hedénec et al., 2023) and 2) NP addition modulates leaf and root litter biota in different ways, because litter chemistry has been showed to be an important driver of organism decomposition pathways (bacterial- or fungal-based), C-use strategy (oligotrophic or copiotrophic), and the stability of micro-food webs (Ferris and Matute, 2003; Wallenstein et al., 2006; Fierer et al., 2007; Li et al., 2022) and NP addition increases biochemistry differences between leaf and root litter residues (Jiang et al., 2018; Chen et al., 2022b; Wang et al., 2022).

2. Materials and methods

2.1. Study site description

The experiment was conducted in a Chinese fir (*Cunninghamia lanceolata*) plantation at the Qianyanzhou Ecological Research Station, Chinese Academy of Sciences, Taihe County, Jiangxi Province, southern China (26°42′N, 115°04′E, 102 m a.s.l.). The mean annual temperature and precipitation at the site were 17.9 ℃ and 1471.2 mm, respectively. The annual atmospheric deposition of N and P at the site was approximately 29 kg N ha^{-1} y^{-1} and 0.56 kg P ha^{-1} y^{-1}, respectively (Zhu et al., 2016). The soil is a typical red soil, and soil pH in the top 0–10 cm layer is 4.4.

2.2. Experimental design

A litter decomposition experiment was set up on three separate hilly slopes (three replicated experimental blocks) in August 2012 (Fig. S1). There were four 3 × 5 m plots at each block, and resulted in twelve plots in total. Within each block, the four plots were separated by at least 14 m, with two plots under ambient NP deposition (i.e., control) and two plots under NP addition (100 kg N ha^{-1} y^{-1} + 50 kg P ha^{-1} y^{-1}). Within each plot, microcosms for leaf and root litter decomposition were nested. The range of spatial dependence is approximately 1 m for soil nematodes (Viketoft, 2013) and generally less than 10 m for microbes (Saetre and Bååth, 2000; Franklin and Mills, 2003). Therefore, we assumed that all plots were independent.

Leaf and root litter from Chinese fir were collected from an unfertilised area of the plantation. Freshly fallen leaf litter was collected using litter traps. As obtaining fresh dead fine roots was challenging, we used living fine roots (diameter ≤ 2 mm) from the 0–10 cm top soil. The roots were washed on a sieve under running water. Both the leaf and root litters were not sterilised and air-dried. In each plot, microcosms of leaf and root litter decomposition treatments were established in polyvinyl chloride (PVC) tubes (inner diameter, 30 cm; length, 40 cm). To examine mass loss dynamics during decomposition, 16 PVC tubes were prepared in each plot, with each PVC tube being employed with either one leaf litter bag or one root litter bag. The experiment was also designed to differentiate between the effects of leaf litter and root litter sources on soil food web. Therefore, the soil O and A horizons at the mesocosm location were removed to minimize potential legacy effects of the former leaf and root litters. The decomposition rate of unsterilised leaf litter on mineral soil in our study was comparable to that of sterilised Chinese fir leaf litter (by oven-dried) placed on the O horizons (Wang et al., 2007). Here, we focused on the diversity and structure of microbes and nematodes, which are less affected than organism biomass by the removal (López-Mondéjar et al., 2015; Liu et al., 2021). Conversion of tropical forests to plantations had resulted in a 31 % decrease in litter thickness in the O horizon and a reduction of C content by up to 70 % in the A horizon (Guillaume et al., 2015; Zhu et al., 2021). Therefore, our findings could provide some insights into understanding the effects of NP enrichment on litter micro-food webs in extensive tropical plantations, which are established on degrade sites or involved in harsh topsoil and litter layers disturbances.

The tubes were vertically inserted into the mineral soil to a depth of 15 cm. Root litter bags (0.05-mm mesh on both sides) containing 50 g of dry roots were placed horizontally at 5 cm depth of the mineral soil in the tubes. The mineral soil was then refilled to cover the root litterbags.

Leaf litter bags (1-mm mesh on the top and 0.05-mm mesh on the bottom) containing 250 g of dry leaves were placed on the surface of the mineral soil in the tubes. The fine mesh of the root litterbags was selected to minimize material loss from the litterbags. The initial mass ratio of leaf litter to root litter was used because the annual leaf litter input in Chinese fir plantations was approximately 5-fold higher than the annual fine root litter production at the intact 0–10 cm soil depth (Yang et al., 2009; Fu et al., 2015). Four small holes were drilled in each tube, 3 cm above the ground, to prevent excessive retention of water inside the tube during the rainy season. The 25-cm extension of the tubes above the soil surface prevented the litter bags from being covered with mud during heavy rains. The top of each tube was covered with a 2-mm mesh to prevent disturbance by mammals or birds. Newly shed leaves were regularly removed from the cover mesh. 0.125 kg ammonium nitrate (NH_4NO_3) and 2.442 kg dihydrogenphosphate (NaH_2PO_4) dissolved in 25 L water were sprayed trimonthly onto the NP plots. Equal amounts of water were sprayed onto the control plots at the time of NP addition treatments.

2.3. Sampling and sample processing

One intact root litter bag and one intact leaf litter bag were sampled from each plot after 2, 7, 12, 18, 24, 36, and 72 months, resulting in 168 samples (7 times×2 treatments×2 litter types×6 replicates). After about two months, mosses began growing on the soils in the PVC tubes and towards the end of the experiments, short herbaceous plants got established in most of the tubes. For the litter bags, we did not observe any ingrown plant material at any sampling time, but there were signs of root growth in contact with the bags, particularly for the root litter bags after 72 months of incubation.

Upon collection, litter bags were placed in plastic bags and returned to the laboratory in a cooler. In the lab, we removed the litter residues from each bag and carefully homogenised and subdivided the litter in two portions. One portion was used for nematode extraction, while the other was further subdivided for analyses of microbial communities (stored at −20 ℃ until analysis), litter C, N, P concentration, mass remaining (oven-dried at 40 ℃), and pH (oven-dried at 40 ℃ and ground using a mixer mill). After 72 months of incubation, the mass loss of both the leaf and root litters reached a limit (Fig. 1). We defined the timing of the early and humus-near stages of decomposition after 2 and 72 months of incubation, respectively (Berg, 2014; Marian et al., 2018). Only litter samples after 2 and 72 months of incubation were used for micro-food webs analysis.

