Biochar enhances multifunctionality by increasing the uniformity of energy flow through a soil nematode food web

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ARTICLE INFO

ABSTRACT

Soil multifunctionality is the consequence of biotic interactions that drive decomposition, nutrient cycling and net primary production. Energy flux describes the energy consumed and transferred among multitrophic groups in the soil food web, which are logically linked to multifunctionality. In a subtropical agroecosystem with an annual sweet potato-oilseed rape rotation, we explored how biochar and synthetic fertilizer jointly affected agroecosystem multifunctionality (e.g., crop production, soil carbon storage and nutrient cycling) and the energetic structure of the nematode food web during two consecutive years. Results showed that biochar increased soil multifunctionality by 37–110% mainly by promoting a uniform energy flow through the soil nematode food web, which was largely due to increased energy fluxes of fungivores and omnivores-carnivores at the expense of decreased energy flux through herbivores. Applying a lower rate of synthetic fertilizer led to non-uniform energy flow in the soil nematode food web, suggesting that nitrogen limitation could offset the stimulatory effect of biochar on soil multifunctionality. This was because biochar induced oligotrophic conditions (a stoichiometry-induced nitrogen limitation), effectively warranting that continuous biochar application would aggravate nutrient limitations to crops, especially when low rates of synthetic fertilizer are applied. Notably, soil nutrient impoverishment could lead to resource reallocation from aboveground shoot to belowground root production, thereby fueling the energy flow through the herbivore channel. Our findings highlight the importance of balancing biochar and synthetic fertilizer applications to sustain a stable energetic structure in soil nematode food webs, which are associated with greater crop production and soil health in subtropical region.

1. Introduction

Soil multifunctionality is an indicator of ecosystem functions such as net primary production and nutrient cycling. Analysis of soil food webs delivers mechanistic understanding of soil multifunctionality because soil biotic interactions are responsible for almost all important ecological processes in terrestrial ecosystems (Delgado-Baquerizo et al., 2020; Luo et al., 2022). Numerous studies on biodiversity-functionality relationships neglected the fact that individual species were organized into guilds connected through multitrophic interactions and structured by energy fluxes among biotic and abiotic components (Wan et al.,

2022b; Buzhdygan et al., 2020). Theoretically, energy dynamics describe the fluxes of energy and matter along distinct pathways in complex soil food web that are directly linked to ecological processes. For instance, the energy flux of herbivores was closely related to plant productivity because 17–36% of the energy-rich substrates produced by plants were consumed by herbivores (Johnson et al., 2016). Similarly, the energy flux of decomposers was generally associated with organic carbon accumulation and nutrient cycling (Schwarz et al., 2017), whereas the energy flux of omnivores-carnivores was directly related to biocontrol functions and indirectly associated with other functions (Barnes et al., 2020). Thus, deciphering the connection between energy

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dynamics of the soil food web and soil multifunctionality will provide a biological mechanism to evaluate the sustainability of agricultural practices.

Conventional agricultural management relies on synthetic fertilizers to reach target crop yields, but does not consider the energetic needs of soil organisms. Biochar, as an emerging soil amendment, provides multiple benefits for soil such as promoting soil carbon sequestration and crop growth by accelerating the energy flux in the soil food web (McCormack et al., 2019; Schmidt et al., 2021). Biochar is well-suited for acidic soils because it neutralizes soil pH while slowing soil organic matter mineralization rate and alleviating phosphorus deficiency (Gul et al., 2015), but it is uncertain how biochar impacts soil multifunctionality in acidic tropical soils at the field scale. Our previous pot study showed neutral or adverse effects of biochar on biotic communities in acidic soil (Liu et al., 2020) because the high carbon to nitrogen ratio (C:N ratio) and porous structure of biochar appeared to decrease nutrient availability, restrict soil biological activity and limit plant growth, which was also reported for crops growing in biochar-amended soils under nutrient-limited conditions (El-Naggar et al., 2019; Briones et al., 2020). Given the high context dependency of biochar effects (Jin et al., 2022), we must take a holistic approach that considers the multiple components and soil biological functions of the agroecosystem. Analysis of energy flux through soil food webs is a way to consider soil organisms and their functions explicitly (Barnes et al., 2018). This approach will allow us to study energy distributions within the soil food web and its relation to soil multifunctionality following biochar amendment.

Biochar amendment is expected to affect the energy flux of soil nematodes, the most abundant soil fauna that occupy multiple trophic levels within the soil food web (Puissant et al., 2021). First, biochar could increase the energy flux through microbivores by increasing soil microbial biomass (Lehmann et al., 2011; Pathy et al., 2020, Fig. 1 path A). Furthermore, the alkaline properties and porous structure of biochar could increase soil pH, improve soil physical structure and thus favor plant growth, which consequently increases the energy flux through herbivores (Domene et al., 2021, Fig. 1 path B). These biochar-mediated alterations in the soil environment could promote the energy flux through omnivores-carnivores by increasing their food resource, potentially balancing the herbivore-dominated energetic structure that was common in agroecosystems receiving synthetic fertilizers (Moore et al., 2005), thereby enhancing ecosystem stability and multifunctionality (Potapov, 2022, Fig. 1 path C). Although biochar is expected to increase the energy flux, the nematode community could experience nitrogen limitation because bioavailable forms like NH_4^+ are absorbed to biochar surfaces and pores, or temporarily immobilized after biochar application (Gul and Whalen, 2016; Dai et al., 2020). Herbivores will benefit, relative to other trophic groups, when nutrient limitation causes a stimulation of plant root growth and rhizodeposition that boosts nutrient solubilizations and mineralization processes (Song et al., 2020). We predict that this would disrupt the energy flux balance, and thereby change the soil multifunctionality (Fig. 1 path D). Therefore, we propose to combine biochar application with a judicious amount of synthetic fertilizer to avoid nitrogen limitations to soil organisms and crops (Fig. 1 path E).

