



Energy flux across multitrophic levels drives ecosystem multifunctionality: Evidence from nematode food webs

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ABSTRACT

Energy flux in food webs, i.e., energy consumption by different trophic groups and describing their energetic structure, has been proposed as a powerful tool to understand the relationships between biodiversity and multiple ecosystem functions (ecosystem multifunctionality). Here we examined how different fertilization regimes affected the energy flux across multitrophic levels of soil nematodes in the paddy rice and upland maize fields. We considered 13 ecosystem functions of four ecological processes related to plant productivity, nutrient cycling processes and drivers, and functional stability, which are central to energy and nutrient flow across trophic levels. To confirm whether multitrophic flux would underpin the relationships between biodiversity and multifunctionality, we compared energy flux with other approaches including taxonomic diversity, functional diversity and community composition. Results showed that organic fertilizer supported 33–340% greater multitrophic energy flux of soil nematode community and enhanced 41–264% of ecosystem multifunctionality in both fields compared with mineral fertilizer treatments. Organic fertilization enhanced ecosystem multifunctionality by favoring energy flux in multitrophic levels of soil nematodes, while fertilization-mediated changes in other facets of biodiversity were less related to multifunctionality. Our study provides empirical evidence that energy flux within food webs can be used to understand the impacts of environmental change drivers on ecosystem multifunctionality.

1. Introduction

For the past two decades, intensive studies have confirmed the pivotal role of biodiversity in determining ecosystem multifunctionality, i.e., the ability of ecosystems to simultaneously provide multiple functions and services (Bardgett and Van Der Putten, 2014; Guerra et al., 2020; Manning et al., 2018; Wagg et al., 2021). Multiple facets of biodiversity have been proposed as predictors of ecosystem multifunctionality, such as taxonomic diversity (Bardgett and Van Der Putten, 2014; Yachi and Loreau, 2007), functional diversity (Cadotte, 2017; Gross et al., 2017), community composition (Mori et al., 2018; Wagg et al., 2014), and biotic associations (Liu et al., 2019). Each biodiversity attribute provides different information to enhance our understanding of potential consequences for ecosystem functioning. For instance,

taxonomic diversity can describe the gains or losses of different taxa present in the community (Jarzyńska and Jetz, 2016), while functional diversity captures whether a community can maintain its functions under environmental changes (Petchey and Gaston, 2006). This field is largely built by examining the effects of these biodiversity facets on ecosystem functioning from single trophic levels or simple food chains. As emerging efforts suggest that incorporation of multitrophic complexity is needed for ecosystem multifunctionality (Barnes et al., 2014; Soliveres et al., 2016), quantifying energy fluxes within multitrophic levels is proposed as a powerful and universal tool for understanding the impacts of environmental factors on ecosystem functioning (Barnes et al., 2018). However, the application of energy flux in soil food web to biodiversity-ecosystem functioning research, particularly in agroecosystems, remains virtually untapped since the theoretical

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framework was proposed (Barnes et al., 2018).

Fertilization as a common agricultural practice alters a wide range of ecosystem functions through driving the belowground soil biodiversity (Bender et al., 2016; Delgado-Baquerizo et al., 2019; Felipe-Lucia et al., 2020). The inputs of nitrogen and organic carbon accelerate energy flux in soil food web by providing nutrients and energy for the growth of organisms in multitrophic levels such as plants, microbes, herbivores, microbivores and predators (Cui et al., 2018; Li et al., 2021; Pollierer et al., 2012; Shaw et al., 2019). For instance, the energy flux through root herbivores determines the carbon inputs from plants to soil food web (Gan and Wickings, 2020), and the energy flux through decomposers can be directly associated with nutrient cycling and storage (Pollierer et al., 2012; Zhang et al., 2021a). Thus, quantifying multitrophic flux in soil food web may provide a powerful approach to mechanistically understand how fertilization influences the ecosystem multifunctionality through bottom-up effects. However, only few studies have addressed the effect of fertilization on ecosystem functions using a multifunctionality approach (Chen et al., 2020; Luo et al., 2018), and it is still unclear how fertilization will influence the energy flux across multiple trophic levels in the soil food web.

To measure the trophic transfer of energy flux through the soil food web, nematode communities are ideal models because they comprise functional groups at multiple trophic levels: herbivores, bacterivores, fungivores, omnivores and predators (van den Hoogen et al., 2019; Wilschut and Geisen, 2021). The food-web energetics approach can be described as an ecological network calculating energy flux between network nodes represented by each functional group. Herbivores, in the primary production-based food chains, are expected to improve the energy transfer into the nematode food webs as they are fostered by the increased root biomass of crops under fertilization (Gan and Wickings, 2020; Pausch et al., 2016). Bacterivores and fungivores, as basal groups consuming microbes, differ in energy transfer efficiency (Vestergård et al., 2019; Zhou et al., 2021), which may alter the distribution pattern of energy flux through food webs (Cui et al., 2018). Omnivores and predators, as top-down controllers of the lower trophic groups in the nematode food webs, are more likely to benefit from organic fertilizers rather than mineral fertilizers (Liu et al., 2016a). These pieces of evidence suggest that quantification of energy flux in the multitrophic networks of soil nematodes may improve our understanding of ecosystem multifunctionality affected by fertilization.