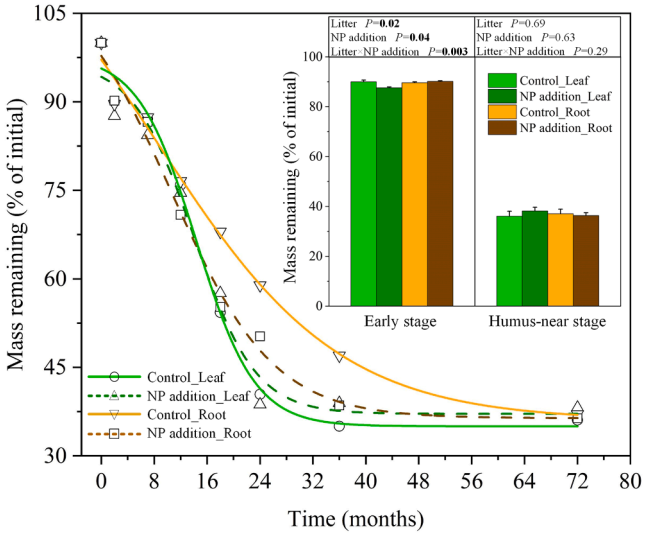

Fig. 1. Dynamics of leaf and root litter mass remaining during 72 months of decomposition. The proportion of litter mass remaining over time was fitted using the sigmoid model (Rovira and Rovira, 2010; Zhou et al., 2012). The inserted figure shows the effects of litter type and NP addition on mass remaining after 2 (early stage) and 72 months (humus-near stage) of incubation. *P*-values from mixed models are inset. Significant *P*-values are presented in bold. Litter mass remaining data before 36 months were from Wang et al. (2022).

2.4. Extraction of nematodes

Nematodes were extracted using Baermann funnels (Barker, 1985). The litter sample was placed in a water-filled funnel for 48 hours at 20 °C. The nematodes, collected from a tube connecting with the funnel were then fixed using a 4 % formaldehyde solution and mounted on microscope slides for further analysis. Individuals were identified at the genus level using a reverse light microscope and allocated to bacterivores, fungivores, herbivores, and omnivores/predators based on mouthpart examination (Yeates et al., 1993). The relative abundances of these four functional groups and the nematode decomposition pathway (fungivores:bacterivores) were quantified (Homet et al., 2023).

2.5. PLFA assay

For the analysis of litter microbial communities, we used phospholipid fatty acids (PLFAs), following methods previously described (German et al., 2011). Briefly, the sample for PLFA analysis was extracted using a chloroform–methanol–phosphate buffer (1:2:0.8). Solid-phase extraction cartridges (LiChrolut Si 60, Merck) were used to separate phospholipids, followed by mild alkaline methanolysis. The free methyl esters of phospholipid fatty acids were analyzed via gas chromatography–mass spectrometry (Varian 3400; ITS-40, Finnigan). PLFAs i14:0, i15:0, a15:0, i16:0, i17:0, a17:0, 16:1ω7cis, 16:1ω9cis, 17:1ω7cis, 18:1ω5c, 18:1ω7cis, cy17:0, and cy19:0 were used as indicators of bacteria. PLFAs 18:1ω9cis, 18:2ω6cis, 18:2ω9cis, and 18:3ω6cis were used as indicators of fungi. We further used the PLFA data to calculate the relative abundances of bacteria and fungi in the microbial community and the fungi:bacteria ratio, also referred to as the microbial community decomposition pathway (de Vries et al., 2006).

2.6. DNA extraction and amplicon sequencing

For analysis of litter microbial communities, we further used DNA analysis. Briefly, the 16S rRNA (primer set 515 F_907 R) and ITS (primer set ITS1F_ITS2R) gene sequences were amplified from bacterial and fungal DNA by PCR using the primers 515 F (GTGCCAGCMGCCGCGG), 907R (CCGTCAATTCMTTTRAGTTT), ITS1F (CTTGGTCATTTAGAGGAAGTAA), and ITS2R (GCTGCGTTCTTCATCGATGC), respectively (Klindworth et al., 2013; Xiong et al., 2017). The PCR amplification products were purified using a QIAquick PCR purification kit (Qiagen), and the DNA concentrations were measured using a NanoDrop ND-1000 (Thermo Scientific). The PCR products were mixed in equimolar ratios. The samples were then barcoded and sequenced using Illumina MiSeq (Illumina Inc., San Diego, CA, USA). For each of these sequences, a length of ≥ 250 bp or a cumulative error of < 1 % was selected to eliminate sequence redundancy. Quality-filtered and combined sequences were clustered into OTUs at a similarity cutoff of 97 % using UPARSE to acquire higher-quality sequences as representative sequences and generate a table of OTUs for each litter sample. The OTUs of bacterial and fungal samples were classified and annotated based on the SILVA 119 database (http://www.arb-silva.de/download/¬archive/qiime/) at 97 % similarity.

We distinguished the microbial life-history strategies into copiotrophs (r-strategists) and oligotrophs (K-strategists) according to previous classifications. The class *Betaproteobacteria* and *Gammaproteobacteria*, and the phyla *Bacteroidetes* and *Firmicutes* were classified as bacterial copiotrophs; the class *Alphaproteobacteria* and *Deltaproteobacteria*, and the phyla *Acidobacteria*, *Actinobacteria*, and

Chloroflexi were considered bacterial oligotrophs (Foulquier et al., 2024). We also estimated bacterial average ribosomal RNA operon (*rrn*) copy number at community level to determine whether the community prefers r- or K-strategy. The *rrn* copy number was obtained from the *rrn*DB database, a publicly available and carefully curated resource providing copy number information for bacteria (Stoddard et al., 2015) (version 5.9, https://rrndb.umms.med.umich.edu/). The mean *rrn* copy number was matched starting from the lowest rank, otherwise higher rank matches were searched. The abundance-weighted *rrn* operon copy number was calculated of all OTUs (Dai et al., 2022). The *rrn* operon copy number was significantly and negatively correlated with the bacterial oligotrophs:copiotrophs (Fig. S2), indicating that the classification of bacterial copiotrophs and oligotrophs was reasonable. Fungal oligotrophs include the phyla *Basidiomycota* and *Chytridiomycota*; fungal copiotrophs include the phyla *Ascomycota* and *Zygomycota* (Li et al., 2021; Wu et al., 2023). Copiotrophs are enriched in environments with abundant labile organic substrates, while oligotrophs are enriched in environments containing recalcitrant C compounds (López et al., 2023). The ratio of oligotrophs:copiotrophs represents microbial C-use strategies. A larger oligotrophs:copiotrophs ratio indicates that the microbial community is inclined to utilise recalcitrant C, less efficient in producing microbial necromass, and thus less mineral-associated organic matter accumulate efficiency (Fierer et al., 2007; Angst et al., 2024).

