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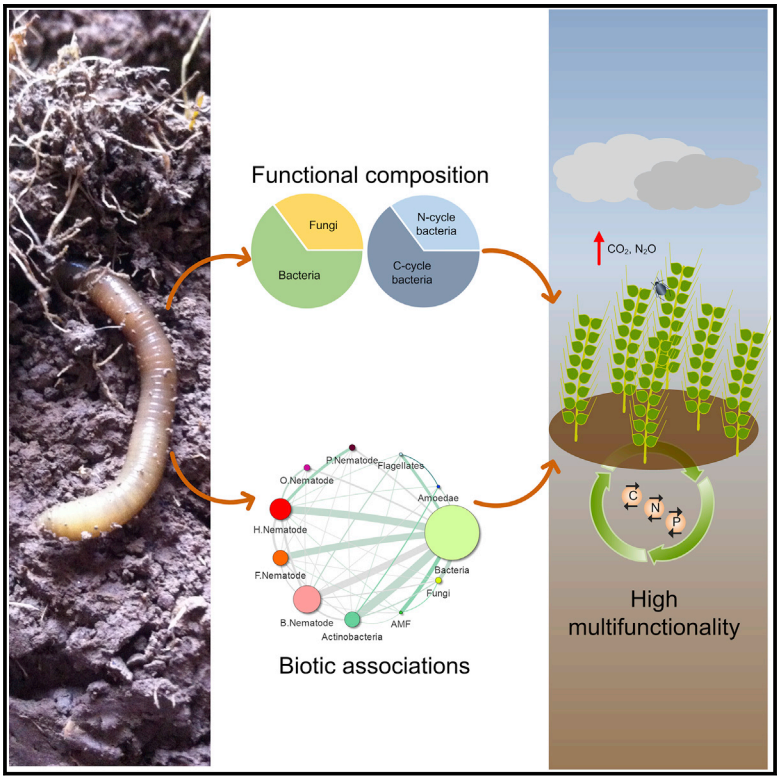
Current Biology

Earthworms Coordinate Soil Biota to Improve Multiple Ecosystem Functions

Graphical Abstract



Highlights

- Earthworms enhanced multifunctionality by indirect rather than direct effects
- Earthworms shifted functional composition toward bacterial-dominated community
- Multifunctionality was unrelated to changes in soil biodiversity, structure, and pH

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In Brief

Liu et al. study a 13-year-old field experiment to show that earthworms are beneficial to agroecosystems from a multifunctional perspective. This work incorporates the concerns of negative effects of earthworms in recently published syntheses and highlights the potential pathways in which earthworms contribute to sustainable agriculture.

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Earthworms Coordinate Soil Biota to Improve Multiple Ecosystem Functions

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SUMMARY

Earthworms have been perceived as benevolent soil engineers since the time of Charles Darwin, but several recent syntheses link earthworm activities to higher greenhouse gas emissions, less soil biodiversity, and inferior plant defense against pests. Our study provides new field-based evidence of the multiple direct and indirect impacts of earthworms on ecosystem functions within an ecological multifunctionality framework (i.e., aggregated measures of the ability of ecosystems to simultaneously provide multiple ecosystem functions). Data from a 13-year field experiment describing 21 ecosystem functions showed that earthworm presence generally enhanced multifunctionality by indirect rather than direct effects. Specifically, earthworms enhanced multifunctionality by shifting the functional composition toward a soil community favoring the bacterial energy channel and strengthening the biotic associations of soil microbial and microfaunal communities. However, earthworm-mediated changes in soil physical structure, pH, and taxonomic diversity were not related to multifunctionality. We conclude that the coordinated actions of earthworms and their associated soil biota were responsible for the maintenance of multifunctionality at high levels in this rice-wheat cropping system. Management of crop residue inputs and reduction of soil physicochemical disturbances should encourage beneficial earthworm effects and support multiple ecosystem services that are vital to sustainable agriculture.

INTRODUCTION

Ecosystem services delivered by agricultural soils, such as food production and nutrient cycling processes, are of increasing importance in the face of the growing world population and global change [1, 2]. These services are not only driven by abiotic factors, such as climate change or land-use conversion, but are influenced considerably by large-bodied, soil-dwelling fauna [3]. Earthworms are well-known "ecosystem engineers" regarded as essential for the maintenance of soil health and plant production in agroecosystems [4]. They are generally assumed to have positive or neutral effects on ecosystem functions, such as soil physical structure and soil organic carbon stock [4, 5]. However, recent meta-analyses revealed a negative effect of earthworms on several ecosystem functions, including increasing greenhouse gas emissions (e.g., CO_2 and N_2O) [5], reducing soil biodiversity [6], and lowering plant resistance to phloem-feeding pests [7]. This indicates that earthworm effects cannot be deduced from studying ecosystem processes individually, because each process may be positively affected, negatively affected, or unaffected by earthworm activities.

A multifunctionality framework may resolve these contradictory reports because it can provide multidimensional, integrated, and simultaneous evaluation of earthworm activity on multiple ecosystem processes (e.g., plant productivity, decomposition processes, and nutrient cycling) [8, 9]. Ecosystem-level multifunctionality can be quantified with the averaging approach (averaging the standardized values of each function) and the threshold approach (counting the number of functions that have passed a threshold), which are used together due to the strengths and weaknesses of each approach [10]. For example, the average approach provides clearly interpretable results when many functions are simultaneously achieving high levels of performance but fails to distinguish intermediate values from multiple functions, some of which are performing at high values and some at low values [11]. The threshold approach captures the number of functions well, even when trade-offs and correlations exist among functions; however, the choice of a threshold value is arbitrary [11]. Because earthworms were historically considered to be an active soil ecosystem engineer [4, 12], their activities are already linked to multiple processes and thus have great potential to be a biological indicator of multifunctionality in agriculture.

Earthworms may influence ecosystem multifunctionality through multiple pathways. First, earthworms are able to directly affect ecosystem functions via grazing on plant roots, releasing

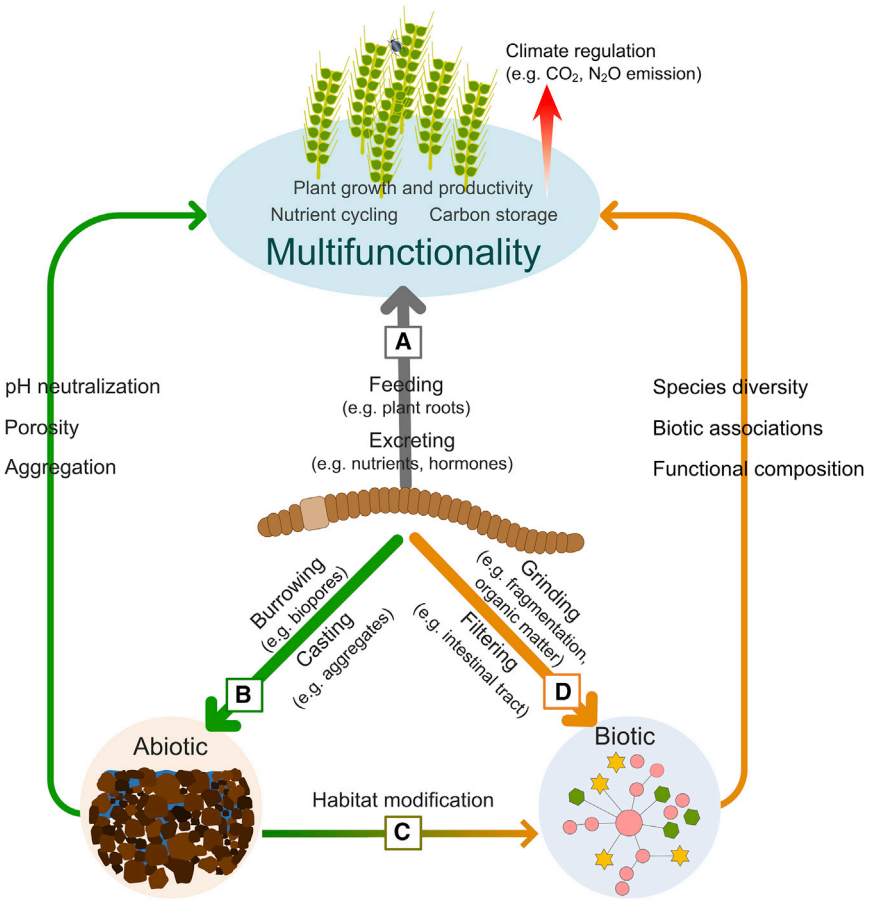

Figure 1. Conceptual Framework Showing the Pathways of Earthworm Contributions to Ecosystem Multifunctionality