Here we adopt a multitrophic perspective to analyze the influence of fertilization on ecosystem multifunctionality considering 13 independent ecosystem functions of four important ecological processes: plant productivity, nutrient cycling processes and drivers, and functional stability. We obtained the data from paddy and upland agroecosystems, which were both treated with organic and mineral fertilizers over three decades. We hypothesized that organic and mineral fertilization would facilitate multitrophic energy fluxes of nematode food webs as compared to no fertilizer treatment. To confirm whether energy fluxes in nematode food webs would be a suitable approach for explaining fertilization-induced changes in ecosystem multifunctionality, we compared multitrophic flux with other biodiversity approaches including multitrophic diversity, functional diversity, and community composition. We hypothesized that multitrophic energy flux would be a reliable determinant of ecosystem multifunctionality in both paddy and upland agroecosystems.

2. Material and methods

2.1. Study sites and soil sampling

The experiments were initiated in 1981 at the Jiangxi Institute of Red Soil, China (116°20'24"E, 28°15'30"N). This region has a subtropical monsoon climate, with a mean annual temperature of 15.7 °C and mean annual precipitation of 1727 mm. The soil is classified as ultisols with a clay-loam texture (Dai et al., 2020). Identical fertilization designs were

conducted in a paddy rice and upland maize field, respectively, about 1000 m apart. Five fertilization treatments were randomly assigned and replicated three times. These fertilization treatments were no fertilizer (CK), 50% mineral fertilizer (50%NPK), 100% mineral fertilizer (NPK), pig manure amendment (OM) and 50% mineral fertilizer combined with pig manure amendment (NPKM). Pig manure was applied as organic amendment, which contained on average 340 g kg⁻¹ of organic C, 28.3 g kg⁻¹ of total N, 10.3 g kg⁻¹ of total P and 9.8 g kg⁻¹ of total K from measured data over three decades. Pig manure and mineral NPK fertilizer were incorporated via tillage every growing season before planting rice and maize. Detailed information of experiment layout, the doses and types of fertilizer were shown in the supporting materials (Table S1). The paddy field was planted with early rice (April to July) and late rice (July to November) cropping, and the upland field was planted with early maize (April to July) and late maize (July to November).

Soil samples and plant roots were collected at the ripening stage of rice and maize in November 2014. In each plot, nine soil cores (3.8 cm in diameter and 20 cm in depth) were randomly collected from the soil plough layer (0–15 cm), and mixed together as one composite sample. Soil samples were quickly taken back to the laboratory and stored at 4 °C. All ecosystem functions and nematode communities that should be analyzed with fresh soils were determined within two weeks, and other ecosystem functions were determined within two months. Dissolved organic C and N were extracted from 10 g fresh soil with 50 mL ultrapure water and determined using a total C/N analyzer (Elementar, Germany). Extractable nitrogen was extracted from 10 g fresh soil with 50 ml 2 M KCl and determined using a continuous flow analyzer (Skalar, Breda, the Netherlands). Soil available phosphorus was extracted by 0.5 M NaHCO₃ and determined using molybdenum blue method (Lu, 2000).

2.2. Nematode identification and community attributes

Nematodes were extracted from 100 g fresh soil samples using a modified Baermann method (Liu et al., 2008). After recording the total number of nematodes, 200 individuals were randomly selected from each sample and identified to genera based on morphological characteristics (Andrássy, 1983; Ahmad and Jairajpuri, 2010; Bongers, 1988; Yin, 1998). Each individual was then assigned into bacterivores, fungivores, herbivores and omnivores-carnivores based on feeding guilds (Yeates and Bongers, 1999). The biomass and colonizer-persister (*cp*) values of identified nematodes were determined using publicly available data (<http://nemalex.ucdavis.edu/Ecology/EcophysiologyParms/EcoParameterMenu.htm>).

2.2.1. Energy flux

We calculated the community energy flux based on the main steps as follows: Step 1, the fresh biomass of each nematode individual was calculated using publicly available data. Step 2, nematode metabolism (F , $\mu\text{g C } 100 \text{ g}^{-1} \text{ dry soil day}^{-1}$) was then calculated according to the formula: $F = \sum(N_t(0.1(W_t/m_t/12) + 0.0159(W_t^{0.75})))$, where N_t , W_t and m_t are the number of individuals, the fresh weight and the *cp* class of taxon t , respectively (Ferris, 2010; van den Hoogen et al., 2019). Step 3, a five-node food web topology was constructed and the feeding preferences of omnivores-carnivores on other trophic groups were assumed according to community density. Step 4, the metabolism of each node was summed by all individual metabolism of the corresponding trophic group. Step 5, we used assimilation efficiencies (e_a) of 0.25 for herbivores, 0.60 for bacterivores, 0.38 for fungivores and 0.5 for omnivores-carnivores according to Barnes et al. (2014) and De Ruiter et al. (1993). Step 6, energy fluxes between nodes were calculated as follows: $F_i = (F + L)/e_a$, where F_i was the energy flux through nematode trophic groups i , F was the energy metabolism of nematode community for respiration and growth, L was the energy loss to higher trophic levels, and e_a is the assimilation efficiency of a given nematode trophic group (Barnes et al., 2018). For example, for the bacterivores, the energy losses

(L) was equal to the energy flux from bacterivores to the omnivores-carnivores following $L = D_{BO} \times F_O$ (Barnes et al., 2014), where D_{BO} was the density-dependent feeding preference of omnivores-carnivores for bacterivores, F_O was the energy flux through omnivores-carnivores. D_{BO} was assigned according to their proportional abundance following $D_{BO=AB} / (AB+AF+AH)$, where AB, AF, AH was the abundance of bacterivores, fungivores and herbivores, respectively (Cui et al., 2018). A calculation example for the bacterivores was provided in the supplementary materials (Excel named calculation example).