2.7. Chemical analyses

The total C and N concentrations of each litter sample were determined using a Vario Max CN elemental analyser (Elementar, Hanau, Germany). The P concentration was determined using an Inductively Coupled Plasma Atomic Emission Spectrometer (Thermo Elemental, Waltham, MA, USA) after high-pressure microwave digestion. The C:N and N:P ratios were calculated. Litter pH was measured in a 1:5 litter: water mixture.

2.8. Data analyses

Diversity indices, including Pielou evenness and Margalef richness (Fontana et al., 2018; Enquist et al., 2019) were determined for the microbial communities based on OTUs and for the nematode communities based on nematode numbers for each genus at the early and humus-near stages, respectively. To test the main and interactive effects of NP addition and litter type on litter C:N, N:P, and pH, and the variables of diversity, relative abundance, decomposition pathway ratio, and microbial C-use strategy ratio at the early and humus-near stages, we used a linear mixed effect model. Litter type, NP addition, and their interaction were defined as fixed factors and block was defined as a random effect. Mixed effect model was run using the "nlme" package in R 4.4.1. Before analysis, the variables were log10-transformed to approximate normal distribution. To investigate whether the effects of NP addition were associated with changes in litter chemistry, the Spearman's rank correlations between the variables with significant main effect of NP addition (both leaf litter and root litter data together) at the early and humus-near stages and the litter chemistry were calculated with the SPSS version 26.0 (IBM Corp, Armonk, NY, USA).

Succession of micro-food web network in leaf and root litters over time was analyzed by constructing co-occurrence networks (Qiu et al., 2021). For each litter type, we analyzed micro-food web network for each sampling time and for ambient NP and NP addition treatment separately. The co-occurrence networks were constructed using Spearman's correlation coefficient > 0.6 and P-values < 0.01. A multiple testing correction using the Benjamini–Hochberg method was applied to adjust the P-values to reduce the false-positive rate (Jiang et al., 2017). Negative associations promote stability of communities (Coyte et al., 2015). The percentage of negative associations was calculated. All network analyses were realized by the Molecular Ecological Network Analyses Pipeline (http://ieg2.ou.edu/MENA/).

3. Results

3.1. Litter residue chemistry

Nutrient enrichment altered litter residue chemistry. For example, NP addition impacted litter C:N, N:P, and pH (Fig. 2). NP addition decreased litter N:P at the early stage, but increased litter C:N at the

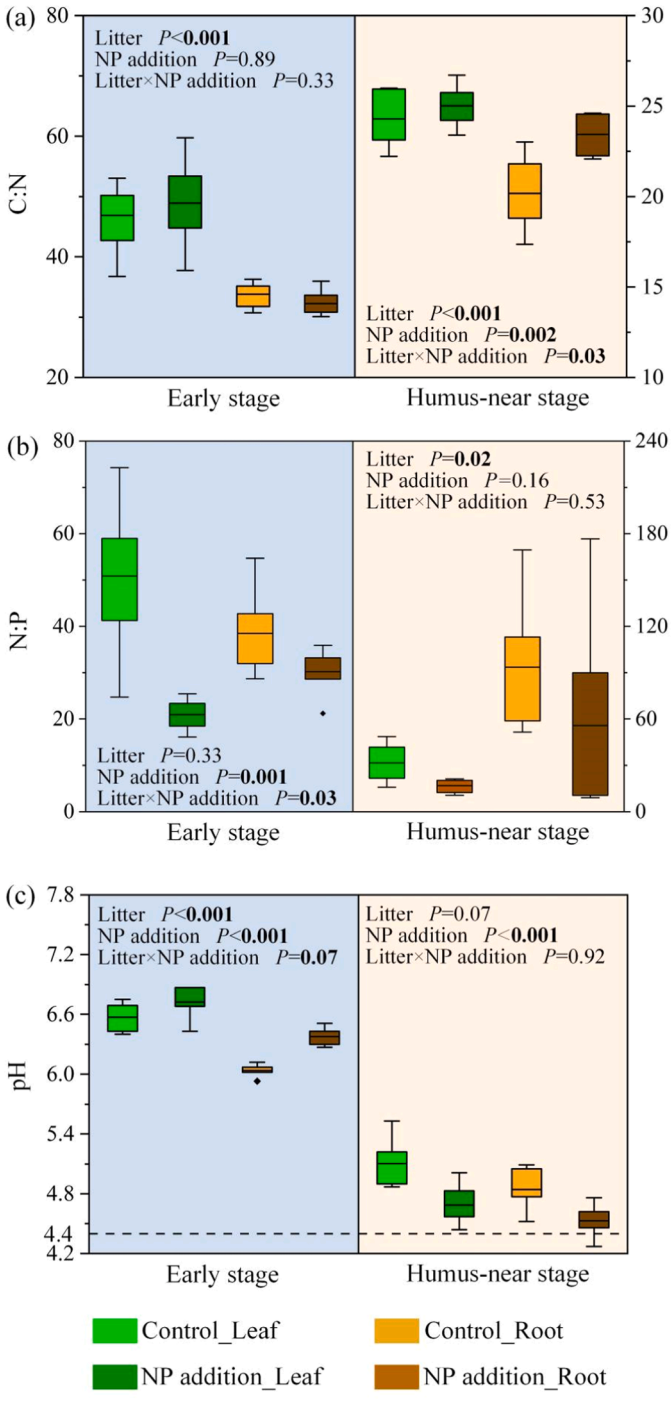

Fig. 2. The response of decomposition residues of litter C:N (a), litter N:P (b), and litter pH (c) at the early and humus-near stages to NP addition and litter type (leaves, roots). Box plots show mean values (lines inside the box) and outliers (black dots). P-values from mixed models are showed in each of the panels. Significant P-values are presented in bold. Data on litter C:N and N:P at the early stage were obtained from Wang et al. (2022). The black dash line in panel (c) represents ambient soil pH (4.4).