Among the pathways, (A) indicates the direct effect of earthworms and (B)–(D) are the indirect effects of earthworms mediated by soil abiotic properties through burrowing and casting activities or biological communities through grinding and filtering processes. All of these effects occur simultaneously during earthworm activities.

mineral nutrients and phytohormones via excrements in support of crop growth (Figure 1A) [13]. Second, earthworm "bioturbation" activities can indirectly influence multifunctionality (e.g., nutrient cycling and plant production) by modifying soil abiotic properties (e.g., soil moisture, pH, and aggregates) through casting and burrowing activities (Figure 1B) [4, 14, 15] and thereby changing soil microbiota, such as microbes and microfauna (Figure 1C) [16–18]. Third, earthworm "digesting" activities can indirectly influence multifunctionality by modifying soil microbiota via gizzard-induced fragmentation and gut-associated filtering processes (Figure 1D) [19]. All of these effects can occur simultaneously, but they have been always examined separately in studies that modify single factors, such as soil pH, soil aggregation, or biological communities. However, to evaluate the importance of earthworm-mediated changes on soil abiotic and biotic properties in a multifunctionality context, it is crucial to disentangle the relative strengths of their direct and indirect effects.

An obstacle to quantify changes in the biotic pathway arises from the oversimplified definitions of soil biological communities. To date, multifunctionality-related studies have almost always linked ecosystem functions to species or taxonomic diversity [8, 19–21] and provided striking evidence that random biodiversity loss resulted in the decline of multiple ecosystem functions in manipulated experiments [9]. However, taxonomic diversity is not the only aspect of biological systems that influences ecosystem functions and generally responds nonrandomly to disturbances compared with experimentally manipulated biodiversity loss [22]. Indeed, shift in soil communities toward certain functional guilds (i.e., functional composition) may have stronger effects on ecosystem functions compared with the roles of taxonomic diversity [23, 24]. For example, previous studies showed that earthworms reduced microbial species diversity [6] but increased the ratio of bacteria to fungi [25], which could transfer a similar amount of nutrients and energy from plants into the soil food web as the original soil microbial community. The associations between taxa across complex and diverse communities (i.e., biotic associations) could be assessed using correlation network analysis, which integrates diversity, community composition, and ecosystem functions [26–28]. This implies that shifts in the biotic associations across multiple trophic groups might be an overlooked but alternative predictor of ecosystem multifunctionality [4, 29].

To clarify the relative strengths of direct and indirect effects of earthworms on multifunctionality, we analyzed data on soil physicochemical properties (aggregate stability and pH), soil community attributes (taxonomic diversity, functional composition, and biotic associations), and 21 ecosystem functions. Data were obtained over 2 consecutive years from a 13-year field experiment under a rice-wheat rotation system, where two methods of straw application (straw mulched on the soil surface or incorporated into the top layer of soil) were applied and where the abundance of a dominated endogeic earthworm *Metaphire guillelmi* was deliberately manipulated (i.e., plots were inoculated with earthworms or earthworms were removed). Soil microbes and microfauna were enumerated, and their community attributes were calculated. The 21 ecosystem functions were grouped into four categories, reflecting both aboveground and belowground ecological processes: (1) plant productivity, (2) plant nutrients, (3) nutrient and carbon cycling processes, and (4) nutrient and carbon cycling drivers [8]. We tested whether earthworm presence would enhance multifunctionality via direct effects (e.g., grazing on plant roots) or indirectly by neutralizing soil pH, promoting soil aggregation and modulating community attributes. We also tested whether the earthworms' indirect

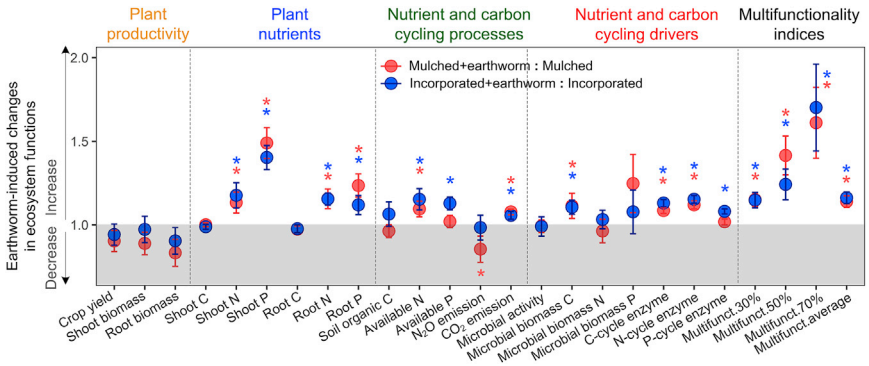

Figure 2. Earthworm Presence Affects Single-Ecosystem Functions and Multifunctionality Indices

Among the multifunctionality indices, multifunct.30%, multifunct.50%, and multifunct.70% were calculated for the threshold approach, whereas multifunct.average was calculated for the averaging approach. Mean ± SD of all variables were expressed as the ratio of the treatments in the presence (straw mulched + earthworm and straw incorporated + earthworm) and absence (straw mulched and straw incorporated) of earthworms (n = 8; 2 years × 2 crop phases × 2 straw applications). A ratio <1.0 with * represents a significant reduction ($p < 0.05$) in the ecosystem function or multifunctionality indices, and a ratio >1.0 with * indicates a significant increase ($p < 0.05$) in the ecosystem function or multifunctionality indices due to the presence of earthworms. See also Tables S1 and S2.

effects on multifunctionality were related to community attributes, such as taxonomic diversity, biotic associations, or to a shift in functional composition of soil communities involved in bacterial- and fungal-energy channel. We found that earthworm presence enhanced multifunctionality by shifting the functional composition of soil communities toward a bacterial-dominated community and thereby strengthening biotic associations within soil food webs. Interestingly, earthworm-mediated changes in soil

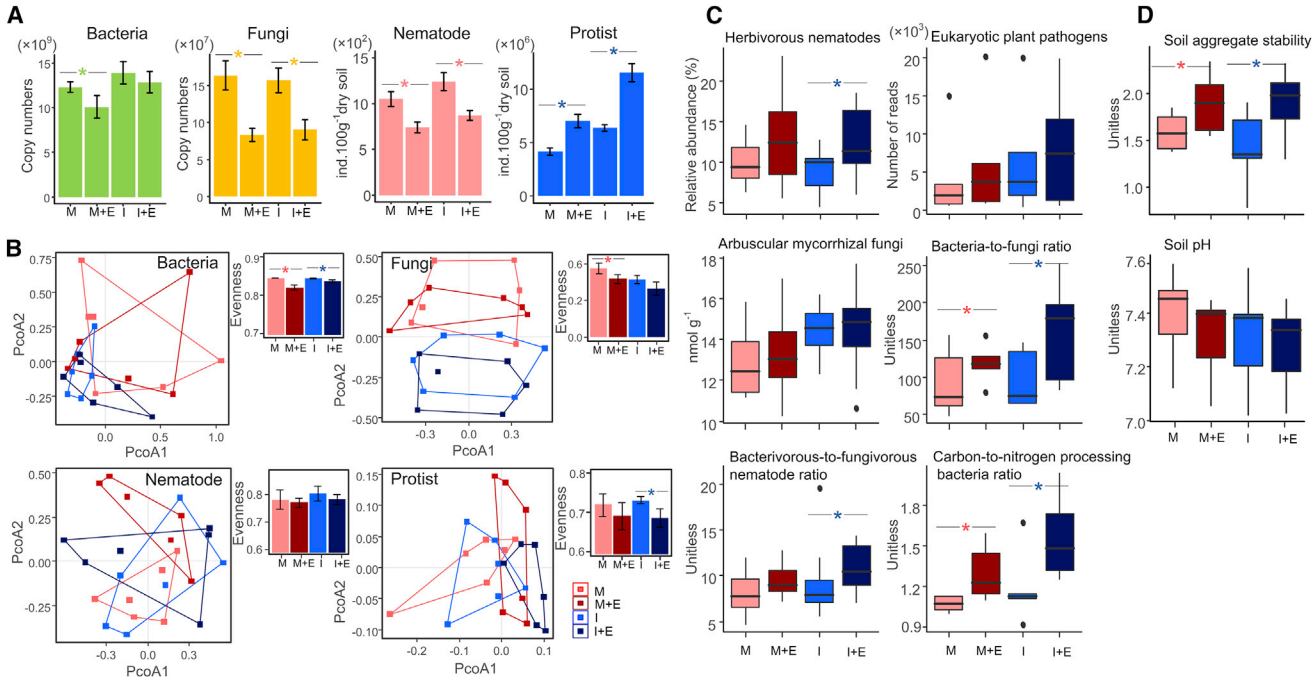

Figure 3. Earthworm Presence Affects Soil Biotic Communities and Abiotic Properties

(A) Abundance of soil biota (bacteria, fungi, nematodes, and protists). Bars represent the mean value, and error bars represent SD.