2.3. Multiple ecosystem functions

A total of 13 ecosystem functions were measured to assess the ecosystem multifunctionality. We grouped these ecosystem functions into four categories, including i) plant productivity (crop yield, aboveground and belowground biomass), ii) soil nutrient cycling processes (total organic C storage, total N storage and C mineralization), iii) soil nutrient cycling drivers (microbial biomass C, microbial biomass N, microbial biomass P and C, N, P cycling enzymes), and iv) soil functional stability. Measurements of each ecosystem function were as follows:

2.3.1. Plant productivity

Parameters including crop yield, aboveground- and belowground biomass were determined from an area of 1 m² in each plot. The roots were dug from about 30 cm depth to collect the belowground biomass. All the measurements were weighted in dry mass (oven-dried at 65 °C).

2.3.2. Soil nutrient cycling processes

Total organic C and total N storage were determined by the Walkley-Black wet digestion method and the Kjeldahl digestion method, respectively (Nelson and Sommers, 1982; Sparks et al., 1996). C mineralization was estimated based on the quantity of CO₂-C, mineralized from fresh soil sample during a short-term incubation (Franzuebbers et al., 1994). Briefly, 50 g fresh soil (three replicates) was placed into a 250 ml jar, which contained a smaller container filled with 0.2 M NaOH solution that needed to be replaced regularly. The jars were sealed and incubated in darkness for 28 days at 22 °C. CO₂ was trapped by an excess of 0.2 M NaOH solution and then determined by titration with 0.1M HCl solution. C mineralization was calculated by the changes in the cumulative CO₂ respiration.

2.3.3. Soil nutrient cycling drivers

Soil microbial biomass carbon (MBC), nitrogen (MBN) and phosphorus (MBP) were assessed by the chloroform fumigation-K₂SO₄ extraction method with the K_{EC} factor of 0.38, the K_{EN} factor of 0.45 and K_{EP} factor of 0.4, respectively (Brookes et al., 1985; Vance et al., 1987). Four carbon-cycling enzymes (including Cellulase, Xylanase, Invertase and β-D-glucosidase), four nitrogen-cycling enzymes (including Urease, Histidase, L-asparaginase and L-glutaminase) and one phosphorus-cycling enzyme (Phosphatase) were determined (Alef and Nannipieri, 1995; Schinner et al., 1996). The invertase, xylanase and cellulase were measured by 3, 5-dinitrosalicylic acid colorimetry. The β-glucosidase was determined by the p-nitrophenol colorimetry. Urease was measured by sodium phenolate colorimetry. Histidase, L-asparaginase and L-glutaminase were determined colorimetrically by ammonia formation. The phosphatase was measured by disodium phenyl phosphate colorimetry (Öhlinger, 1996).

2.3.4. Soil functional stability

Soil functional stability was measured according to Griffiths et al. (2000). For each soil sample, aliquots of 10 g fresh soil (on a dry weight base) were placed into a 150 ml jar. The jars were randomly assigned to two treatments: control (no perturbation applied) and heat perturbation (50 °C for 24 h). All jars were amended with 100 mg barley grass powder (C: N = 10: 1) and then sealed and incubated in the dark for 7 days at 22 °C. CO₂ was trapped by an excess of 0.2 M NaOH solution and then

determined by titration with 0.1M HCl solution. Functional stability was calculated by the changes in short-term decomposition of the perturbation treatment compared with the control: Stability index (%) = (CO₂ treatment/CO₂ control) × 100%.

We used the averaging approach and threshold approach to quantify the ecosystem multifunctionality (Byrnes et al., 2014; Jing et al., 2015). To obtain a quantitative multifunctionality index, all individual ecosystem function variables were standardized to have a minimum of zero and a maximum of 1 as follows: $EF = (rawEF_i - rawEF_{min}) / (rawEF_{max} - rawEF_{min})$, with EF indicating the final (transformed) ecosystem function value and raw EF indicating raw (untransformed) ecosystem function values (Van Der Plas et al., 2016). Following this, the standardized variables were then averaged to obtain the multifunctionality index (Wagg et al., 2014). The averaging approach could clearly assess ecosystem services when multiple functions were at high values or low values simultaneously. However, the averaging approach couldn't distinguish individual values from many functions, if some functions were at high values and others are at low ones (Byrnes et al., 2014). To address this problem, an alternative approach like the threshold approach was used to capture the number of functions that had passed a critical threshold of 'functionality' in a given plot, even when trade-offs among multiple functions existed at high and low values (Byrnes et al., 2014). Every observation of each function was transformed into a percentage of the maximum performance of the function. Each threshold represented the number of functions working beyond that level of functional performance. Here, we used three threshold values (30%, 50% and 70%) to characterize multifunctionality, because they included low, medium and high percentiles which could be representative parameters (Li et al., 2019; Liu et al., 2019; Ratcliffe et al., 2017). For example, when calculating the multifunctionality 30% index for each plot, a 30% threshold estimated the number of ecosystem functions whose value exceeded 30% for that function observed across all plots.