In a 3-year field experiment with biochar and synthetic fertilizer treatments, we quantified the soil nematode energetic structure and linked this to the nematode-associated functions, namely soil multifunctionality, which were related to support crop productivity, soil organic carbon storage and nutrient cycling. In contrast to earlier multifunctionality studies in agroecosystems receiving compost or manure amendments rich in labile carbon and nutrients (Luo et al., 2018), we used biochar that contained some labile carbon but low available nutrients, and modified the available nutrient level by adding synthetic fertilizer. We hypothesized that biochar amendment would enhance soil multifunctionality by balancing the energetic structure of soil nematode food web (Fig. 1). Specifically, a biochar-amended soil with abundant available nutrients from synthetic fertilizer would have higher soil pH, better physical structure and more available nutrients, resulting in
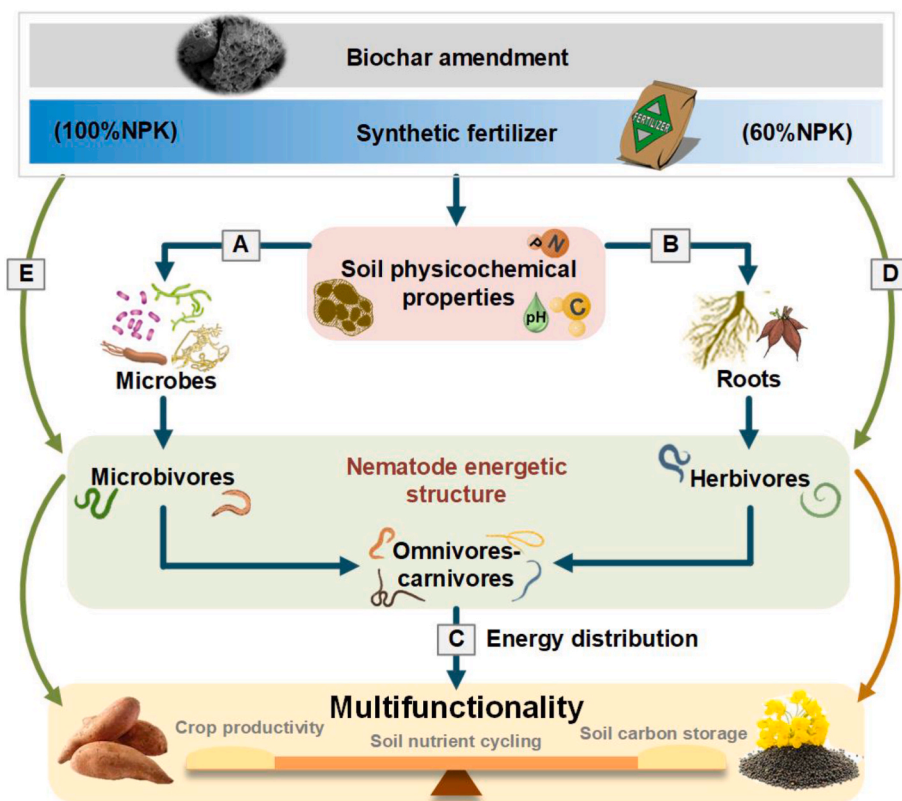

Fig. 1. A conceptual framework showing how soil multifunctionality in sustainable agriculture emerges as the result of the underlying food web structure, which is fueled by energy and nutrients coming from biochar and fertilizer applications to the agroecosystem. Biochar could increase the energy flux through microbivores and herbivores by promoting soil microbial biomass (A) and plant growth (B), respectively. These changes would increase the energy flux through omnivores-carnivores and alter nematode energetic structure, then enhancing soil multifunctionality (C). When nutrients were limited, plant root growth and rhizodeposition would be stimulated, more energy flux through herbivores would disrupt the energy flux balance and change soil multifunctionality (D). Conversely, when nutrients were sufficient, more energy flux by microbivores would favor soil multifunctionality (E).

uniform energy flow through the soil nematode food web (Wan et al., 2022a, 2022b). However, a biochar-amended soil that received less synthetic fertilizer (i.e., 40% lower synthetic fertilizer rate than the local recommendation, here 60% NPK), the beneficial effect of biochar on soil multifunctionality would be limited because a restricted amount of available nutrients and poor soil fertility restricts crop growth.

2. Material and methods

2.1. Site and experiment design

The field experiment is located in Jiangxi Province, China (116°20′24″E, 28.3°15′30″N), which has a typical subtropical monsoon climate with mean annual precipitation of 1537 mm, annual evaporation of 1100–1200 mm, and mean annual temperature of 18 °C. The soil, classified as Ferralsols according to World Reference Base (IUSS Working Group WRB, 2014), has a clay loam texture with 246 g sand kg^{-1}, 358 g silt kg^{-1}, and 396 g clay kg^{-1}. Initially, this acidic soil (pH 4.5) contained 8.4 g kg^{-1} soil organic carbon (SOC), 0.95 g kg^{-1} total nitrogen (N), 0.49 g kg^{-1} total phosphorus (P), 18 g kg^{-1} total potassium (K), 21 mg kg^{-1} available P (Bray-2 extractable nutrients), 160 mg kg^{-1} available K (1 mol L^{-1} NH$_4$OAC extractable nutrients). The field experiment was established in May 2015 with a crop rotation of sweet potato (*Ipomoea batatas* L., from May to October) and oilseed rape (*Brassica napus* L., from October to next May). The experiment followed a randomized complete block design with 4 replicates of each treatment. Each plot was 4 m × 7 m and isolated by a trench with 40 cm wide and 20 cm deep. The treatments selected for this study were: i) 100% synthetic fertilizer (100%NPK), ii) 60% synthetic fertilizer (60%NPK), iii) 100%NPK with biochar, and iv) 60%NPK with biochar.

Synthetic fertilizer and biochar were incorporated into the soil (20 cm depth) with a spike-tooth harrow as basal amendments before planting crops in each growing season. The 100%NPK rate was the local conventional fertilizer recommendation with urea (117 kg N ha^{-1}), calcium superphosphate (44 kg P ha^{-1}) and potassium chloride (149 kg K ha^{-1}). Biochar, prepared by pyrolysis of straw at 350–500 °C in a vertical kiln made of refractory bricks at the Sanli New Energy Company, China, was applied at a rate of 1500 kg ha^{-1}, which contained 376 g kg^{-1} total C, 9.4 g kg^{-1} total N, 9.4 g kg^{-1} total P, 17 g kg^{-1} total K and pH 9.0. The specific surface area of biochar by the Brunauer-Emmett-Teller (BET) method (BELSORP-max II) was 2.3960 m^2 g^{-1}, the micropore surface area was 3.0610 m^2 g^{-1}, the total pore volume was 0.1735 cm^3 g^{-1}, and the biochar adsorption rate was 3.2 mg NH$_4^+$-N g^{-1} and 0.3 mg NO$_3^-$-N g^{-1}.

2.2. Soil and plant sampling

Soil and plant samples were collected at the ripening stage of sweet potato (October 2016 and 2017) and oilseed rape (May 2017 and 2018). Five soil cores (3.5 cm diameter) from the plow layer (0–20 cm depth) were randomly collected and mixed into one composite sample per plot. Fresh soils were sieved (<2 mm) immediately to remove plant residues and stones, followed by nematode community analysis, or stored at 4 °C until soil physicochemical and biological properties were analyzed, as explained below. Storage roots of sweet potato and seed of oilseed rape were harvested manually from each plot to measure crop yield. Five randomly selected plants were collected from each plot to measure the fresh mass of all shoot components (total aboveground of sweet potato, sum of stems, leaves and pods of oilseed rape) and non-food roots (fine roots of sweet potato, all roots of oilseed rape). After this, approximately 100 g of the fresh, non-edible shoot and root subsamples were dried (80 °C for 48 h) to calculate the dry mass of shoots and roots, before nutrient analysis for the N, P and K concentrations.