(B) Principal coordinate analysis (PcoA) of the soil biota. The evenness index was calculated as Pielou's index, with a lower value indicating a higher heterogeneity.

(C) Components of functional composition of soil biota. The carbon-to-nitrogen processing bacteria ratio is the ratio of bacteria active in the carbon cycle versus nitrogen cycle. Outliers are plotted as points.

(D) Soil abiotic properties (aggregate ability and pH). The taxonomic diversity of soil biota is shown in Figure S1.

The bacterial and fungal abundance data in (A) and bacteria-to-fungi ratio in (C) were derived from qPCR results, and the bacterial, fungal, and protist community data in (B) were derived from MiSeq sequencing data (Table S3). Soil nematodes were morphologically identified to the genus level (Table S4). The protist abundance in (A) was counted under microscope. The eukaryotic plant pathogen and carbon-to-nitrogen processing bacteria ratio were predicted from MiSeq sequences according to the FUNGuild database (Table S5) and the FAPROTAX database (Table S6), respectively. Treatments were straw mulched (M), straw mulched + earthworm (M + E), straw incorporated (I), and straw incorporated + earthworm (I + E). * indicates significant difference ($p < 0.05$) between M and M + E treatments or between I and I + E treatments.

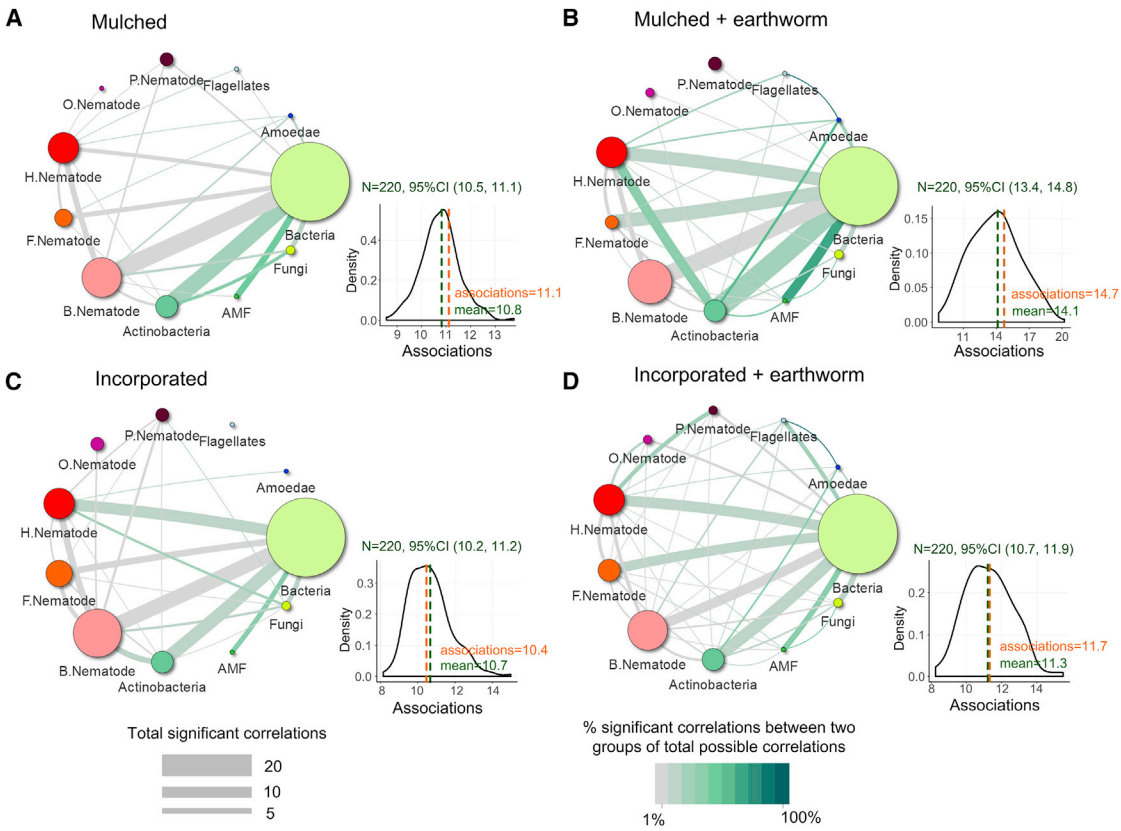

Figure 4. Network Visualization of the Biotic Associations with Earthworm Presence

Significant correlations ($p < 0.05$) were divided by the total number of possible biotic correlations to obtain the average "associations" strength between two groups [26]. Line width indicates the number of significant correlations, whereas line color and transparency indicate the correlation strength, as shown in the figure legend. The size of each circle is proportional to the number of taxa in that group. See also Figure S2. Sensitivity analyses were conducted to test whether the results of correlation network are sensitive to the sample size. We reduced 25% of the sample size and used 75% of the sample size ($n = 9$) to re-analyze the correlation between two groups. When we selected 9 samples from the total 12 samples, there were 220 different combinations of the correlation network for each treatment. We calculated associations of each combination by dividing all significant correlations ($p < 0.05$) by all possible correlations. The density plots indicate the distribution, 95% confidence interval, and mean (green dashed line) of 220 associations, which were compared with the associations of total 12 samples (yellow dashed line). For each treatment, the associations of the total 12 samples was in the range of 95% confidence interval of the 9 samples, suggesting that the network results are robust to sensitivity analysis of sample size. The left-hand panels show biotic associations in straw mulched (A) and incorporated treatments (C) without earthworms. The right-hand panels show biotic associations in straw mulched (B) and incorporated treatments (D) with earthworms. See also Table S7. B.Nematode indicates bacterivorous nematodes. F.Nematode indicates fungivorous nematodes. H.Nematode indicates herbivorous nematodes. O.Nematode indicates omnivorous nematodes. P.Nematode indicates predatory nematodes.

physical structure, pH, and taxonomic diversity did not change multifunctionality. Earthworms coordinate soil biological communities to achieve high levels of multifunctionality, revealing the pivotal roles of earthworm populations for sustainable agriculture.

RESULTS

Earthworm Presence Changed Ecosystem Functions

Earthworm presence increased all four multifunctionality indices (i.e., multifunct.30%, multifunct.50%, and multifunct.70% in the threshold approach and multifunct.average in the averaging approach) and more than half of the single-ecosystem functions (52% of the 21 ecosystem functions; $p < 0.05$; least significant difference [LSD] test; Figure 2). The multifunctionality indices were significantly higher in treatments with straw incorporation than straw mulching and did not differ between the rice- and wheat-cultivation phases ($p < 0.05$; LSD test; Table S1). Among the 21 ecosystem functions, 43% were not affected by earthworms, including crop yield, shoot biomass, root biomass, shoot carbon, root carbon, soil organic carbon, microbial activity, microbial biomass nitrogen, and microbial biomass phosphorus (Figure 2). Only N_2O emissions in the straw-mulched treatments decreased due to earthworm presence ($p < 0.05$; LSD test; Figure 2).