2.4. Data analysis

We first explored the taxonomic diversity of nematodes, estimated with the Shannon index, using function *diversity* in the *vegan* package (Oksanen et al., 2013). We also normalized taxonomic diversity and energy flux of herbivores, bacterivores, fungivores and omnivores-carnivores using Z-score transformation. Then, the normalized values of all trophic groups were averaged to represent the multitrophic diversity and multitrophic energy flux, respectively (Delgado-Baquerizo et al., 2020; Soliveres et al., 2016). Non-metric multidimensional scaling (NMDS) was used to assess the community similarity based on the relative abundance of identified nematodes (Legendre and De Cáceres, 2013). As a way of describing the functional characterization of soil nematodes in each plot, we calculated functional diversity (RaoQ) based on three trait categories: (1) trophic groups including bacterivores, fungivores, herbivores and omnivores-carnivores; (2) colonizer-persister (*cp*) representing the species susceptibility to environmental changes; (3) biomass (Li et al., 2020; Mulder and Maas, 2017). Functional diversity was calculated using the *FD* package in R (Laliberté et al., 2014).

Before further analysis, all data were tested for normality using the Kolmogorov-Smirnov method, and tested for equality of error variances using Levene's test. If prerequisites were violated, ecosystem functions and nematodes data were square-root-transformed to meet the requirements of normality and homoscedasticity of errors. Variables of ecosystem functions (crop yield, aboveground and belowground biomass, total organic C storage, total N storage, C mineralization, MBC, MBN, MBP and C, N, P cycling enzymes and functional stability index) and community attributes (multitrophic diversity, functional diversity and multitrophic energy flux) were analyzed using linear mixed-effects models with fertilization, land type, and their interactions as fixed factors and replicate as random factors. The linear mixed-effects models

were analyzed with the *nlme* package (Pinheiro et al., 2017). An ANOVA with post-hoc LSD test was conducted to assess the significant difference among treatments using *emmeans* package (Lenth, 2019). A heat-map was generated using *GGally* (Schloerke et al., 2018) and *ggplot2* packages (Wickham, 2011) to analyze the correlations between each of the 13 ecosystem functions and community attributes (multitrophic diversity, functional diversity, community composition and multitrophic energy flux).

Piecewise structural equation model (Piecewise SEM) was conducted to identify the direct and indirect effects of fertilization, soil nutrients (dissolved organic carbon and dissolved organic nitrogen, extractable nitrogen and available phosphorus) and community attributes (multitrophic energy flux, multitrophic diversity, functional diversity and community composition) on ecosystem multifunctionality (average) using the *piecewiseSEM* package (Lefcheck, 2016). Before modeling, all data were normalized by Z-score transformation. We performed a principal component analysis to calculate the composite variables of fertilization, soil nutrients and community composition in the Piecewise SEM analysis. The principal component analysis for fertilization was calculated based on the nutrient input of carbon, nitrogen, phosphorus and potassium, while the community composition was calculated based on the relative abundance of nematode individuals. We used the first principal component, which explained 23–80% of the total variance in each group, as the indicator of fertilization, soil nutrients and community composition. A maximum-likelihood χ^2 goodness-of-fit test was used for the model fitting. For the χ^2 test, an accepted model should have a *p* value > 0.01 (Schermerle-Engel et al., 2003). Further, we used linear mixed-effects models (LMM) to demonstrate the effects of fertilization on ecosystem multifunctionality, which were mediated by five predictors (soil nutrient, multitrophic diversity, functional diversity, community composition, and multitrophic energy flux). LMM was analyzed with the *lme4* package (Bates et al., 2007). In the analyses of

Piecewise SEM and LMM, soil nutrients and community attributes were assigned as fixed factors, whereas land types (paddy and upland) and replicate were assigned as random factors.

3. Results

3.1. Organic fertilization increased ecosystem functions

Organic fertilization (OM and NPKM) increased ecosystem multifunctionality (i.e., multifunctionality30%, multifunctionality50% and multifunctionality70% as well as multifunctionality average) compared with no fertilizer treatments in paddy and upland fields, while mineral fertilization (50%NPK and NPK) only increased the multifunctionality in paddy field (*p* < 0.05; Fig. 1 and Table S2). Organic fertilization supported 198% higher multifunctionality than mineral fertilization in the upland field (*p* < 0.05; Fig. 1 and Table S2). Generally, organic and mineral fertilization increased the measured ecosystem functions by 92–100% and 39–46%, respectively, compared with no fertilizer treatment (*p* < 0.05; Fig. 1 and Table S2).