2.3. Physicochemical and biological analyses

2.3.1. Soil physicochemical and biological properties

Soil pH was measured in a 1:2.5 ratio of soil to deionized water with pH meter. Soil aggregates were separated using the wet-sieving method and three aggregates size classes were obtained, including macroaggregate (>250 μm), microaggregate (250-53 μm) and the silt and clay fraction (<53 μm) (Six et al., 1998). The mean weight diameter (MWD) was used to represent soil structure stability, which was calculated by the equation $MWD = \sum x_i \times w_i$, where x_i was the mean diameter of each fraction size and w_i was the relative weight of correspondence size (Kemper and Rosenau, 1986). Soil organic C and soil total N were determined by the Walkley-Black's wet digestion method and the micro-Kjeldahl digestion (Lu, 2000), respectively. Soil mineral nitrogen (Mineral N) was the sum of NH$_4^+$-N and NO$_3^-$-N, which was extracted with 1:5 ratio of soil: 2 M KCl solution and measured on an Auto Analyzer (AA3, Bran and Luebbe, Germany). Soil microbial biomass carbon (MBC) and nitrogen (MBN) were assessed by the chloroform fumigation-K$_2$SO$_4$ extraction method (Brookes et al., 1985; Vance et al., 1987). Bacterial and fungal biomass carbon were estimated from a phospholipid fatty acids analysis (PLFA) using the modified Bligh-Dyer method (Bossio and Scow, 1998). The detailed protocol of analysis for MBC, MBN, bacterial and fungal biomass carbon were provided in supplementary materials.

2.3.2. Ecological functions

Ecological functions were assessed from the cycling rates and pools of energy and matter, since carbon and nutrients pools could act as surrogate of the soil ecosystem state (Manning et al., 2018; Garland et al., 2021). According to this criteria, 13 ecological functions were selected to quantify the biogeochemical process and ecosystem productivity, and further grouped into three categories: i) crop yield of each product including storage roots of sweet potato, seed of oilseed rape and the N, P and K concentration in harvested biomass, ii) soil organic carbon storage including dissolved organic carbon (DOC), labile organic carbon (LOC), particulate organic carbon (POC), soil basal respiration, enzymes associated with carbon-cycling reactions, iii) soil nutrient cycling including available phosphorus (AP), soil net nitrogen mineralization rate (N mineralization rate), enzymes associated with nitrogen and phosphorus-cycling reactions.

The storage roots of sweet potato and seed of oilseed rape were separately ground to a fine powder on a ball mill and analyzed for product nutrient concentrations. Plant N, P and K were determined by digestion with H$_2$SO$_4$ and H$_2$O$_2$, after which N concentrations was measured by the Kjeldahl method, P concentrations were measured by the molybdenum-blue method and K concentrations were measured using flame photometer analysis (Lu, 2000).

DOC was extracted from 10.0 g fresh soil with 50 ml ultrapure water and determined by a TOC-analyzer (SPD-20A). LOC was determined by oxidation with 333 mM KMnO$_4$ according to the method of Blair et al. (1995). POC was determined by dispersion in 5 g L^{-1} sodium hexametaphosphate according to the methods of Cambardella and Elliott (1992). Soil basal respiration was measured by weighing fresh soil equivalent to 5 g dry mass into jars, and measuring CO$_2$ accumulation by gas chromatography after incubating at 25 °C for 5 h (Creamer et al., 2014). The activities of enzymes associated with carbon, nitrogen and phosphorus-cycling reactions were measured using microplate fluorometric techniques (Bell et al., 2013). AP was extracted with 0.03 M NH$_4$F-0.025 M HCL and determined by the molybdenum-blue method (Lu, 2000). The N mineralization rate was estimated by laboratory incubation method (Evans et al., 2001). The detailed protocol of analysis for N mineralization rate and enzyme activities were provided in supplementary materials.

2.3.3. Nematode extraction, identification and analysis

Nematodes were extracted from 100 g of fresh soil by the modified

Baermann method (using pans instead of funnels for better recovery of nematodes from a shallow soil layer) followed by sugar centrifugal flotation (Liu et al., 2008). After counting, nematodes were killed in 60 °C hot water bath for 5 min to elongate the body before it was fixed in 4% formaldehyde, for ease of identification. About 200 individuals per sample were selected at random for identification to the genus level with a 40× magnification microscope (Olympus B×50). Based on feeding guilds and colonizer-persister values (*cp*), nematodes were classified into bacterivores, fungivores, herbivores, omnivores-carnivores (Yeates et al., 1993). Nematode abundance was expressed as the number per 100 g dry soil.

2.3.4. Nematode energetic structure

We calculated the average fresh biomass of each nematode individuals using the Nemaplex database (http://nemaplex.ucdavis.edu/Ecology/EcophysiologyParms/EcoParameterMenu.htm). Assuming nematode dry weight was 20% of the fresh weight, and carbon in body tissues was 52% of the dry weight, the component of C partitioned into production (P_C) of individuals was calculated as $52\% \times 20\% W_t/100$ (µg C g^{-1}), where W_t represented the fresh biomass (Mulder et al., 2005). The total P_C value per day (µg C 100 g^{-1} dry soil day^{-1}) was calculated after assuming the life-cycle duration of soil nematodes was 12 times the *cp* scale (van den Hoogen et al., 2019), using the formula: $P_C = \sum(N_t(0.1(W_t/m_t/12)))$, where N_t, W_t and m_t were the number of individuals, the fresh biomass and the *cp* class of taxon t respectively. According to the allometric power dependence of metabolism and body size of soil organisms (West et al., 1997), we calculated total carbon used in respiration (R_C, µg C 100 g^{-1} dry soil day^{-1}) of soil nematodes with the formula: $R_C = \sum(N_t(a(W_t^b)))$, where a was the relative molecular weights of C and O in CO_2 (12/44 = 0.273), using a coefficient of 0.058 to further convert the value of a to estimate C respiration in µg per day (Klekowski et al., 1972), and b was close to 0.75 according to Atkinson (1980).

The energy flux of nematode (F, µg C 100 g^{-1} dry soil day^{-1}) was the sum of the production P_C and the respiration R_C and calculated according to nematode metabolic footprints: $F = \sum(N_t(0.1(W_t/m_t/12) + 0.0159(W_t^{0.75})))$, where N_t, W_t and m_t were the number of individuals, the fresh biomass, and the *cp* class of taxon t, respectively (Ferris, 2010; Wan et al., 2022a). We assumed the energetic relations in the nematode food web were in a steady-state system, which allowed us to calculate the C flux to each trophic groups from nematode energy flux, $F_i = (F + L)/e_a$, where F_i was the C flux according to the biomass of nematode trophic group i, L was the energy loss to higher trophic levels via consumption, and e_a was the diet-specific assimilation efficiency, defined as the proportion of food consumed that was used for respiration and tissue production (Jochum et al., 2017). According to published data (Hunt et al., 1987), we used assimilation efficiencies of 0.60 for bacterivores, 0.38 for fungivores, 0.25 for herbivores and 0.5 for omnivores-carnivores.