Earthworm Presence Affected Soil Community Attributes and Soil Abiotic Properties

Earthworms changed soil community composition and decreased the evenness of soil communities (Figures 3A and 3B). Earthworm presence slightly reduced the number of taxa of bacteria, fungi, archaea, and nematodes and slightly increased the number of protist taxa (Figure S1). Earthworms changed the pathway of preferential energy flow through the soil food web by increasing the bacteria-to-fungi ratio and the bacterivore-to-fungivore ratio of the nematode consumers, as

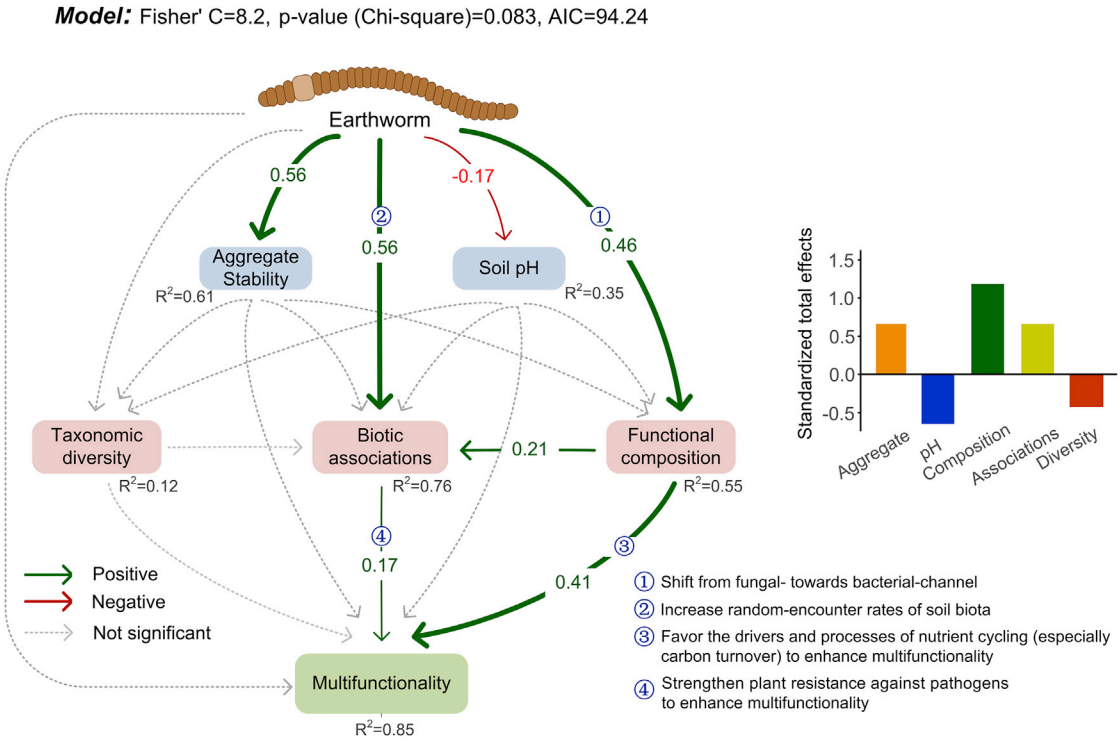

Figure 5. Piecewise Structural Equation Model (Piecewise SEM) Describing the Direct and Indirect Effects of Earthworms on Ecosystem Multifunctionality

In the model, a direct effect of earthworms on multifunctionality is indicated by a single-headed arrow pointing from earthworm to multifunctionality, whereas the indirect effects of earthworms on multifunctionality are mediated by soil community attributes (taxonomic diversity, functional composition, and biotic associations) and soil abiotic properties (aggregate and pH). We quantified the earthworm as 0 for the treatments without earthworms (mulched and incorporated) and 1 for the treatments with earthworms (mulched + earthworm and incorporated + earthworm). The width of arrows is proportional to the strength of path coefficients (standardized coefficients). Continuous green and continuous red arrows indicate positive and negative relationships, respectively, whereas dashed black arrows indicate no significant relationship. The p (chi-square) > 0.05 indicates that the model fitted the data very well. The standardized total effects (direct plus indirect effects) from the model (right panel) indicate the effect size of the relationship, which also confirmed a good model fit. Multifunctionality in the figure was presented as average approach (multifunct.average), which had a better model fit (lower AIC values) than the threshold approaches (multifunct.30%, multifunct.50%, and multifunct.70%). We also split the data into two groups (with and without earthworm) or other two groups (straw mulched and straw incorporated) and conducted separate piecewise SEM analysis for each group. See also Figure S3.

well as the ratio of bacteria active in the carbon cycle versus nitrogen cycle. These increments were higher in treatments with straw incorporation than straw mulching ($p < 0.05$; LSD test; Figure 3C). At the same time, earthworms strengthened biotic associations across trophic groups, as indicated by correlation network analyses (Figures 4 and S2; Table S7). In addition, earthworms improved soil physical structure by increasing soil aggregate stability ($p = 0.003$; LSD test); meanwhile they had no effect on soil pH (Figure 3D).

Direct and Indirect Effects of Earthworms on Ecosystem Multifunctionality

The piecewise structural equation model revealed that indirect effects mediated by functional composition (standardized coefficient = 0.11–0.22) and biotic associations (standardized coefficient = 0.08–0.10) contributed to multifunctionality (Figures 5 and S3). Such effects were much greater than the direct effects of earthworm as well as indirect effects of the taxonomic diversity and abiotic factors (Figures 5 and S3). The strength of biotic associations increased along changes in functional composition (Figures 5 and S3). Earthworms increased aggregate stability, but such physical properties were unrelated to multifunctionality (Figure 5).

The linear mixed-effect model confirmed that the indirect effects of earthworms on multifunctionality were significantly mediated by functional composition and biotic associations, whereas taxonomic diversity, aggregate stability, and pH had no effect on multifunctionality (Tables 1, S3, and S4). Several predictors in the functional composition parameter, namely the bacteria-to-fungi ratio, the bacterivorous-to-fungivorous nematode ratio, and the ratio of carbon cycle versus nitrogen cycle bacteria, significantly contributed to multifunctionality (Tables 1 and S4). Both functional composition and biotic associations were correlated positively with multifunctionality indices and many ecological functions, especially the drivers and processes connected to nutrient cycling (Figure 6).

DISCUSSION

By integrating key ecological properties, such as plant growth, carbon, and nutrient cycling, as well as greenhouse gas emissions, we confirmed that earthworm presence enhanced

Table 1. Linear Mixed-Effect Model (LMM) Results Showing the Indirect Effects of Earthworms on Ecosystem Multifunctionality, Mediated by Soil Community Attributes (Taxonomic Diversity, Functional Composition, and Biotic Associations) and Soil Abiotic Properties (Aggregate Stability and pH)

Source	Estimate	p Value
Predictors of All Variables	R2 0.86, AIC −26.5	
Aggregate stability	−0.123	0.277
Soil pH	−0.334	0.052
Taxonomic diversity	−0.014	0.829
Functional composition	0.366	<0.001
Biotic associations	0.198	0.040
Predictors of Functional Composition	R2 0.73, AIC −26.0	
Herbivorous nematodes	−0.014	0.923
Bacteria-to-fungi ratio[a]	0.321	0.003
Bacterivorous-to-fungivorous nematode ratio[a]	0.287	0.049
Carbon-to-nitrogen processing bacteria ratio[a]	0.354	0.002

Multifunctionality in the table was calculated as multifunct.average, which had a better model fit (higher R^2 with lower AIC values) than the threshold approaches (multifunct.30%, multifunct.50%, and multifunct.70%; Table S8). The four multifunctionality indices were correlated ($r = 0.56–0.79$; $p < 0.001$), and each was positively correlated with some of the individual ecosystem functions (Figure S4). R^2 denotes the proportion of variance explained. In the model, crop phase and sampling year were assigned as random factors, whereas the predictors were allocated as fixed factors. Herbivorous nematodes, bacteria-to-fungi ratio, bacterivorous-to-fungivorous nematode ratio, and carbon-to-nitrogen processing bacteria ratio were included as the predictors of functional composition, and eukaryotic plant pathogens and arbuscular mycorrhizal fungi were excluded from the model due to their non-significant response to earthworm presence (see Figure 3C). The carbon-to-nitrogen processing bacteria is the ratio of bacteria active in the carbon cycle versus nitrogen cycle. Models of splitting data to two groups (straw mulched and straw incorporated) with regard to straw application regimes generated similar results and were presented in Table S9.[a]Predictors were finally selected, standardized (0–1 transformation), and averaged into a composite index to represent functional composition, due to their significant response to the multifunctionality in the model.

ecosystem multifunctionality and promoted more than half of the 21 individual ecosystem functions, indicating an overall positive effect of earthworms on ecological processes in this rice-wheat system. Earthworms' contribution to multifunctionality occurred primarily by indirect processes rather than direct effects. The indirect effects of earthworms on functional composition and biotic associations were more important for multifunctionality than the indirect effects mediated by taxonomic diversity and the abiotic properties (i.e., soil aggregate stability and pH). It was notable that earthworms did not affect taxonomic diversity (i.e., the composite index of taxonomic diversity), and this parameter was relatively unimportant for multifunctionality in this rice-wheat system. Biodiversity loss was often accompanied by a decrease in ecosystem multifunctionality [21, 30], especially when the species diversity loss was caused by strong disturbances, such as global change drivers [8, 20, 31, 32] or land-use intensification [33]. We supposed that the added straw likely buffered earthworm-induced changes in biodiversity [34], because the organic substrates from the straw could maintain the earthworm population and minimize their antagonistic relationship with free-living microbiota or plant roots via competition, predation, or bioturbation effects [35, 36].