3.2. Fertilization affected community attributes of soil nematodes

Organic fertilization supported 33–340% higher multitrophic energy flux than mineral fertilization in both fields, whereas mineral fertilization did not enhance the multitrophic energy flux compared with no fertilizer treatments (*p* < 0.05, Fig. 2d and Table S3). The multitrophic diversity was 30–227% higher under organic fertilization treatment than mineral fertilization treatments in both fields (Fig. 2a). Organic fertilization increased multitrophic diversity as compared to no fertilizer treatment in upland field, but showed no significant effects in the paddy field (*p* < 0.05, Fig. 2a and Table S3). The functional diversity was increased in organic fertilizer treatments compared to no fertilizer

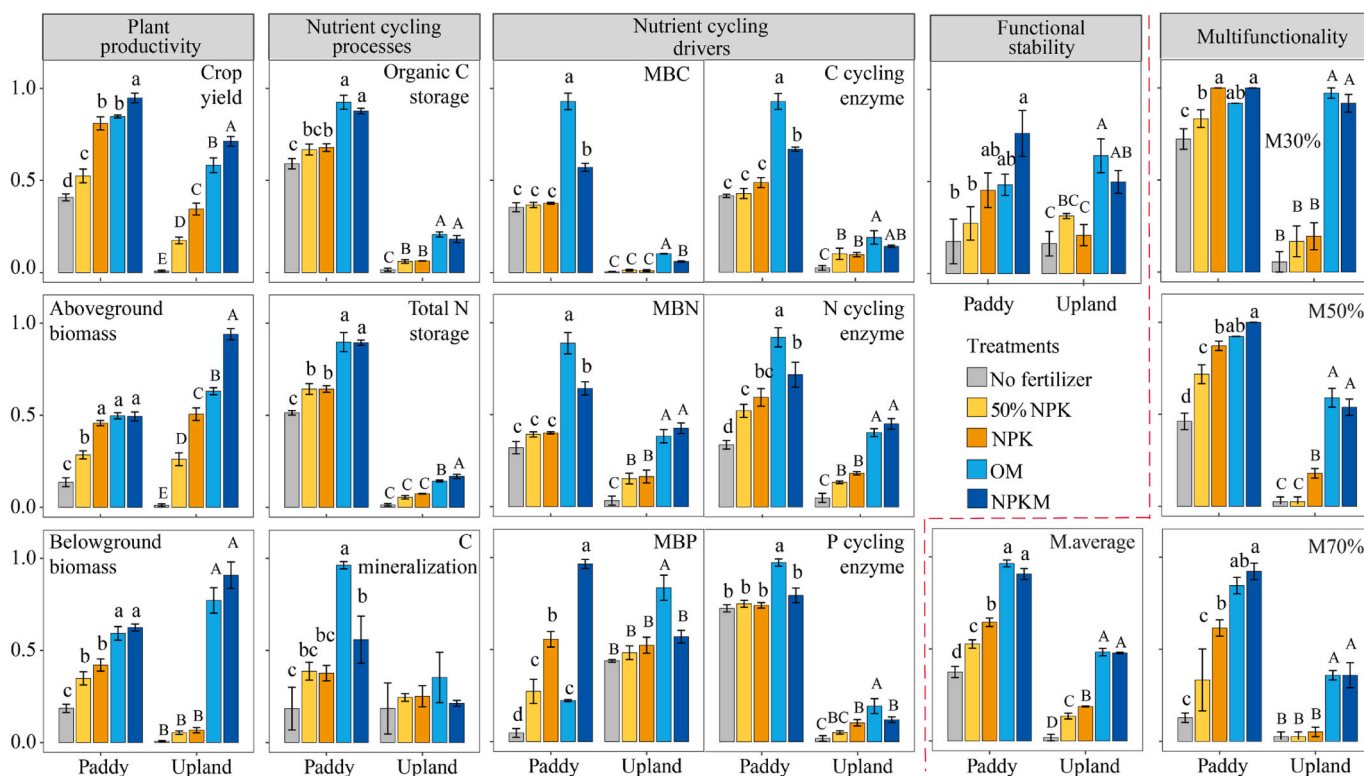


Fig. 1. Effects of long-term fertilization on plant productivity, nutrient cycling processes and drivers, functional stability, and multifunctionality indices in the paddy and upland fields. Individual function variable was standardized using 0–1 transformation. M30%: Multifunctionality30%, M50%: Multifunctionality50%, M70%: Multifunctionality70%, M.average: Multifunctionality (average). Different letters above the bars indicate significant difference among treatments at *p* < 0.05 (LSD's test) in paddy (a, b, c) and upland (A, B, C) field.