Higher trophic groups were not considered in our study owing to limited data availability. We assumed the energy flux to omnivores-carnivores resulted in no energetic loss to higher trophic levels, so it was equal to the energetic demand (F_O) of this trophic group. In a nematode food web, where omnivores-carnivores fed on other nematode groups, the energy that they obtained from other trophic groups was assumed to depend on the density within the community (Cui et al., 2018). We calculated the energy loss of bacterivores, fungivores and herbivores to omnivores-carnivores as follows: $L = D_{iO} \times F_O$, D_{iO} was the density-dependent feeding preference of omnivores-carnivores for trophic group i, assigned according to the relative abundance of each of the lower trophic groups. Then, the energy flow uniformity in the soil nematode community (unitless) was determined from the ratio of the mean summed energy flux through each energy channel (e.g., the energy that goes from a bacterivore to an omnivore-carnivore is an energy channel) to the standard deviation of these mean value (Buzhdygan et al., 2020; Wan et al., 2022a). Energy flow uniformity was an indicator of the energy distribution of different channels in nematode food web.

2.4. Statistical analysis

2.4.1. Assessing soil multifunctionality

Soil multifunctionality was quantified by the averaging approach and the multiple-threshold approach. For the threshold approach, we first transformed the plot-level values of each soil function into 0 to 1 with the formula $EF_i = (rawEF_i - min(rawEF))/(max(rawEF) - min(rawEF))$, where EF_i and $rawEF_i$ were the final (transformed) and untransformed function value in plot i respectively and min($rawEF$) and max($rawEF$) were the minimum and maximum untransformed function value in each sampling phase, respectively. The multifunctionality of plot i was estimated from the number of functions where EF_i was greater than the selected threshold level of maximum functioning. We set the maximum level of functioning of each EF as the 95th percentile of the standardized EF value, to avoid the influence of outliers on the estimate. Multiple-threshold multifunctionality was then calculated as the number of functions surpassing a series of consecutive thresholds (from 10 to 90% at 10% intervals) of the maximum for each function (Byrnes et al., 2014). The multifunctionality of averaging approach for plot i was calculated as the average value of the transformed function values in that plot (van der Plas et al., 2016). We assumed the ratio of the multifunctionality indices in the biochar amended treatment (100%NPK + biochar and 60%NPK + biochar) and synthetic fertilizer treatment (100%NPK and 60% NPK) represented changes in multifunctionality due to the biochar amendment under 100%NPK and 60%NPK respectively (a ratio <1.0 represents reduction effect and a ratio >1.0 represents increase effect).

2.4.2. Data analyses

All statistical analyses and figures were generated in R version 4.1.3 (R Core Team, 2022). Prior to analysis, all data were checked for normality with the Shapiro-Wilk test and for homogeneity of variance using Levene tests. The significant differences in soil properties, crop biomass, soil functions and multifunctionality indices between with biochar and without biochar treatments were tested by independent *t*-test. In each crop phase, the soil nematode energy flux, crop biomass, soil physicochemical and biological properties, and soil multifunctionality were analyzed using linear mixed-effects models, in which the reduced synthetic fertilizer, biochar amendment, and their interactions were fixed factors and years were random effects. The energetic structure was visualized as a five-node food web model with network-wide metrics (including biomass and energy flux) using the 'igraph' package (Gauzens et al., 2019). Edge width and node size in network-wide metrics were scaled by the mean of all fluxes to consumers and their biomass, respectively.

To synthesize the information on carbon to nitrogen stoichiometry ratio of soil ecosystem, a principal component analysis (PCA) was performed using the data sets of soil carbon and nitrogen parameters (including SOC, MBC, Mineral N^{-1}, MBN^{-1}, C:N, MBC:MBN and N:C enzyme ratio) from four sampling times. MBN and Mineral N were reciprocal transformed to make unidimensional indicators, i.e., each indicator was positively correlated to the C:N ratio, and all these data were normalized by min-max transformation. The scores on the first coordinate PC1 were used as a proxy for the carbon to nitrogen stoichiometry ratio. Further, linear regression analysis was used to test the relationship between carbon to nitrogen stoichiometry ratio and energy flow uniformity as well as soil multifunctionality.

Partial least squares path model (PLS-PM) was done with the 'plspm' R package (Sanchez et al., 2015) to infer the potential direct and indirect effects of biochar amendment, soil physicochemical properties, soil microbial biomass carbon, root biomass, total energy flux, energy composition and energy flow uniformity on soil multifunctionality under high synthetic fertilizer inputs and low synthetic fertilizer inputs.

Before modeling, data were grouped by sampling phase and normalized by min-max transformation within the group, then all data were divided into two groups based on synthetic fertilizer level. Soil physicochemical properties, microbial carbon and energy composition were latent variables, soil physicochemical properties were indicated by soil pH, soil structure stability and C:N stoichiometry (indicated by the scores of PC1 in Fig. 5). Microbial carbon was reflected by bacterial carbon and fungal carbon. The energy composition was measured by energy flux through bacterivores, fungivores, herbivores and omnivores-carnivores. The quality of the PLS-PM was evaluated by examining the goodness of fit (GoF) index, and by examining the coefficients of determination (R^2) of the latent variables which indicated the amount of variance of the dependent variables explained by their independent latent variables.

3. Results

3.1. Soil functions

Biochar amendment significantly increased high threshold (>70%) soil multifunctionality when the crop received a high synthetic fertilizer input (100%NPK) ($p < 0.05$), but decreased the 90% threshold multifunctionality when it had a low synthetic fertilizer input (60%NPK) (Fig. 2; Table S1). These changes in the multifunctionality indices were consistent across the sweet potato and oilseed rape phases. With 100% NPK, biochar amendment increased 13 single functions by 31% in the sweet potato and by 46% in the oilseed rape phases but reduced single functions by 15% in the sweet potato and by 23% in oilseed rape phases ($p < 0.05$, Fig. 2; Table S1). When 60%NPK was applied, biochar amendment increased single functions by 15% in the sweet potato and by 23% in oilseed rape phases and reduced 38% of the single functions in these crop phases ($p < 0.05$, Fig. 2; Table S1).

3.2. The energetic structure of the soil nematode food web

Biochar-amended soils supported a higher energy flux through soil nematodes when 60%NPK was applied ($p < 0.05$; Fig. 3a). Specifically, biochar amendment increased the energy flux through fungivores regardless of the synthetic fertilizer input ($p < 0.05$; Fig. 3b; Tables S2 and S4). Biochar amendment with 60%NPK also increased the energy flux of herbivores and omnivores-carnivores ($p < 0.05$; Fig. 3b; Tables S2 and S4). Biochar treatments supported a higher energy flux from organic substrates to fungivores and from fungivores to omnivores-carnivores. However, the positive effect of biochar on the energy flux from food resources to herbivores and from herbivores to omnivores-carnivores were only observed with the 60%NPK input (Fig. 4). Biochar amendment with 100%NPK increased the relative energy flux through fungivores and omnivores-carnivores but decreased relative energy flux through herbivores, whereas biochar and 60%NPK input reduced the relative energy flux through bacterivores and increased relative energy flux through fungivores ($p < 0.05$; Fig. S3). Greater energy flow uniformity was found with biochar than without biochar when soil received 100%NPK ($p < 0.05$; Fig. 3c).