Earthworm-induced changes in functional composition and biotic associations, both indirect effects, contributed to enhance ecosystem multifunctionality. In the absence of earthworms, the energy flow through the soil food web occurred through the slower cycling, fungal-dominated energy channel, but earthworm presence shifted the flow toward a faster, bacterial-dominated energy channel. Earthworm presence may favor the bacterial-dominated processes through several mechanisms. First, earthworm burrowing activity strongly reduced fungal biomass (Figure 3A), likely by disrupting fungal mycelium networks and reducing the growth of fungal hyphae by mixing and homogenizing the soil [6, 25, 36]. Second, the ingestion, fragmentation, and mixing of residues with soil by earthworms accelerated the release of labile organic substrates that can be metabolized by bacteria [25, 37], resulting in a higher ratio of carbon to nitrogen-processing bacteria (Figure 3C). Straw incorporation was complementary to the earthworm-mediated effects on functional composition, compared to straw mulched on the soil surface, and produced a higher bacteria-to-fungi ratio as well as greater enhancement of ecosystem multifunctionality. This could be due to the fact that the inoculated earthworms prefer feeding on a mixture of soil and semi-decomposed organic materials, and incorporation of straw with soils can facilitate the colonization and decomposition of bacteria [4].

We also observed that earthworm-induced shifts in functional composition of soil communities were accompanied with strengthening biotic associations in the correlation network analysis. This could be due to the increase in groups of smaller species with more rapid growth and higher carrying capacity (e.g., bacterial-dominated energy channel), as indicated by higher ratios of bacteria to fungi and bacterivorous to fungivorous nematodes. This is consistent with the notion that successional shifts in species composition following disturbance are always associated with changes in connections of soil communities [38]. Besides, earthworm burrowing activity could reduce the natural barrier to interactions, resulting in greater heterogeneity among soil biota (Figure 3B, lower evenness) or shift in the overlap niches of soil biota (Figure 3A) [39]. Earthworm-induced effects on biotic associations were stronger when straw was mulched on the soil surface than when incorporated into the soil, probably due to greater foraging activities by earthworms coming to the surface to search for food and associated activities like burrowing and casting [40]. Network structure could provide a conceptual basis for linking food-web structure with ecosystem functioning by depicting and partitioning energy flows among species and trophic groups in the soil ecosystem [41, 42]. However, how earthworms would influence the energy flows within this network structure need to be explored in future studies.

Despite a high level of multifunctionality, earthworms exerted neutral or negative effects on crop productivity and carbon sequestration [43]. Earthworms are generally thought to increase plant productivity, such as crop yield and shoot biomass [44]; however, the direction and magnitude of these effects depends on ecological factors, including soil type, plant, and earthworm

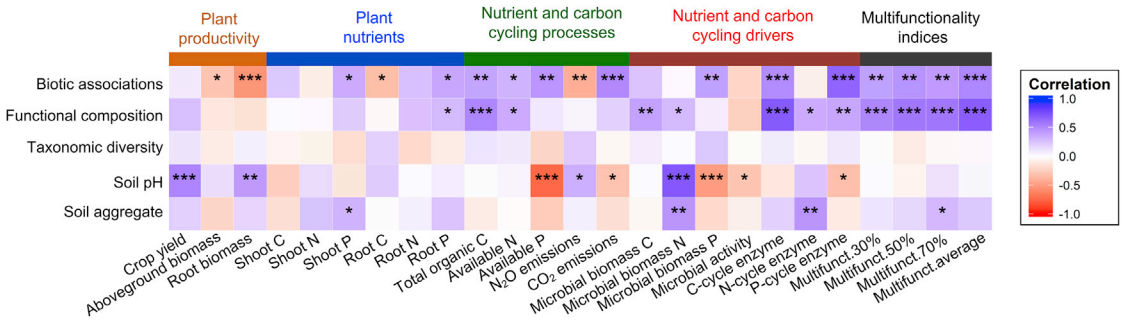

Figure 6. Pairwise Correlation Coefficients between Ecosystem Functions and the Earthworm-Mediated Predictors, including Soil Community Attributes (Taxonomic Diversity, Functional Composition, and Biotic Associations) and Soil Abiotic Properties (Aggregate Stability and pH)

Among the multifunctionality indices, multifunct.30%, multifunct.50%, and multifunct.70% were calculated for the threshold approach, whereas multifunct.average was calculated for averaging approach. Label colors reflect their classification: yellow, plant productivity; blue, plant nutrients; green, nutrient and carbon cycling processes; red, nutrient and carbon cycling drivers; and black, multifunctionality indices. Detailed descriptions of each ecosystem function and earthworm-mediated predictors were summarized on Table S10. The color of the square indicates a positive (blue) or negative (red) correlation, and the color intensity indicates the strength of the correlation. Correlations between two variables were significant at ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$.

species [4]. For example, stimulatory effects of earthworm would be weaker in soils with a sandy texture than in clay soils [44], and endogeic earthworms might affect plant productivity negatively by root abrasion and ingestion [36, 45]. Besides, earthworms are also important regulators of the terrestrial carbon sink through their involvement in carbon mineralization and stabilization processes [5, 46, 47]. Their activity accelerated soil organic carbon turnover, such as increased microbial biomass carbon, carbon-cycle enzyme activity, and CO_2 emissions (Figure 2). However, they could also increase carbon stabilization by converting substrates into stabilized clay-organic matter complexes and water-stable macro-aggregates [48, 49]. This trade-off agrees with the negligible effects of earthworms on soil carbon storage of our long-term study as well as the findings of a meta-analysis [5]. Additionally, it seems that the reduction in N_2O in the presence of earthworms accompanied with higher CO_2 emissions, which differed from the suggestion that earthworm-induced N_2O emissions could be an inevitable side effect of increased CO_2 emissions from the increased decomposition and mineralization processes [5]. Because the meta-analysis was based on experiments without plants [5], more experimental works are needed to verify the earthworm-mediated net soil greenhouse gas emissions across various plant ecosystems [50].

Our work provides the first empirical evidence that earthworm activity enhanced agroecosystem multifunctionality via shifting the soil food web toward the bacterial channel and strengthening biotic associations across trophic groups. In this double-cropping system, although earthworm-mediated outcomes did not enhance provisioning functions relative to human demand (i.e., ecosystem services) like crop production, they definitely improved the supporting and regulating ecosystem functions like nutrient and carbon cycling, which are fundamentally critical for human sustainability. It has been argued that measurement of multifunctionality may obscure mechanistic relationships between individual functions and their specific drivers [46]. The multifunctional approach, however, is well suited to provide a holistic understanding of earthworm effects [10]. Our results suggest that earthworms have great potential to modify pivotal components of the soil food web that control many essential ecosystem functions required for sustainable agriculture. We encourage more studies conducted in well-managed, long-term agroecosystems to generalize the earthworm-multifunctionality relationship.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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- METHOD DETAILS
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  - Soil aggregate and pH measurement
  - Microbes
  - Protists
  - Nematodes
  - Estimating taxonomic diversity
  - Estimating functional composition
  - Estimating biotic associations
  - Ecosystem multifunctionality
- QUANTIFICATION AND STATISTICAL ANALYSIS
- DATA AND CODE AVAILABILITY

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.cub.2019.08.045.