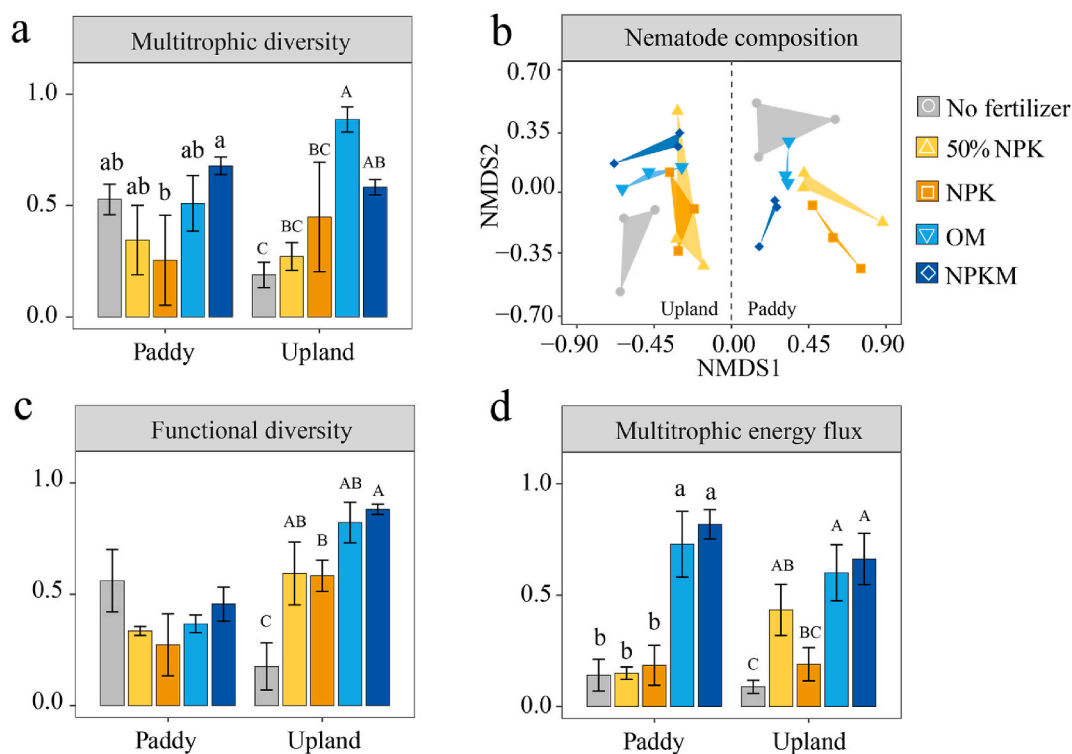


Fig. 2. Effects of long-term fertilization on multitrophic diversity (a), community composition (b), functional diversity (c) and multitrophic energy flux (d) of soil nematodes in the paddy and upland fields. The individual variable was standardized using 0–1 transformation. Different letters above the bars indicate significant differences among treatments at $p < 0.05$ (LSD's test) in paddy (a, b, c) and upland (A, B, C) fields. Stress value (0.14) in the nonmetric multidimensional scaling analysis indicates the goodness-of-fit of the model, with a lower value corresponding to a better model fit.

treatment in upland field ($p < 0.05$, Table S3), but remained similar among all fertilization treatments in paddy field (Fig. 2c). The NMDS analysis further revealed that fertilization changed nematode

community composition in both agroecosystems (stress = 0.14, Fig. 2b). Specifically, organic fertilizer supported a more divergent community than mineral fertilizer, and this fertilization-driven discrepancy was

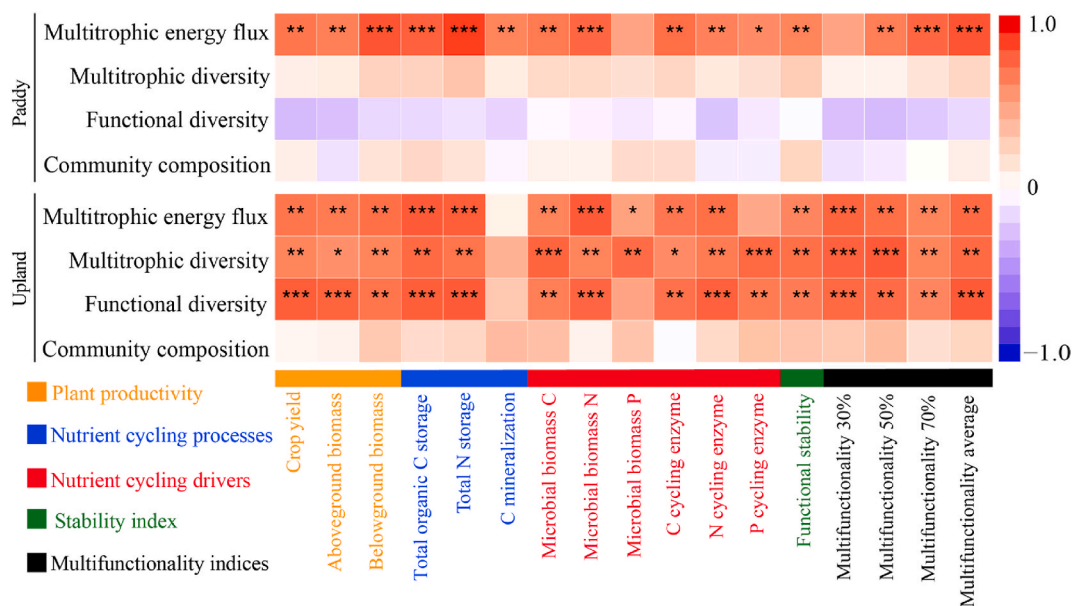


Fig. 3. Pairwise correlation coefficients between ecosystem multiple functions and nematode community attributes (multitrophic energy flux, taxonomic diversity, functional diversity and community composition) in the paddy and upland fields. The values for community composition were calculated from the first principal component of the PCA analysis with the nematode community. Label colors reflected the classification of ecosystem multiple functions: orange, plant productivity; blue, soil nutrient cycling processes; red, nutrient cycling drivers; green, soil functional stability; and black, multifunctionality indices. The color of the square indicated a positive (red) or negative (blue) correlation, and the color intensity indicated the strength of the correlation. Correlations between two variables were significant at $***p < 0.001$, $**p < 0.01$, and $*p < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

more obviously in paddy rice than upland maize field.