humus-near stage. At the early stage, litter pH was higher (6.7 and 6.2 for leaf and root litter residues, respectively). At the humus-near stage, litter pH became acidic (4.8 and 4.7 for leaf and root litter residues, respectively), but was still higher than the ambient soil pH of 4.4 ($F=46.5$, $P < 0.001$). NP addition increased litter pH at the early stage and decreased it at the humus-near stage. A significant interaction between NP addition and litter type resulted in stronger effects on leaf litter N:P and root litter pH at the early stage, as well as on root litter C:N at the humus-near stage.

3.2. Characterization of litter micro-food webs

Overall, the effects of litter type were much stronger than those of NP addition at both the early and humus-near stages, as indicated by the number of significantly altered variables (Table 1) and the magnitude of those responses (Figs. 3–5). However, the effects of NP addition were still outstanding, significantly altering two and six variables at the early and humus-near stages, respectively.

At the early stage, NP addition significantly decreased fungal richness by 13 % (Table 1). The decline in fungal richness was associated with a decrease in *Basidiomycota* richness (Fig. S3b). NP addition significantly increased bacterial oligotrophs:copiotrophs by 18 %, mainly by affecting leaf litter bacterial oligotrophs:copiotrophs (Fig. 5c, Table 1). Moreover, nematode richness was affected by a significant interaction between NP addition and litter type (Table 1), with NP addition having negative effects on root litter but positive effects on leaf litter. Therefore, no significant main effect of NP addition was noted on nematode richness across leaf and root litter types. Besides, NP addition decreased the percentage of negative correlations of leaf (-26 %) and root (-8 %) litter micro-food web networks (Table S2).

At the humus-near stage, NP addition had more profound effects on the micro-food webs (Table 1). The evenness of the bacterial community declined by 3 %, along with a decrease in the dominance of *Betaproteobacteria* and *Chloroflexi* (Fig. S4, Table S3). A 12 % decline in fungal richness was associated with a decrease in two less dominant fungal phyla (*Basidiomycota* and *Chytridiomycota*) and one rare taxon of *Glomeromycota* (Fig. S5, Table S4). The increase in fungal predominance (+19 % relative abundance of fungi and +22 % fungi:bacteria) was due to the increases in the dominant *Ascomycota* (Fig. S5, Table S4). A decline in *Basidiomycota* and *Chytridiomycota* further decreased fungal oligotrophs:copiotrophs by 68 % (Fig. S5, Table S4). However, NP addition had no significant effect on fungivores. In contrast, NP addition significantly increased only the relative abundance of herbivores (+107 %), with *Filenchus*, *Nothotylenchus*, and *Paratylenchus* being enriched (Fig. S6, Table S5). Moreover, NP addition had stronger positive effects on root litter fungi:bacteria compared with those of leaf litter fungi:bacteria (Fig. 5a, Table 1), but stronger negative effects on leaf litter fungal oligotrophs:copiotrophs than those on root litter oligotrophs:copiotrophs (Fig. 5d, Table 1). However, the response directions of fungal evenness and bacterial oligotrophs:copiotrophs to NP addition differed between litter types (Figs. 3b, 5c, and Table 1). Therefore, NP addition had no significant effect on these two variables (Table 1). Besides, NP addition increased the percentage of negative correlations of root litter micro-food web network (+85 %), but slightly decreased the percentage of negative correlations of leaf litter micro-food web network (-3 %) (Table S2).

3.3. Relationships between litter residue chemistry and variables of micro-food webs

Four of the eight significantly affected response variables to NP addition were associated with litter residue pH, but none of these eight variables were related to litter residue C:N and N:P (Fig. S7). At the early stage, fungal richness decreased with increasing litter pH because higher litter pH was harmful to four fungal phyla (*Basidiomycota*, *Glomeromycota*, *Rozellomycota*, and *Zygomycota*) (Table S6). At the humus-near stage, a higher relative abundance of fungi with a lower oligotrophic proportion was related to lower litter pH because copiotrophs (*Zygomycota*) were enriched at a lower pH (Table S6).

4. Discussion

Recent studies have investigated how plants in tropical and subtropical forests respond to global change scenarios (Manu et al., 2022; Yang et al., 2022a). However, little is known about how the belowground biota drives plant dynamics in these forests (de Paula et al., 2021). Using NP addition as a global change agent, our results suggest that nutrient enrichment affects soil biota across multiple trophic levels associated with litter decomposition in subtropical plantation, with consequences for the replenishment of nutrients, SOC dynamics, and plant performance in these ecosystems.

Table 1
Results from mixed effects models evaluating the statistical significance of effects of litter type (leaves, roots) and NP addition on diversity, relative abundance, decomposition pathway ratio, and microbial C-use strategy ratio of the micro-food webs at the early and humus-near stages.