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AUTHOR CONTRIBUTIONS

M.L. and F.H. designed the research; X.C., X.G., Y.J., and W.F. performed the research; T.L., X.L., and X.G. analyzed the data; and T.L., M.L., I.M.L., J.K.W., B.S.G., and M.B. wrote the paper.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological Samples		
Endogeic earthworm species (*Metaphire guillelmi*)	Natural isolate from a double cropping system of summer rice and winter wheat, Nanjing, Jiangsu, China	N/A
Software and Algorithms		
R v3.4.1	https://www.r-project.org/	N/A
R package *vegan*	https://cran.r-project.org/web/packages/vegan/vegan.pdf	N/A
R package *visNetwork*	https://cran.r-project.org/web/packages/visNetwork/visNetwork.pdf	N/A
R package *igraph*	https://cran.r-project.org/web/packages/igraph/igraph.pdf	N/A
R package *lme4*	https://cran.r-project.org/web/packages/lme4/lme4.pdf	N/A
R package *car*	https://cran.r-project.org/web/packages/car/car.pdf	N/A
R package *piecewiseSEM*	https://cran.r-project.org/web/packages/piecewiseSEM/piecewiseSEM.pdf	N/A
Other		
FAPROTAX database	http://www.loucalab.com/archive/FAPROTAX/	N/A
FUNGuild database	http://www.stbates.org/guilds/app.php	N/A

LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, Manqiang Liu (liumq@njau.edu.cn). This study did not generate new unique reagents.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The study was conducted at the experimental field station of Nanjing Agricultural University (118°85′E, 32°02′N). This region has a humid subtropical climate with average annual precipitation of 1106 mm and mean atmospheric temperature of 16.0°C. The soil at the field station is classified as *Orthic Acrisol* (FAO classification) and has a sandy loam texture. Before the experiment was initiated, the field was used for rice cultivation (*Oryza sativa L.*) in the summer and wheat (*Triticum aestivum L.*) in the winter for at least 100 years. At the time the experiment was initiated, the arable soil layer (0-20 cm) had pH (H_2O) of 8.25, and it contained 5.86 g kg^{-1} soil organic C, 0.7 g kg^{-1} total N, 0.66 g kg^{-1} total P, 6.0 mg kg^{-1} available P and 47.1 mg kg^{-1} available K. Because P is not retained in this sandy loam soil, the inherent soil P fertility is low and P fertilizer is added to the field to increase P availability for the crop.

The experiment was established in 2001 planted with rice (*Oryza sativa L.*) in the summer and wheat (*Triticum aestivum L.*) in the winter. Both rice phase and wheat phase were grown in an upland field. Four treatments were randomly assigned to one of 12 plots in three blocks (= three replicates per treatment). Each plot was 2.8 m × 1.0 m and separated by 0.15 m wide concrete buffers extending 0.6 m into the soil and 0.2 m above the soil surface. The four treatments were: (i) straw mulched on the soil surface without earthworms (mulched), (ii) straw mulched on the soil surface with earthworms (mulched + earthworm), (iii) straw incorporated into soil without earthworms (incorporated) and (iv) straw incorporated into soil with earthworms (incorporated + earthworm). The straw input was based on local straw-returning practice to supply adequate organic substrates (e.g., food resources) to maintain the earthworm population. Straw was maize residues (7500 kg ha^{-1}) containing 7.96 g N kg^{-1}, 2.85 g P kg^{-1}, 10.67 g K kg^{-1} and 45.8 C/N that were added at the beginning of each crop growing phase. Straw was cut into 2-5 cm length, and then homogeneously mixed into the top layers (20 cm depth) when incorporated or left on the soil surface (mulched) the crop was planted. Fertilizers were applied in both rice and wheat phases. All plots received urea (210 kg N ha^{-1}), superphosphate (46 kg P ha^{-1}) and potassium chloride (87 kg K ha^{-1}) during each crop phase. Before seeding, the arable soil layer (0-20 cm) was ploughed manually with a nail harrow to avoid damaging the earthworms.

In 2001, the electro-shocking method combined with hand-sorting method were used to substantially reduce the indigenous earthworm population, including the cocoons, juveniles and adults [51]. Then we added the endogeic earthworm species (*Metaphire guillelmi*) into plots with earthworms at a rate of 70 g fresh weight m^{-2}, which was equivalent to the earthworm biomass in local fields. The straw mulch retains moisture, prevents soil erosion and supplies extra substrates for earthworms, as this kind of earthworm lives mainly in the plow layer (20 cm depth, where crop roots grow in abundance) and sometimes it forages below the soil surface. During

the experiment, earthworms were added if necessary, i.e., when their biomass had declined to less than 60 g fresh weight m^{-2}. Earthworm populations were monitored after every harvest, two times per year [40]. Typically, the soil was disturbed during that period by tillage to prepare for seeding the next crop, so hand-sorting a 1 m^2 block to estimate earthworm population density produced minimal disturbance [40]. The inoculated earthworm species is a native species and nearly 100% dominant in the local arable fields. During the two-year sampling period (2014-2015), very few earthworms (< 15 ± 3 g m^{-2}) were observed in non-earthworm plots, but plots with earthworms had a constant biomass of about 65 ± 10 g fresh weight m^{-2}.

METHOD DETAILS

Collecting the soil cores

We collected soil samples at the ripening stages of wheat (May) and rice (October) in 2014 and 2015. In each plot, eight soil cores (2.5 cm in diameter) from the 0-20 cm soil layer were collected randomly and mixed together to generate one composite sample per plot. There were 12 plots × 2 (two crop phases) × 2 (two years) = 48 soil samples. Soil cores were collected within 2 h and then stored at 4°C. All analyses that required fresh material were done within two weeks of sampling, and all other analyses were done within two months of sampling.

Soil aggregate and pH measurement

As an indicator of soil structural stability, the water-stable aggregate distribution was determined by a modified wet sieving procedure [52]. Briefly, 50 g of air-dried soil was immersed in water for 10 min and was then sieved through a sequence of sieves (< 0.053 mm, 0.053-0.25 mm, 0.25-2 mm, and > 2 mm). Aggregates were separated by moving each sieve up and down 3 cm manually with 50 repetitions for 5 min. The aggregate size classes were dried overnight at 65°C and weighed. The mean weight diameter (MWD) was used to represent soil aggregate stability, it was calculated as mean aggregate diameter weighted by its relative weight [53]: $MWD = \sum_{i=1}^{n} xi * wi$, where *xi* is the mean diameter of each fraction size and *wi* is the relative weight of correspondence size. Soil pH was measured using a pH meter after shaking a soil-water suspension (1:5w/v) for 30 min.

Microbes

We measured the abundance of microbes using quantitative PCR (qPCR) (i.e., bacteria and fungi) and phospholipid fatty acids (PLFA) analysis (i.e., bacteria, fungi, actinomycetes and arbuscular mycorrhizal fungi). Relative abundance of the major species in microbial communities (i.e., bacteria, fungi, archaea and protist) was determined with Illumina MiSeq sequencing (Table S3). The PLFA analysis was conducted in 2014 and 2015 (four sampling times, n = 12) but the qPCR and Illumina MiSeq sequencing were analyzed in 2015 only (two sampling times, n = 6). We used qPCR and MiSeq data for all the bacteria and fungi analysis (including abundance and community structure), and PLFA data for the correlation network. Because the PLFA described the relative amounts of certain functional groups (e.g., iron reducers, sulfate reducers, or fermenters) better than the OTU results [54]. It matches well with the rationale of our network structure, since we used aggregated groups consisting of species that are known to share a common function in the network analysis. Besides, the larger sample size of PLFA generated a more robust network analysis than the smaller sample size of MiSeq.