3.3. Relative strengths of community attributes in explaining ecosystem functions

The multitrophic energy flux was significantly correlated with 85% of the measured functions and positively related to all multifunctionality indices in both fields, except multifunctionality30% in the paddy field (Fig. 3). Such positive linkages across multitrophic levels were stronger than those at individual trophic groups, except bacterivores (Fig. S1). The multitrophic diversity and functional diversity were significantly correlated with 92% and 85% of the ecosystem functions and all multifunctionality indices in the upland field, but didn't relate to these indices in the paddy field (Fig. 3). Community composition showed no significant relationships with the measured functions and multifunctionality indices in both fields (Fig. 3).

The piecewise SEM revealed that multitrophic energy flux (path coefficient = 0.34) and soil nutrients (path coefficient = 0.35) had direct positive effects on ecosystem multifunctionality, which explained 37% of the total variation in ecosystem multifunctionality (Fig. 4). Additionally, fertilization was positively related to functional diversity and multitrophic diversity, but such community attributes had no direct effect on multifunctionality. The multitrophic energy flux had a positive relationship with multitrophic diversity, indicating that multitrophic diversity would indirectly affect multifunctionality through multitrophic energy flux. The linear mixed-effect model confirmed that the effects of fertilization on ecosystem multifunctionality were significantly mediated by soil nutrients and multitrophic energy flux, whereas multitrophic diversity, functional diversity, and community composition were not directly related to ecosystem multifunctionality (Table 1).

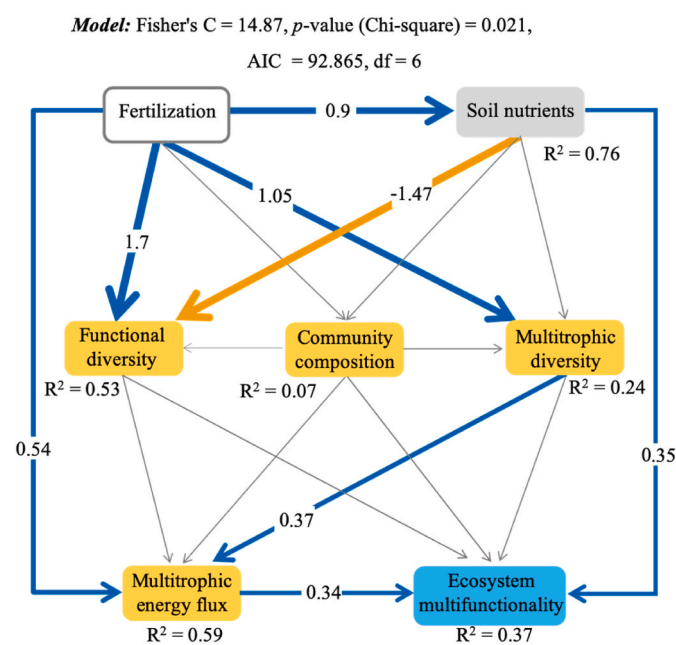


Fig. 4. Piecewise structural equation model (Piecwise SEM) describing fertilization effects on ecosystem multifunctionality (average). In this model, community attributes (multitrophic energy flux, multitrophic diversity, functional diversity, and community composition) and soil nutrients were assigned as fixed factors, whereas land types (paddy and upland) and replicate were assigned as random factors. R² denotes the proportion of variance explained from fixed factors in the Piecwise SEM. Arrow thickness was scaled proportionally to the standardized path coefficients (numbers on arrows). Solid blue and yellow arrows indicate positive and negative relationships respectively, whereas grey arrows indicate no significant relationship. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1

Linear mixed-effect model (LMM) results showing the effects of fertilization on ecosystem multifunctionality (average), mediated by soil nutrients, and soil community attributes (multitrophic energy flux, multitrophic diversity, functional diversity, and community composition). In the model, land types (paddy and upland) and replicate were assigned as random factors, whereas soil community attributes and soil nutrients were assigned as fixed factors. R² denotes the proportion of variance explained from fixed factors.

Predictors	Estimate	<i>p</i> value
	R ² 0.37, AIC -9.76	
Soil nutrients	0.422	< 0.001
Multitrophic energy flux	0.341	0.003
Multitrophic diversity	0.115	0.711
Functional diversity	0.007	0.955
Community composition	-0.012	0.697

4. Discussion

By integrating key ecological properties, such as plant productivity, carbon, and nutrient cycling, as well as functional stability, our results confirmed that organic fertilization enhanced ecosystem multifunctionality, indicating an overall positive effect of organic fertilizer on ecological processes in the paddy rice and upland maize agroecosystems. Organic fertilizer contributed to multifunctionality primarily by increasing soil nutrients thereby facilitating multitrophic energy flux in nematode food webs, suggesting that energy flux in ecological networks provided a powerful approach for understanding the impacts of anthropogenic drivers on ecosystem multifunctionality.