Decomposition stage	Variable		Litter type			NP addition		Litter type × NP addition	
			F	P		F	P	F	P
Early stage	Diversity	Bacterial evenness	7.63	**0.01**	L	0.91	0.35	0.25	0.62
		Bacterial richness	12.58	**0.003**	R	0.85	0.37	1.10	0.31
		Fungal richness	1.09	0.31		5.28	**0.03** ↓	0.00	0.98
		Nematode richness	15.17	**0.001**	L	2.56	0.13	6.22	**0.02**
	Relative abundance	Bacteria	10.93	**0.005**	L	0.20	0.66	0.13	0.72
	Microbial C-use strategy ratio	Bacterial oligotrophs:copiotrophs	45.34	**< 0.001**	L	7.47	**0.01** ↑	59.80	**< 0.001**
		Fungal oligotrophs:copiotrophs	99.31	**< 0.001**	R	0.09	0.77	0.14	0.71
Humus-near stage	Diversity	Bacterial evenness	47.79	**< 0.001**	L	10.07	**0.006** ↓	17.37	**< 0.001**
		Fungal evenness	90.18	**< 0.001**	L	3.11	0.10	5.44	**0.03**
		Fungal richness	47.46	**< 0.001**	L	5.18	**0.04** ↓	0.73	0.41
	Relative abundance	Bacteria	415.11	**< 0.001**	L	4.32	0.05	1.28	0.27
		Fungi	470.82	**< 0.001**	R	11.49	**0.003** ↑	8.73	**0.008**
		Fungivores	15.35	**0.002**	L	0.00	0.98	1.24	0.29
		Herbivores	11.66	**0.004**	R	11.23	**0.004** ↑	2.46	0.14
		Omnivores/predators	1.93	0.18		2.25	0.15	11.18	**0.004**
	Decomposition pathway ratio	Fungi:bacteria	429.32	**< 0.001**	R	4.80	**0.04** ↑	1.60	0.22
		Fungivores:bacterivores	9.41	**0.01**	L	0.02	0.90	1.59	0.24
	Microbial C-use strategy ratio	Bacterial oligotrophs:copiotrophs	6.05	**0.02**	L	1.05	0.32	3.02	0.10
		Fungal oligotrophs:copiotrophs	114.95	**< 0.001**	L	29.48	**< 0.001** ↓	1.48	0.24

The F-ratios are presented with their levels of significance. Significant P-values are presented in bold. L, leaf litter with higher value; R, root litter with higher value; ↑ NP addition significantly increased value; ↓ NP addition significantly decreased value. The results of these non-significant differences are presented in Table S1.

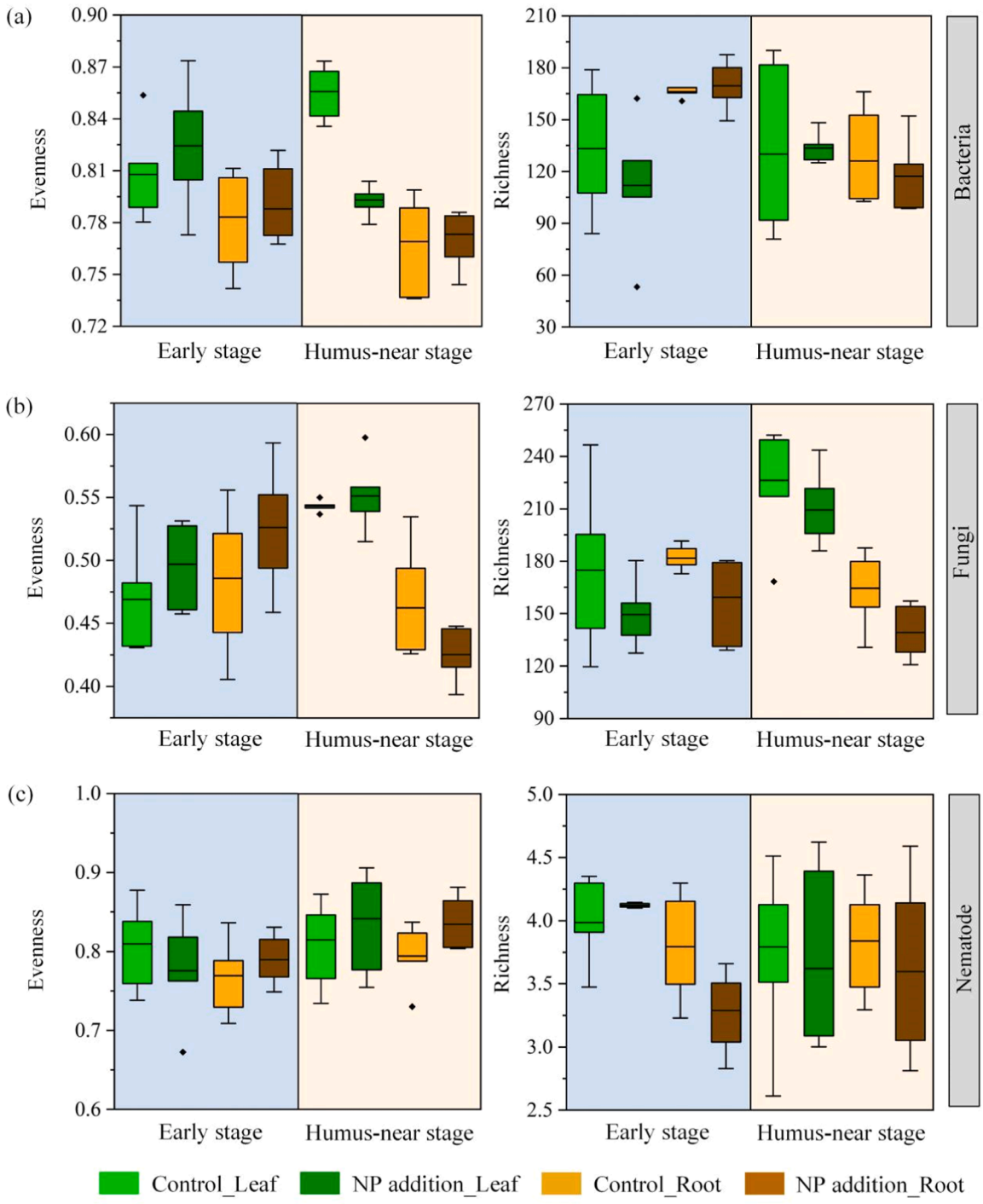

Fig. 3. The response of diversity indices for bacterial (a) and fungal OTUs (b), and nematodes (c) at the early and humus-near stages to NP addition and litter type (leaves, roots). Box plots show mean values (lines inside the box) and outliers (black dots). Results of main effects (litter type and NP addition) and their interactions are showed in Tables 1 and S1.