PLFA analysis

Microbial biomass was estimated from total extractable phospholipid fatty acids (PLFA) using the modified Bligh-Dyer method, then PLFA profiles were used to determine microbial community composition. Lipids were extracted from soil samples that were first lyophilized and stored at −70°C. Then, 5.0 g soil was extracted using a single-phase chloroform-methanol-aqueous buffer system. Next, phospholipids were subjected to mild alkaline methanolysis to form fatty acid methyl esters (FAMEs). The FAMEs were separated and quantified using an Agilent 6890 series capillary gas chromatograph. Identification and quantification of FAMEs were conducted using the MIDI software with MIDI microbial calibration standards. The following fatty acids were used as biomarkers for bacterial biomass: i14:0, 14:0, i15:0, a15:0, 15:0, i16:0, 16:1ω11c; 16:1ω7c; 16:0, i17:0, a17:0, 17:1ω8c, cy17:0, 17:0, 2-OH16:1, 18:1ω7c, cy17:0, 17:0, 2-OH16:1, 18:1ω9c, 18:1ω7c, 18:1ω5c and 18:0. PLFA 10Me16:0, 10Me17:0, 11Me18:1ω7c, 10Me18:0 and cy19:0ω8c were used as indicator for actinomycetes. PLFA 18:2ω6.9c was considered as an indicator for fungal biomass. PLFA 16:1ω5c was used as specific indicator for arbuscular mycorrhizal fungi (AMF).

qPCR and Illumina MiSeq sequencing

Total soil DNA was extracted from 0.5 g soil using the FastDNA Spin Kit for Soil and the FastPrep Instrument (MP Biomedicals, Santa Ana, CA, USA). All steps were carried out following the manufacturer's instructions. Quality and quantity of extracted soil DNA were certified with 1% agarose gel electrophoresis and Nanodrop-2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), respectively. Abundances of total prokaryotes and eukaryotes were quantified using qPCR targeting V3 region of 16S (with primer pairs 515F-GTGYCAGCMGCCGCGGTAA and 907R-CCGTCAATTCCTTTRAGTTT) and V4 region of 18S (with primer pairs TAReuk454FWD1-CCAGCASCYGCGGTAATTCC and TAReukREV3-ACTTTCGTTCTTGATYRA) rRNA gene, respectively [55, 56]. Briefly, 20 μL volume reaction was prepared using the PowerUp SYBR Green Master Mix (Life Technologies, Grand Island, NY, USA) with 0.2 μM of each primer and 50 ng of DNA template. Three technical replicates were prepared for each sample. Cycling conditions included an initial uracil-DNA glycosylase activation at 50°C for 2 min, denaturing at 95°C for 2 min, then 40 cycles of 95°C for 15 s and 72°C for 30 s, followed by a dissociation curve step of slowly heating the PCR mixtures from 55°C to 95°C. Fluorescence was

recorded after the extension phase of each cycle. Quantitation of unknown samples was achieved using standard curves generated from known concentrations of plasmid containing respective inserts for each set of primers.

For microbial community (bacteria, archaea, fungi and protist) analysis, triplicate PCR reactions for each DNA sample were pooled and purified using QIAquick Gel Extraction Kit (QIAGEN, Chatsworth, CA, USA). Approximately equimolar amounts of the PCR products from each sample were combined prior to amplicon sequencing using an Illumina Miseq platform at Shanghai Genesky Biotechnologies (Shanghai, China) and unprocessed sequences were deposited in the NCBI SRA database under the accession number SRP111087. Sequence data was processed and analyzed using QIIME software package [57]. Briefly, sequences < 200 bp with an average quality score < 25 and ambiguous characters were discarded. After chimeras and singletons were detected and removed, OTUs were clustered on the basis of 97% similarity with the UCLUST algorithm and classified using Greengenes database (http://greengenes.lbl.gov/) for bacteria and archaea, and PR^2 database for fungi and protist (http://ssu-rrna.org).

Protists

A most probable number (MPN) procedure was used to quantify protists [58]. Briefly, a 96-well cell culture plate with 100 μl 0.1 g L^{-1} tryptic soy broth (TSB) as substrate was prepared. Then 3.0 g field-moist soil was suspended in 30 mL sterile Neff's modified amoebae saline solution (NMAS) and gently shaken at 180 rpm for 20 minutes. Next, 50 μl soil suspensions were added to the first well of each dilution series. Three-fold dilution series with TSB and NMAS at 1:9 v/v were prepared for each soil sample. The plates were incubated in darkness at 22°C. Each well was observed with an inverted microscope at 400 × magnification and scored for the presence/absence of naked amoebae, flagellates and ciliates after 7, 14 and 21 d. Data were expressed as the number of individuals per 100 g dry soils. As ciliates were observed at low abundance and not in all plots, they were excluded from subsequent analyses.

During the MiSeq sequencing process, eukaryotic OTUs were assigned to species level with PR^2 database. After curation, protist OTUs were extracted from the whole eukaryotic OTU table to represent the community structure of soil protists.

Nematodes

Nematodes were extracted from 100 g field-moist soil using a modified Baermann method [59]. The soil was placed as a thin layer (2-4 mm) on a milk filter above a wire mesh pan (15 cm diameter) and totally submerged in water. The nematodes were allowed to migrate through the filter into the water for 48 h at 22°C, which resulted in relatively clean suspensions for nematode counting and identification. The suspensions were filtered through two 25 μm sieves, washed into a Petri dish and stored at 4°C until counting (total nematodes per 100 g dry soil) and identification of about 150 specimens per sample to the genus level based on morphological characteristics (Olympus BX50 microscope at 400-1000 × magnification) [60] (Table S4). All identified nematodes were then assigned to five feeding groups (bacterivore, fungivore, herbivore, omnivore and predator) [61].

Estimating taxonomic diversity

Five components were selected as indicators of species diversity: bacterial OTUs, fungal OTUs, archaeal OTUs, protist OTUs, and nematode genus richness. The OTUs for bacteria, fungi, archaeal and protist were derived from Illumina MiSeq sequencing results (Table S3), while nematode genus richness was based on the identified nematode genera. We also averaged the five standardized (0-1 transformation) diversity components into a composite index of soil taxonomic diversity [9, 32].

Estimating functional composition

As a first step, six components of soil functional composition were considered: herbivorous nematodes, eukaryotic plant pathogens, arbuscular mycorrhizal fungi, bacteria-to-fungi ratio, bacterivorous-to-fungivorous nematode ratio, and carbon-to-nitrogen processing bacteria ratio. Since bacteria-to-fungi ratio, bacterivorous-to-fungivorous nematode ratio, and carbon-to-nitrogen processing bacteria ratio contributed largely and significantly to multifunctionality (Table 1), these three components were standardized (0-1 transformation) and averaged into a composite index of functional composition. The bacteria-to-fungi ratio was calculated with the qPCR results of bacterial and fungal abundance. Arbuscular mycorrhizal fungi was quantified from PLFA results. The carbon-cycle bacteria and nitrogen-cycle bacteria were predicted from Illumina MiSeq sequencing results according to the FAPROTAX database (http://www.loucalab.com/archive/FAPROTAX/) (Table S6). This is a manually constructed database that maps prokaryotic taxa to ecologically relevant functions like nitrification, denitrification or fermentation [62]. Eukaryotic plant pathogens were predicted from Illumina MiSeq sequencing results according to the FUNGuild database (http://www.stbates.org/guilds/app.php) (Table S5), an online tool that can be used to taxonomically parse eukaryotic OTUs to 12 ecological guilds [63].

Estimating biotic associations

Our concept of the biotic associations induced by earthworms required consideration of the co-occurrence pattern within soil communities (microbes, protists and nematodes). It was defined as 'the percentage of significant correlations between two groups of total possible correlations', with higher values indicating stronger biotic associations [26]. We built the correlation network for aggregated groups (bacteria, fungi, AMF, actinomycetes, bacterivorous nematodes, fungivorous nematodes, herbivorous nematodes, omnivorous nematodes, predatory nematodes, flagellates and amoebae). We used aggregated groups consisting of species that are known to share a common function or the same feeding group. With this approach, we could indicate their role in the soil food web [26]. For network analysis, a Spearman's rank correlation approach was used to visualize the correlations between all individual members of the aggregated groups. All the positive correlations ($p < 0.05$) between groups were visualized.

Ecosystem multifunctionality

We used 21 ecosystem functions reflecting basic ecological processes both belowground and aboveground to assess the ecosystem multifunctionality (Table S10). We grouped these ecosystem functions in four categories, including i) plant productivity (crop yield, shoot biomass and root biomass), ii) plant nutrients (shoot carbon, shoot nitrogen, shoot phosphorus, root carbon, root nitrogen and root phosphorus), iii) nutrient and carbon cycling processes (soil organic carbon, available nitrogen, available phosphorus, N_2O emissions and CO_2 emissions), and iv) nutrient and carbon cycling drivers (microbial activity, microbial biomass carbon, microbial biomass nitrogen, microbial biomass phosphorus, carbon-cycle enzyme, nitrogen-cycle enzyme and phosphorus-cycle enzyme). Among them, plant productivity and nutrients could be assigned to 'ecosystem service multifunctionality', as they are more relevant for applied research and human demand, whereas nutrient and carbon cycling processes and drivers could be assigned to 'ecosystem function multifunctionality', because they are drivers of ecosystem functioning [10].