3.3. Biochar amendment influences the energetic structure of the soil nematode food web and soil multifunctionality

The C:N stoichiometry was higher with biochar amendments (100% NPK + Biochar and 60%NPK + Biochar) than with synthetic fertilizer alone (100%NPK and 60%NPK) (Fig. 5a). Higher C:N stoichiometry was associated with higher values of SOC, MBC, soil C:N, MBC:MBN and N:C enzyme ratio and lower mineral N and MBN concentration (Fig. 5a). Furthermore, C:N stoichiometry was positively correlated with the energy flow uniformity of nematode communities and soil multifunctionality with an input of 100%NPK, but was not correlated to energy flow uniformity and negatively correlated with soil multifunctionality when 60%NPK was applied (Fig. 5b and c). The energy flow uniformity of nematode communities was positively related to soil multifunctionality at high thresholds (>50%) (Fig. S7).

Biochar amendment affected energy flow uniformity and soil multifunctionality by driving the C:N stoichiometry and other
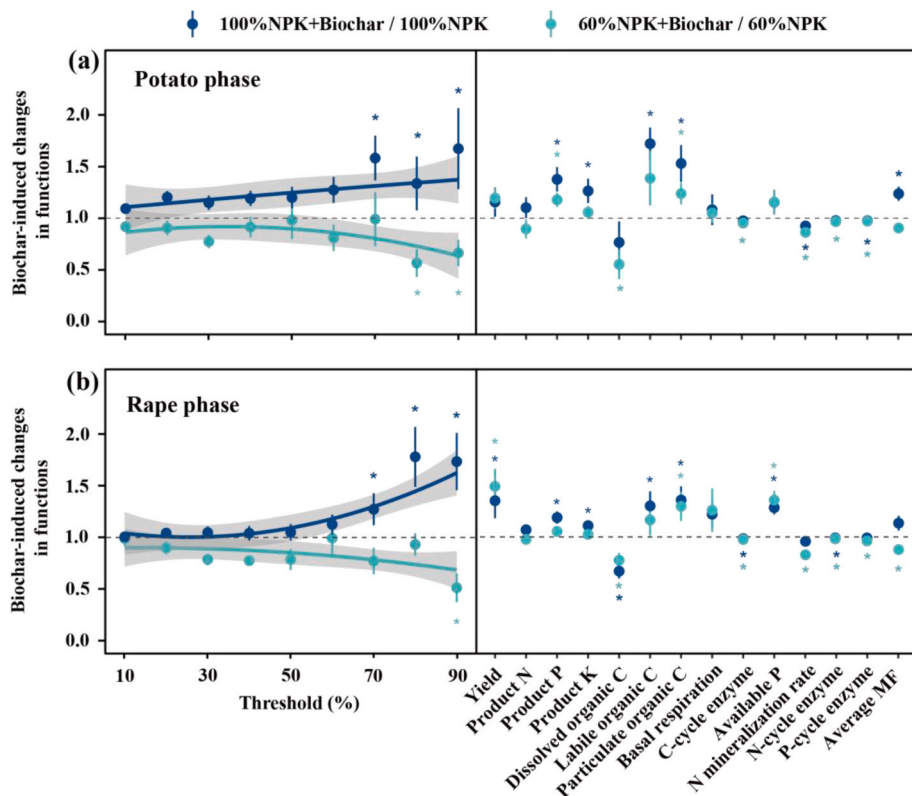