4.1. The effects of NP enrichment on diversity and structure of litter micro-food webs

Contrary to our first hypothesis, we found no evidence that NP addition had a stronger effect on bacteria-based decomposition pathway (Table 1). Instead, our results showed that NP addition consistently shifted litter micro-food webs to low fungal richness during the decomposition process. The NP addition-induced decline in litter fungal richness is consistent with findings from a bulk soil study in other tropical forests (Ma et al., 2022). The different responses of bacterial and fungal communities to NP addition may be attributed to their divergent demands on nutrient. Previous study showed that soil fungi typically have lower nutrient demands than soil bacteria (Leff et al., 2015). The increase in nutrient availability caused by NP addition may disadvantage soil fungi with large genomes and low guanine-cytosine content (Zhang et al., 2023). After 6-years of decomposition, our study further demonstrated that NP addition increased litter fungi:bacteria and decreased litter bacterial evenness. This shift direction of litter fungi: bacteria in response to NP addition is opposite to that in bulk soil after 3-years of decomposition from the same experiment design (Fu et al.,

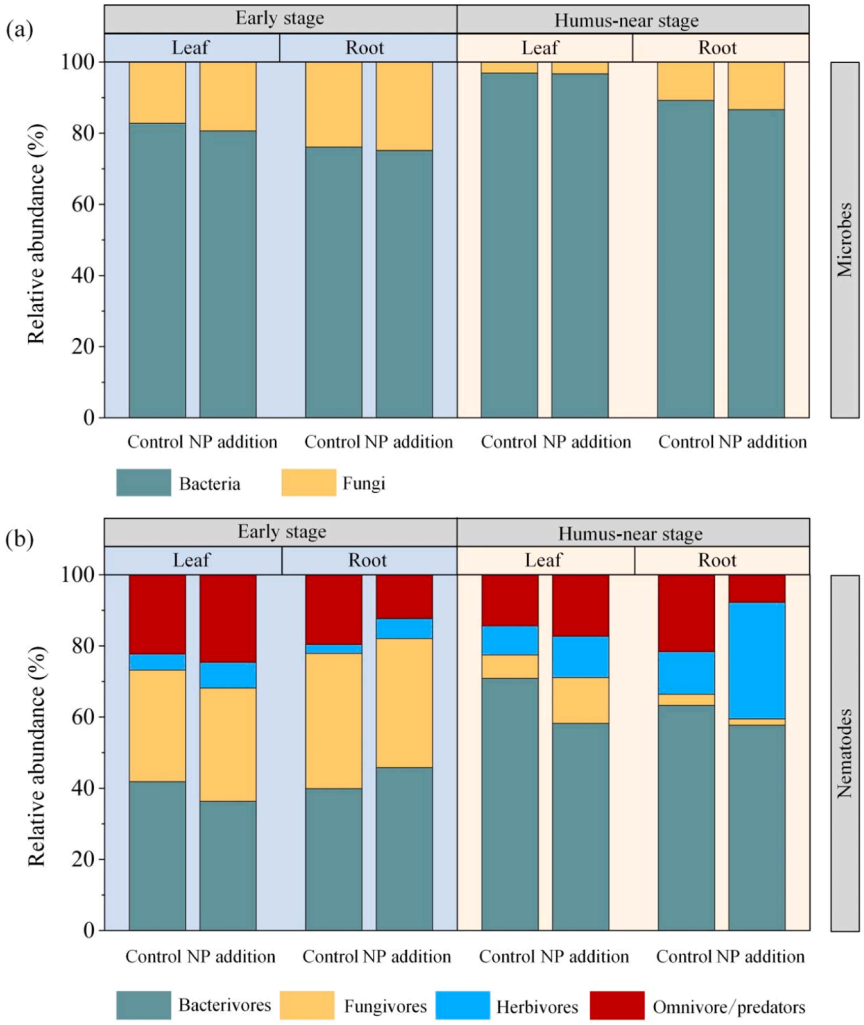

Fig. 4. The response of the relative abundance of microbial PLFAs (a) and nematode feeding groups (b) at the early and humus-near stages to NP addition and litter type (leaves, roots). Results of main effects (litter type and NP addition) and their interactions are showed in Tables 1 and S1.

2017), despite the shift direction of litter bacterial evenness is consistent with the observation from a study on the bulk soil biota of two tropical forests (Ma et al., 2022). Moreover, NP addition significantly altered the relative abundance of herbivores compared with other nematode functional groups in our study, likely because herbivores are more widely and uniformly distributed (Liu et al., 2019). In contrast, two previous studies (one in from the same experiment design) on bulk soils from tropical and subtropical forests showed no significant effects of NP addition on herbivore predominance (Zhao et al., 2014; Fu et al., 2017). Collectively, these findings suggest that litter nematode communities, in the aboveground leaf litter (Chen et al., 2021) and the belowground root litter, are more vulnerable to nutrient enrichment than those of bulk soil nematode communities.

In line with a recent report on soils from alpine ecosystems (Hu et al., 2024), we found that litter pH overrode litter stoichiometry in regulating the diversity and structure of the litter biota (Fig. S7). At the early stage, the pH increase induced by NP addition inhibited fungal richness; however, at the humus-near stage, the pH decrease caused by NP addition promoted the relative abundance of fungi and fungi:bacteria. This is likely because acidification enhances the competitiveness of fungi. Existing evidences showed that soil fungi have much higher tolerance to osmotic stress, compared with soil bacteria (Griffiths et al., 1998) and show greater resistance to the increasing concentrations of Al^{3+} ions due to soil acidification (Piña and Cervantes, 1996; Chen et al., 2015). Thus, soil fungi generally reach maximal growth in acidic environments (Rousk et al., 2009). Surprisingly, at the humus-near stage, litter pH was still higher than the ambient soilpH. Although we did not have data from bulk soil, the strong influence of pH on the biotic communities and functions observed in the present and previous studies (Thakur et al., 2014; Li et al., 2023a) suggests that the biota of humus-near litters may still be different from that in soil. Along with the notion that litter biota is likely more vulnerable to NP enrichment than bulk soil biota, we argue that bulk soil biota is insufficient as a general driver of soil biota-function relationships under global change.