Different methods have been used to estimate the relationship between diversity and ecosystem multifunctionality, including the single functions approach [32], turnover approach [64], averaging approach [8, 9, 32], and threshold approach [8, 11, 32]. No single method represents multifunctionality unequivocally, although the averaging approach and the threshold approach, which are always used together considering their strengths and weaknesses, are generally considered to represent ecosystem multifunctionality. In this study, we used three distinct methods to characterize multifunctionality: the single functions approach, threshold approach and averaging approach. For the threshold approach, we first standardized the plot-level values of each ecosystem function using 0-1 transformation. We set the maximum functioning level as the 95th percentile of the standardized values, to avoid the influence of outliers on the estimated value. We calculated the multifunctionality index with the threshold approach for each plot as number of functions whose value exceeded 30, 50 and 70% of the 95th percentile for that function observed across all plots [8] (hereafter, multifunct.30%, multifunct.50% and multifunct.70%). For the averaging approach, each ecosystem function was 0-1 standardized like the threshold approach. The maximum value was defined as the 95th percentile to avoid the influenced of outliers on the estimation procedure [8]. The multifunctionality of averaging approach for each plot was estimated as the average value of the standardized values in that plot [8, 11], hereafter referred to as multifunct.average.

Plant productivity

All the crops in each plot were harvested manually to measure standing aboveground biomass and the roots were dug from about 40 cm depth to collect the root biomass. Grain of rice and wheat were gathered and weighed (dry mass) after harvest to quantify crop yield. The biomass of roots and aboveground materials was expressed as g m^{-2}.

Plant nutrients

The aboveground plant materials and root materials were separately ground to a fine powder on a ball mill and analyzed for plant carbon, nitrogen and phosphorus using the techniques outlined for soils (below).

Nutrient and carbon cycling processes

Soil organic carbon was measured by the Walkley-Black procedure. Soil available nitrogen (sum of ammonium and nitrate nitrogen) was determined using a flow injection auto analyzer (SEAL-AA3, Germany). Available phosphorus concentration was determined by the Olsen-P extraction. A chamber (40 × 25 × 60 cm) was used to collect gases before the crop was 60 cm tall, and then a chamber (40 × 25 × 60 cm) and an extension chamber (40 × 25 × 50 cm) were stacked over the crop to collect gases. Gas samples (20 mL) were collected in the morning (8:30 −11:30) at intervals of 10 min (i.e., 0 min, 10 min, 20 min and 30 min after chamber closure). The fluxes of N_2O and CO_2 from each plot were collected once a week during the crop growing season. The air temperature inside the chamber was measured with a mercury thermometer. Gas samples were analyzed within 24 h of sampling using a gas chromatograph (Agilent 7890A, USA). Cumulative emissions during the observation period were sequentially accumulated from the fluxes between every two adjacent intervals of the measurements. The fluxes and cumulative N_2O and CO_2 emissions were calculated as follows [65]: Cumulative gas emissions = $\sum(F_{i+1} + F_i)/2) \times (t_{i+1} - t_i) \times 24$, where F indicates N_2O or CO_2 flux, i refers to the sampling times and t is the sampling date.

Nutrient and carbon cycling drivers

Microbial activity (soil basal respiration) was measured by weighing fresh soil (equivalent to 5 g dry mass) into jars, and then measuring CO_2 accumulation by gas chromatography after incubating at 25°C for 12 h [66]. Microbial biomass carbon, nitrogen, and phosphorus were measured by the direct chloroform-fumigation extraction method with correlation factors of K_{EC} = 0.45, K_{EN} = 0.54 and K_{EP} = 0.4 [67–69]. Three carbon-cycle enzymes (invertase, cellulase and β-D-glucosidase), two nitrogen-cycle enzymes (urease and protease) and one phosphorus-cycle enzyme (alkaline phosphatase) were analyzed in this study [70]. Among them, invertase and cellulase were determined by 3, 5-dinitrosalicylic acid colorimetry. The β-D-glucosidase was measured by the *p*-nitrophenol colorimetry. Urease was determined by the sodium phenolate colorimetry. Protease was measured by the folin-phenol colorimetry. Alkaline phosphatase was determined by disodium phenyl phosphate colorimetry.

QUANTIFICATION AND STATISTICAL ANALYSIS

We performed a principal coordinate analysis (PcoA) to visualize the dissimilarity of soil communities among different treatments. We also calculated the Pielou's evenness index (*J*) [71]: $J = H'/ln\ S$, where H' is the Shannon-Wiener index and S is the total number of species. This index indicates the evenness or heterogeneity of soil biota among different treatments, where lower values indicate higher heterogeneity.

For the single functions approach, we correlated (Pearson's correlation) each of the 21 ecosystem functions with each of the five predictors (aggregate stability, pH, taxonomic diversity, functional composition and biotic associations) to visualize specific relationships (Figure 6). For the averaging approach and threshold approach, we first correlated (Pearson's correlation) the multifunctionality indices (multifunct.30%, multifunct.50%, multifunct.70% in the threshold approach and multifunct.average in the averaging approach) with each ecosystem function to visualize their relationships (Figure S4). We then used linear mixed-effect model (LMM) to demonstrate the indirect effects of earthworms on ecosystem multifunctionality, which were mediated by five predictors (taxonomic diversity, functional composition and biotic associations, aggregate stability and pH). In this and subsequent models, crop phase and sampling year were allocated as random factors due to repeated-measurements within the experiment. We fitted two models of LMM, one is a full model with five predictors, the other a model with four functional composition predictors (Table 1). Before fitting the model, the variance inflation factor (VIF) was estimated of each predictor to check the multicollinearity [72]. All $\sqrt{vif} < 2$, suggesting independence among the predictors in these two models. Moreover, we split the data to two groups (straw mulched and straw incorporated) and conducted separate LMM for each group to test how multifunctionality was estimated by the five predictors within each straw treatment (Table S9).

Although the four multifunctionality indices were correlated ($r = 0.56$-0.79, $p < 0.001$) (Figure S4), the LMM results showed that multifunct.average generates a higher determinant coefficient (R^2) in the regression models (Table S8). Thus, the average approach described more of the variability in multifunctionality than the threshold approaches for this dataset and multifunct.average was selected as the indicator of multifunctionality in the subsequent piecewise structural equation modeling (piecewise SEM) analysis (Figure 5). Piecewise SEM was used to analyze hypothetical pathways that may explain how earthworm impacted the ecosystem multifunctionality [73]. It allowed us to partition direct and indirect effects of one variable in relation to other variables and to estimate the strength of multiple effects. The first step was to build *a priori* model based on the known effects and relationships among earthworms, soil community attributes (taxonomic diversity, functional composition and biotic associations), soil abiotic factors (aggregate stability and pH) and multifunctionality. In the model, crop phase and sampling year were allocated as random factors and treatments were coded as 0 without earthworm (mulched, incorporated) and 1 with earthworm (mulched + earthworm, incorporated + earthworm). Model fitting was using a maximum likelihood $\chi 2$ goodness-of-fit test. Under the $\chi 2$ test, a good model should have a p value > 0.05 [74]. Furthermore, we calculated the standardized total effects of distance from soil community attributes and soil abiotic factors on multifunctionality. The net influence that one variable has upon another is calculated by summing all direct and indirect pathways between the two variables [20]. If the data fits the model well, the total effect should be approximately the bivariate correlation coefficient for that pair of variables [20]. Alternative piecewise SEM were tested to confirm that the model structure was robust. Specifically, we split the data into two groups (with and without earthworm) and conducted separate piecewise SEM analysis for each group (Figures S3A and S3B). We also split the data into two residue groups (straw mulched and straw incorporated) and conducted piecewise SEM for each group (Figures S3C and S3D). In all models, crop phase and sampling year were assigned as random factors.

All statistical analyses and figures were produced with R software version 3.4.1 [75]. Specifically, PcoA and Pielou's evenness index were calculated with the *vegan* package. Network co-occurrence was constructed and analyzed with the *visNetwork* package [76] and *igraph* package [77]. LMM was analyzed with the *lme4* package. The multicollinearity of each predictor was checked using the *vif* function in the *car* package. Piecewise SEM was performed using the *piecewiseSEM* package [78].

DATA AND CODE AVAILABILITY

The datasets generated during this study are available at Mendeley Data http://dx.doi.org/10.17632/pwtz4pctsk.2.