Fig. 2. Changes in multi-threshold multifunctionality indices and soil functions in the sweet potato (a) and oilseed rape (b) phase induced by biochar amendment under high synthetic fertilizer and reduced synthetic fertilizer. Mean ± SD of all variables were expressed as the ratio of the treatments in the biochar amendment (100%NPK + biochar and 60%NPK + biochar) and synthetic fertilizer (100%NPK and 60% NPK), respectively (n = 8, i.e., 2 years × 4 replicates). A ratio <1.0 with asterisks represents a significant reduction ($p < 0.05$) in the soil function or multifunctionality indices, and a ratio >1.0 with asterisks indicates a significant increase ($p < 0.05$) in the soil function or multifunctionality indices due to the biochar amendment. C-cycle enzyme, enzyme associated with carbon-cycling reactions; N-cycle enzyme, enzyme associated with nitrogen-cycling reactions; P-cycle enzyme, enzyme associated with phosphorus-cycling reactions; MF, soil multifunctionality.
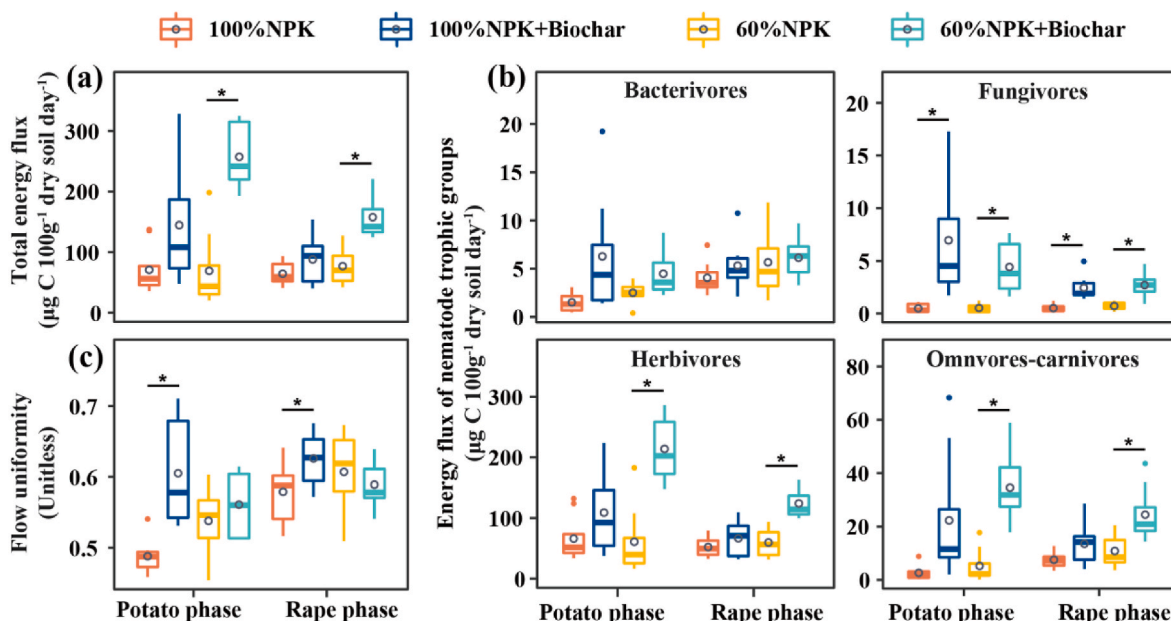
Fig. 3. Changes in the energy flux through total soil nematodes (a) and through each trophic group (b) (bacterivores, fungivores, herbivores, omnivores-carnivores) and the energy flow uniformity (c) in the sweet potato and oilseed rape phase induced by biochar amendment. Box plots represent the lower quartile, median and upper quartile values. The lines and circles in the boxes represent the median and mean values of all variables (n = 8, i.e., 2 years × 4 replicates). Asterisk indicates significant differences in treatment effects ($p < 0.05$).
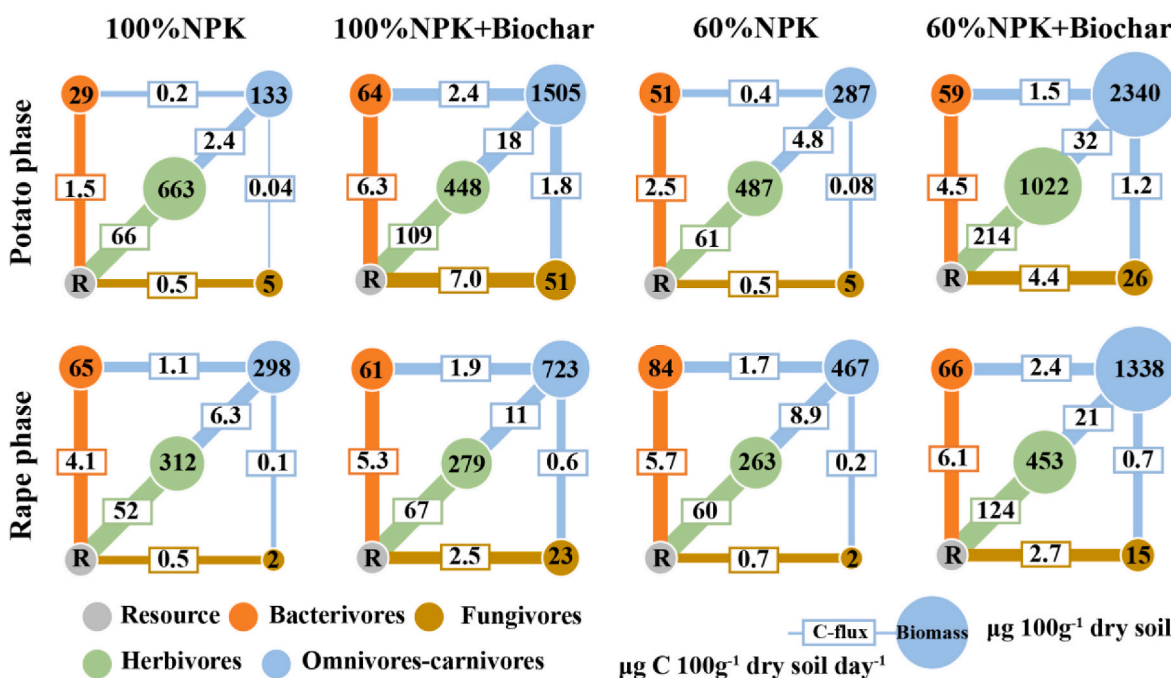

Fig. 4. Changes in carbon flux and biomass distribution between nematodes in the sweet potato and oilseed rape phase induced by biochar amendment. For each treatment, a five-node food web was constructed with bacterivores (orange), fungivores (brown) and herbivores (green) receiving energy from basal resources, omnivores-carnivores (blue) receiving energy from other nodes (n = 8, i.e., 2 years × 4 replicates). Numbers on the arrows represented energy flux (µg C 100 g^{-1} dry soil day^{-1}). The size of nodes corresponded to the fresh biomass (µg 100 g^{-1} dry soil).

physicochemical and biological properties (soil pH, structure stability, microbial carbon) as well as root biomass when 100%NPK and 60%NPK were applied. Altogether, the soil and root variables explained 79% of the variation in energy flow uniformity and 44% of the variation in soil multifunctionality with an input of 100%NPK (Fig. 6a). Similarly, all soil and root variables explained 40% of the variation in energy flow uniformity and 34% of the variation in soil multifunctionality when 60%

NPK was applied (Fig. 6c). Inputs of 100%NPK and 60%NPK with biochar was positively related to soil physicochemical properties, which was positively correlated to microbial carbon. And the microbial carbon was positively correlated to the energy composition of nematodes. However, biochar-induced changes in soil physicochemical properties could also exert positive effects on root biomass at 60%NPK, changes in the root biomass have negative effects on energy composition and
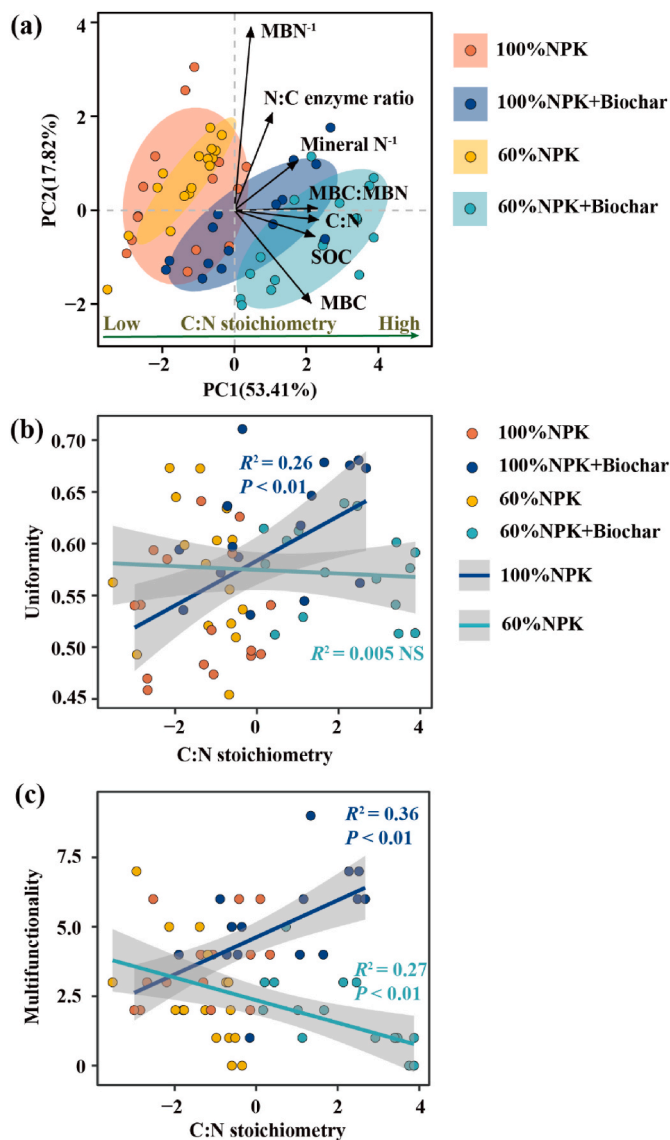
Fig. 5. Principal component of analysis (PCA) based on carbon to nitrogen stoichiometry ratio (C:N stoichiometry, including SOC, MBC, Mineral N^{-1}, MBN^{-1}, C:N, MBC:MBN, N:C enzyme ratio) (a). Relationship between C:N stoichiometry (indicated by the scores of PC1 in panel a) and energy uniformity of nematode communities (b) and multifunctionality (multifunct. 80%) (c). Colored lines indicate different synthetic fertilizer levels (100%NPK, 60%NPK) (n = 32, i.e., 2 treatments × 2 years × 2 crops × 4 replicates). Shaded areas around the lines show the 95% confidence intervals. NS, no significant. C:N, soil organic carbon to nitrogen ratio; N:C enzyme ratio, enzyme associated with nitrogen-cycling reactions to carbon-cycling reactions ratio.