4.2. The asymmetric effects of NP enrichment on leaf and root litter micro-food webs

In agreement with our second hypothesis, our results showed nutrient-induced asymmetric effects on leaf and root litter micro-food webs (Table 1). For the leaf litter micro-food web, NP addition increased the bacterial oligotrophs:copiotrophs at the early stage and decreased the fungal oligotrophs:copiotrophs at the humus-near stage. At the early stage, NP addition accelerated the leaf litter decomposition more than root litter in our study, leading to the accumulation of recalcitrant C (Cotrufo et al., 2015). This process favored the proliferation of oligotrophic bacteria and increased the bacterial oligotrophs: copiotrophs in leaf litter residue. At the humus-near stage, NP addition induced a greater decrease of pH in leaf litter residue than in root litter residue. This is because a greater decrease of pH led to a more

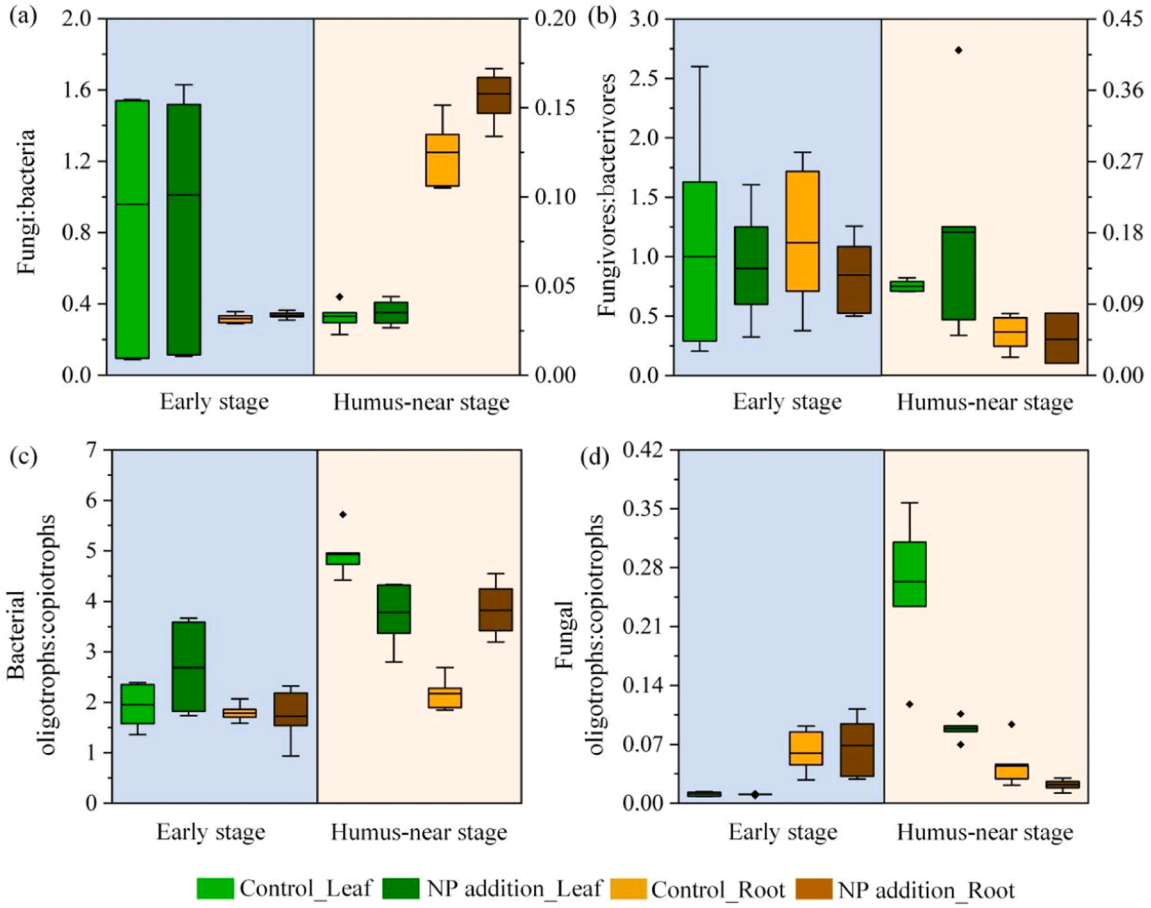

Fig. 5. The response of decomposition pathways for microbial (a) and nematode (b) communities and microbial C-use strategy ratio for bacterial (c) and fungi (d) communities at the early and humus-near stages to NP addition and litter type (leaves, roots). Box plots show mean values (lines inside the box) and outliers (black dots). Results of main effects (litter type and NP addition) and their interactions are showed in Tables 1 and S1.

pronounced decrease of fungal oligotrophs (*Basidiomycota* and *Chytridiomycota*) and a more pronounced increase of fungal copiotrophs (*Zygomycota*) (Table S6). For the root litter micro-food web, the lack of changes in microbial life-history strategies by NP addition at the humus-near stage may be attributed to the relatively small change in pH caused by NP addition. Instead, NP addition affected the microbial community decomposition pathway of root litter at the humus-near stage, as indicated by an increase of fungi:bacteria. This is likely because a lower pH favors fungal growth (Rousk et al., 2009). The pH of root litter residue, which was consistent lower than that of leaf litter residue, was further lowered by NP addition.

NP enrichment improved the stability of the root litter micro-food web but decreased that of the leaf litter micro-food web at the humus-near stage (Table S2). Fungi, acting as a "stabilizer", play a pivotal role in maintaining the stability of community association networks (Yang et al., 2022b). Moreover, in terms of microbial interactions within co-occurrence network, an increase in fungal relative abundance can further enhance stability of the microbial network (Yue et al., 2023). Thus, the different effects of NP addition on the stability of leaf and root litter micro-food webs could be attributed to the stronger positive influence of NP addition on the relative abundance of fungi in root litter residue at the humus-near stage.