Our first hypothesis was partially supported because organic fertilizer enhanced multitrophic energy flux of nematode food webs, but mineral fertilizers did not. It is most likely that long-term manure application could increase the carbon uptake by microbes, fostering energy transfer to nematode food webs (Schmidt et al., 2020). Generally, organic fertilizers like animal manure support many more numbers of nematodes than mineral fertilizers because they supply more carbon to the soil (Liu et al., 2016a), resulting in greater utilization of exogenous carbon by nematode food webs. Given that more energy flux at lower trophic levels can confer greater stability to food webs (Rip and McCann, 2011), our study, from an energy flux perspective, confirms that organic fertilizer supports a much more structured nematode food web.

As we expected in the second hypothesis, multitrophic energy flux had a strong and positive relationship with ecosystem multifunctionality in the paddy rice and upland maize ecosystems. Our result confirms that a multitrophic approach generally shows a stronger positive relationship with ecosystem multifunctionality compared with a single trophic group, which is consistent with the recent findings (Delgado-Baquerizo et al., 2020; Potapov et al., 2019). A single trophic group might poorly represent trophic complexity, since some trophic groups could counteract the effect on ecosystem functioning induced by others (Soliveres et al., 2016). For example, a greater abundance of predators could dampen the positive effects of bacterivores on ecosystem multifunctionality (Fig. S1b). Besides, soil community with greater energy flux generally has a lower risk of functional loss (Barnes et al., 2018), leading to the absence of negative effects from species loss on multiple ecosystem functions (Liu et al., 2016a; Turnbull et al., 2014). Although the paddy rice and upland maize agroecosystems differed greatly in water management practices and crop varieties, resulting in distinct nematode community patterns, these discrepancies generated similar relationships between multitrophic energy flux and ecosystem multifunctionality. Furthermore, the Piecwise SEM result suggests that the positive effect of multitrophic energy flux on multifunctionality is still maintained after accounting for soil nutrients and other key community factors, indicating that multitrophic energy flux is a robust predictor of ecosystem multifunctionality irrespective of ecosystem types that differ greatly in environmental conditions.

Other community attributes also related positively with

multifunctionality, although the pattern was different between the paddy and upland fields. Each biodiversity attribute can provide distinct information about fertilization effects on soil community, thereby enhancing our understanding of potential consequences for ecosystem functioning. A positive relationship was observed between multifunctionality and multitrophic diversity as well as functional diversity in the upland field (Fig. 3), indicating the strong effects of these community attributes on ecosystem multifunctionality. Such linkages arise from the complementary use of existing resources as well as interactions between coexisting species in a community, and have received support from many field observations and experiments (Gross et al., 2017; Le Bagousse-Pinguet et al., 2019; Schuldt et al., 2018; Wagg et al., 2014). However, multitrophic diversity and functional diversity were not related directly to ecosystem multifunctionality in paddy field. This does not indicate that the biodiversity attributes have no critical effects on ecosystem functioning, but suggests that the relationship between biodiversity and ecosystem multifunctionality could be affected by ecosystem types with strong environmental filters. In this study, flooding for 3–4 months is expected to serve as a strong anthropogenic filter that affects sensitive nematode species, such as *Pratylenchus* and *Mesorhynchitis* (Kurm et al., 2019; Liu et al., 2016b; Yan et al., 2021), which may help to explain a lower diversity in the paddy field than in the upland field (Fig. 2). We argue that flooding was a stronger filter than crop variety in driving nematode community assembly in the paddy and upland agroecosystem. Our finding is consistent with a previous study showing that nematode communities (especially herbivores) are different between the paddy and upland fields, even when the same rice variety is cultivated in both fields (Okada et al., 2011). Additionally, according to the mass ratio hypothesis, the dominance by species with particular traits would counteract the negative effect of species loss on ecosystem functioning (García-Palacios et al., 2017; Yang et al., 2017). This further emphasizes that community composition is a critical attribute in regulating ecosystem functioning, suggesting that particular species or trophic groups could be determinant drivers of ecosystem processes (White et al., 2020). Therefore, we suppose that a strong anthropogenic filter like flooding may constrain soil biodiversity, resulting in a biased relationship between biodiversity and ecosystem multifunctionality. The contrasting findings in paddy field matches with recent studies reporting that the biodiversity and multifunctionality relationships could be positive, neutral, or negative in various ecosystems (Saleem et al., 2019; Zhang et al., 2021).

Our work provides the first empirical evidence that organic fertilization enhanced agroecosystem multifunctionality by facilitating energy flux across trophic levels in soil food web, while mineral fertilizers are not able to increase multitrophic energy flux as well as the ecosystem multifunctionality in the paddy and upland cropping systems. However, whether these results show a generalized pattern remains to be determined, because there are too limited studies demonstrating the effect of fertilization on multitrophic energy flux as well as ecosystem multifunctionality. Nevertheless, our work conducted in two agroecosystems highlights that quantification of energy flux in ecological networks opens a new perspective to study the relationship between biodiversity and ecosystem multifunctionality. Therefore, we encourage many more researches to explore the energy dynamics across environment and community, as well as integrating above- and belowground systems. The energy flux model may serve as a powerful predictor for multiple ecological consequences of anthropogenic activities.

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.soilbio.2022.108656>.

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