positive effects on total energy flux. Furthermore, the total energy flux and energy composition had negative and positive effects on energy flow uniformity of nematode food web respectively, and the energy flow uniformity was positively correlated with soil multifunctionality (80% threshold). Among all biotic variables in predicting soil multifunctionality, the energy flow uniformity had the highest positive effects, and the root biomass had the highest negative effects (Fig. 6b and d).

4. Discussion

4.1. Biochar uniforms the energetic structure of nematode food web

As hypothesized, applying biochar together with the recommended

rate of synthetic fertilizer (100%NPK) increased the energy flow uniformity of the soil nematode food web. We found that biochar enhanced the energy flux through fungivores in all synthetic fertilizer treatments, and this was related to a greater fungal biomass carbon concentration (Fig. S6). This is consistent with previous studies showing that biochar is a source of organic substrates preferred by fungi, relative to bacteria (Bahram et al., 2018; Pollierer et al., 2012). In addition, biochar can be an alternative habitat that is colonized by fungi because it induces an oligotrophic environment (Thakur and Geisen, 2019). Several factors might be associated with lower soil nutrient content of biochar and in biochar-amended soil. First, biochar derived from plant residues tends to have a low nitrogen content (Bolan et al., 2022). Second, biochar surfaces can absorb mineral nitrogen, within the nano-sized and micron-sized pores, and the sorption of NH_4^+ on the negatively charged functional groups on external surfaces of biochar particles (Saarnio et al., 2013) and NO_3^- to positively charged moieties on biochar (Ren et al., 2020). Third, biochar-induced increase in soil pH of the acidic soil enhances crop growth, which increases the competition for nitrogen between soil microbes and plants (Peng et al., 2011). Overall, biochar addition stimulated more direct carbon transfer to fungi, and indirectly increased the energy flux to fungivores through bottom-up effects.

We were surprised that biochar decreased the relative allocation of energy flux to herbivores when combined with the higher synthetic fertilizer rate, but this trend reversed when biochar-amended soil received the reduced synthetic fertilizer input. Other reports indicated that biochar favored plant colonization with beneficial microorganisms that induced plant resistance, which lowered the energy flux through herbivores (Huang et al., 2015; Kolton et al., 2017). The energy flux through herbivores was also impacted by their food resources (Jiang et al., 2020). We found that biochar plus low synthetic fertilizer input increased root biomass (Fig. S4), which could provide more food resources for herbivores. One possible mechanism was that plants enhanced nutrient uptake by promoting resource allocation to roots in the context of belowground nutrient limitations (Poorter et al., 2012). Our field experiment confirmed that biochar increased the soil C:N ratio, which limited the nitrogen supply to crops that had lower than recommended synthetic fertilizer inputs. This feedback probably changed the resource allocation between shoots and roots (Shipley and Meziane, 2002), consequently more carbon was allocated to roots and ultimately increased the energy flux through herbivores.

4.2. Biochar-induced changes in nematode energetic structure enhance soil multifunctionality

As hypothesized, we found that biochar with the high synthetic fertilizer input increased soil functions at high threshold levels. Biochar had positive, neutral or negative effects on single functions, and reduced a few functions such as dissolved organic carbon and nitrogen mineralization rate. We found the energy flow uniformity of the soil nematode food web was positively related to soil multifunctionality at a high threshold (>50%) (Fig. S7). Biochar applied with the high rate of synthetic fertilizer increased the relative allocation of energy flux to fungivores and omnivores-carnivores but decreased that to herbivores (Fig. S3), which evenly distributed the energy flow through different pathways in the soil nematode food web. A soil food web with higher energy flow uniformity has balanced resources for all trophic groups, which makes an energy-efficient communities that minimizes energy loss (Buzhdygan et al., 2020; Ghedini et al., 2018). Our results demonstrated the multitrophic energy flux complementarity, energy through different trophic groups of soil food web jointly increased soil multifunctionality.