4.3. *The potential influence of NP enrichment on plant growth through litter biota*

Here, we showed three mutually compatible pathways through which the litter biota modulates plant growth in subtropical forests under NP addition (Fig. 6). First, the NP addition-induced higher relative abundance of herbivores at the humus-near stage (Fig. 4b, Table 1) indicates a harmful structure of the nematode community for plant productivity, because a higher proportion of herbivorous nematodes would increase damage to roots (Thakur et al., 2014). Second, the oxidation of organic N driven by fungi is a pivotal process that produces plant-available N for plant growth in subtropical coniferous forests (Zhu et al., 2015). Higher fungal biodiversity was associated with higher mobilisation of organic N (Digby et al., 2010; Li et al., 2019). Then, NP addition-induced decline in fungal richness at both early and humus-near stages (Fig. 3b, Table 1) indicates inhibition of litter N release for plant uptake during the decomposition process. Third, NP addition-induced shifts in the decomposition pathway and microbial C-use strategy may decrease soil fertility over a longer period by reducing SOC formation efficiency. For example, microbial necromass directly contributes to SOC (Ludwig et al., 2015; Liang et al., 2017, 2019; Wang et al., 2021), and fungal turnover is slower than bacterial turnover (Glassman et al., 2018). The higher fungal-dominant microbial community induced by NP addition (Fig. 4a, Table 1) suggests a lower production efficiency of microbial necromass. Moreover, a higher bacterial oligotrophs:copiotrophs is less efficient in producing bacterial necromass, resulting in less mineral-associated organic matter accumulation, and a lower fungal oligotrophs:copiotrophs confers a lower conversion efficiency of recalcitrant plant residue C into more stable microbial necromass C (Fierer et al., 2007; Glassman et al., 2018; Shao et al., 2021). The higher prevalence of K-strategy bacteria communities at the early stage and lower prevalence of K-strategy fungal communities at the humus-near stage induced by NP enrichment further indicates a

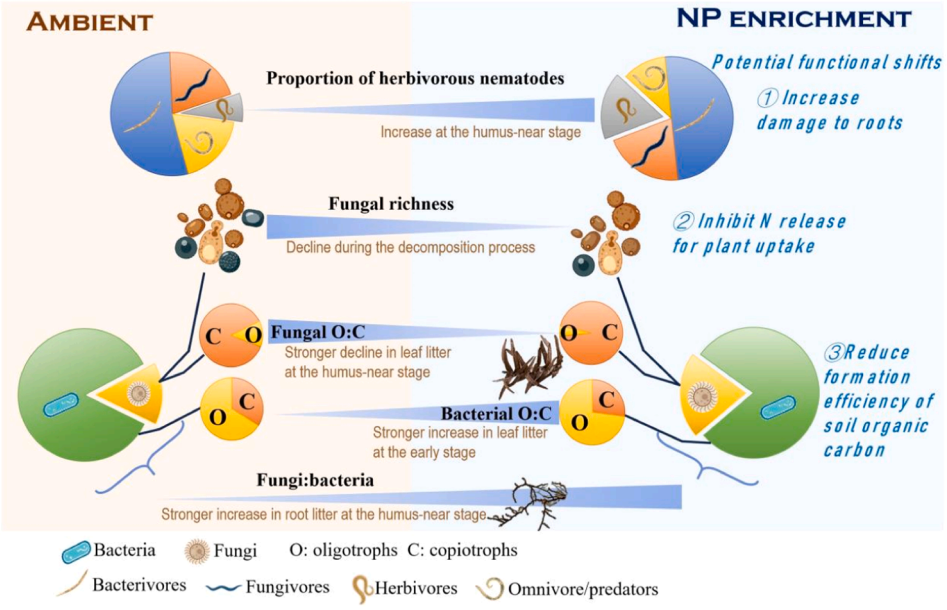

Fig. 6. Pathways through which the litter biota modulates plant growth under NP enrichment. The regular and italic fonts indicate the observed changes in litter biota and the hypothesized functional shifts, respectively. Changes in litter biota caused by NP enrichment and negatively affect plant growth through three pathways. First, NP enrichment-induced higher herbivore dominance at the humus-near stage would increase damage to roots. Second, NP enrichment-induced decline in fungal richness during the decomposition process may inhibit litter N release for plant uptake. Third, NP enrichment-induced increase bacterial oligotrophs:copiotrophs (stronger increase in leaf litter residues) at the early stage, and decline in fungal oligotrophs:copiotrophs (stronger decline in leaf litter residues) and increase in fungi:bacteria (stronger increase in root litter residues) at the humus-near stage may decrease soil fertility over a longer time period by reducing the formation efficiency of soil organic matter.

lower formation efficiency of the stable C pool (Fig. 5c, d, and Table 1). Collectively, the three pathways indicate that shifts in the litter biota caused by NP enrichment could have negative effects on plant growth. Therefore, in addition to plant adaptation to native infertile soils (Coley et al., 1985) and increasing leaf pest pressure (Campo and Dirzo, 2003), our results present new mechanisms that contribute to the weak effects of NP enrichment on plant growth in tropical and subtropical forests.

5. Conclusions

Our study revealed that NP addition can largely alter the microbes and microfauna associated with litter decomposition in subtropical plantation by shifting the litter food webs to lower fungal richness and higher relative abundance of fungi and herbivorous nematodes, especially at the humus-near stage. Globally, 75–135 Pg dm of leaf and root litters are continuously deposited in the soil every year (Matthews, 1997). Given that litter habitats hold a significant portion of belowground biodiversity and that most previous studies of belowground biota have largely focused on bulk soil, this work advances our understanding of belowground biota-function relationships under global change. Furthermore, our results showed that NP addition modulates leaf and root litter micro-food webs in different ways. NP addition had stronger effects on leaf litter microbial life history but stronger effects on root litter microbial community decomposition pathway. Therefore, our findings encourage models to include the responses of root and leaf litter biota together with those of soil biota to generate realistic predictions of how the structure and function of belowground biota will respond to ongoing environmental changes.

CRediT authorship contribution statement

Gao Decai: Writing – review & editing. **Kou Liang:** Writing – review & editing, Methodology. **Chen Fusheng:** Writing – review & editing. **Wang Yuxin:** Writing – review & editing, Methodology, Investigation. **Kardol Paul:** Writing – review & editing, Methodology. **Meng Shengwang:** Writing – review & editing, Methodology. **Dai Xiaoqin:** Writing – review & editing, Methodology. **Delgado-Baquerizo Manuel:** Writing – review & editing, Methodology. **Wang Huimin:** Writing – review & editing, Methodology. **Shao Hui:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis. **Fu Xiaoli:** Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Conceptualization.

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. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.foreco.2025.122545.

Data availability

Data will be made available on request.

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