Biochar amendment combined with low synthetic fertilizer input had low multifunctionality and low energy flux uniformity across trophic groups resulting from high energy flux through the herbivores. This might occur due to imbalance in the carbon to nitrogen stoichiometry because nitrogen limitation caused by the high soil C:N ratio was
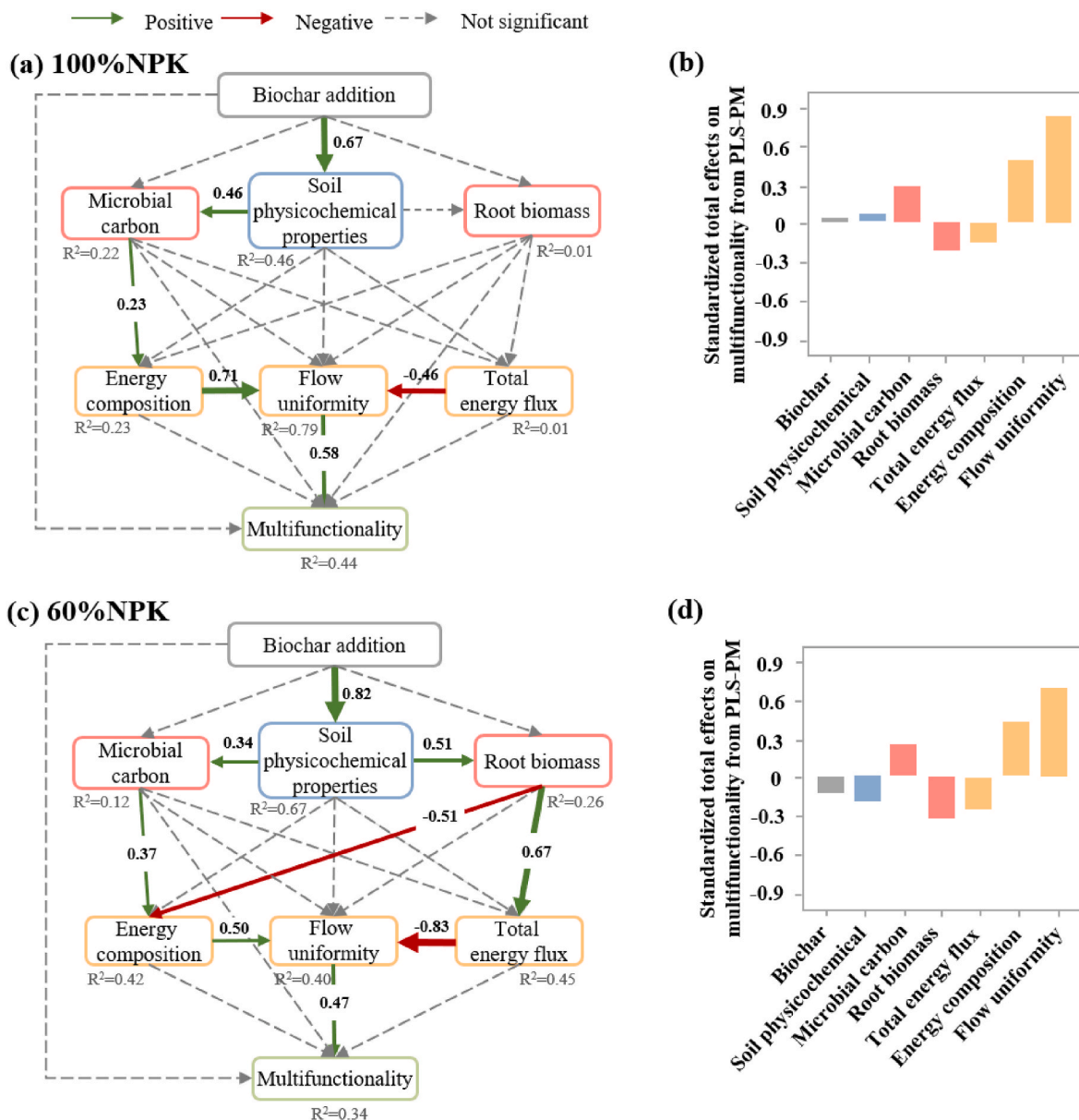
Fig. 6. The partial least squares path models (PLS-PM) illustrating the direct and indirect effects of biochar amendment, soil physicochemical properties, soil microbial biomass carbon, root biomass, total energy flux, energy composition and flow uniformity on multifunctionality and standardized total effects under high synthetic fertilizer level (a, b; GoF = 0.45; n = 32) and reduced synthetic fertilizer level (c, d; GoF = 0.51; n = 32). Soil physicochemical properties, microbial carbon and energy composition are latent variables, soil physicochemical properties were indicated by soil pH, soil structure stability and C:N stoichiometry (indicated by the scores of PC1 in Fig. 5). Microbial carbon was reflected by bacterial carbon and fungal carbon. The energy composition was measured by energy flux through bacterivores, fungivores, herbivores and omnivores-carnivores. The red and green arrows indicate negative and positive flows of causality, respectively. Numbers on the arrowed lines indicate normalized path coefficient. The dotted gray arrows represent non-significant path relationships. R^2 besides the latent variables are the coefficients of determination. The GoF index represents the goodness of fit. Multifunctionality in the figure was presented as threshold approaches (multifunct. 80%).

strongly associated with greater energy flux through herbivores. The stoichiometry balance of soil carbon and nitrogen potentially optimized the energy structure of the food web by enhancing carbon assimilation efficiency and balancing the energy distribution among trophic levels (Vrede et al., 2004; Zhang et al., 2022). Although biochar amendment could be a carbon source that boosts energy flow uniformity, high soil C: N stoichiometry resulting from insufficient nitrogen fertilizer could impede this positive effect (Fig. 5). As a consequence, soil C:N stoichiometric imbalance could interfere with trophic functions that relied on soil carbon and nutrient cycling, especially in acidic tropical soil with a very high weathering rate and high nutrient-limitation (Wang et al., 2019). When using biochar as soil amendment, it would be important to add sufficient synthetic fertilizer to avoid nutrient limitations that might

interfere with the long-lasting beneficiary effects of biochar. Our study clearly indicated that the effect of biochar application on the energy dynamics of the soil food web is strongly determined by nitrogen availability, which affects the position and distribution of energy within the soil food web and ultimately altering soil multifunctionality.

5. Limitations and outlook

Energy fluxes reveal interactions within food webs, but it is challenging to precisely quantify the energy dynamics across multiple trophic levels in soil ecosystem (Gauzens et al., 2019). This study could provide limited insight into soil energy dynamics because we calculated energy fluxes through the nematode food web, a simplistic proxy of a

realistic soil food web. Future work should consider the higher trophic groups of the soil food web, such as macro- and mesofauna, which are not available for the current study but critical for systematic understanding energy flux through the whole soil food web. In addition, parameters in the energetic models were based in part on published data, which might not necessarily be accurate for the nematode food web in a subtropical agroecosystem. As such, the results allow comparison between a few agricultural treatments, they cannot be generalized to understand energy fluxes in other agroecosystems (Jochum et al., 2021). Advanced methods such as molecular gut analysis, fatty-acid analysis and stable-isotope analysis are needed to determine assimilation efficiencies and confirm the multidimensional trophic niches that affect the energy structure of the soil nematode food web (Potapov et al., 2021).

6. Conclusions and implications

We concluded that biochar combined with synthetic fertilizer could enhance soil multifunctionality at high thresholds in a subtropical agroecosystem, mainly through strengthening the energy flow uniformity of the nematode food web. Higher energy flow uniformity under biochar amendment was associated with increased energy flux of fungivores and decreased that of herbivores, and this trend would be counteracted when combining the currently advocated reducing synthetic fertilizer input. Accompanied with the reducing available nutrient input, biochar amendment intensified the nitrogen limitations in light of crop demands and stimulated root vigorous growth. This, in turn, would boost the energy flux through herbivores, and such a trend would be aggravated with time. Critically, the present study further proved that the suitable soil carbon-to-nitrogen stoichiometry would be fundamental to sustaining multiple agroecosystem services. In particular, our research highlighted the importance of systematic considering multitrophic soil biota and multifunctionality particularly when adopting the new soil sustainable managements. Collectively, our study could provide new insights into how biochar influence ecosystem multifunctionality in subtropical region, would be useful for strengthening the knowledge of the functional mechanism for biochar, and would provide a basis for guiding the sustainable utilization of biochar in these regions. Given the nutrient dependency of biochar effects, a future research priority is to underline the ecological stoichiometry as a corner stone relating to multifunctionality for regenerative agricultural management under the future scenarios of food demands and global change.

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.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2023.109056.

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