Diversity of soil faunal community as influenced by crop straw combined with different synthetic fertilizers in upland purple soil

Xiuhong Xie1,2,3, Xuefeng Wang4, Zhixin Dong1,2,3 & Bo Zhu1,2

Soil fauna play a crucial role in sustaining agro-ecosystem functions. Crop straw is recommended for application to agricultural fields to improve soil quality. However, the effects of crop straw combined with different synthetic fertilizers on the soil faunal community remain unclear, and knowledge regarding purple soil is limited. Using the conserved cytochrome c oxidase I (COI) gene as markers, we examined the responses of the soil faunal community to different fertilization in upland purple soil of southwestern China. The accuracy of the morphological and molecular methods in characterizing soil nematodes was compared. Our results showed that different fertilization treatments significantly changed the soil faunal community structure (Adonis test, $R^2 = 0.43$, $P = 0.011$). Sixteen biomarkers were identified according to LEfSe (linear discriminant analysis effect size). The diversity and species number of soil fauna were closely related to soil organic matter (SOM) and total phosphorus (TP) ($P < 0.05$). This study indicates that crop straw return can improve the soil fertility and diversity of soil fauna in purple soil. Additionally, the morphological approach and molecular method based on the COI gene can be considered as complementary approaches in characterizing soil nematode community.

Soil fauna are an important part of the food webs in the soil ecosystem and play multiple roles as consumers and decomposers. They directly or indirectly take part in sustaining ecosystem functions, including litter decomposition, soil pollution abatement, plant community dynamics, maintenance of soil structure, and carbon, nitrogen and phosphorus cycling. Soil fauna are highly diverse, which are generally divided into microfauna, mesofauna, macrofauna and megafauna based on different body widths. They are sensitive to changes in their habitat such as fertilizer use, tillage, and multiple species, i.e., earthworms and oribatids can be used as biological indicators to indicate changes in soil fertility and quality.

Crop straw return to agricultural fields is an important measure to improve soil fertility and increase yield, since the straw contains necessary ingredients for plants to grow, including calcium, magnesium, nitrogen, phosphorus, potassium and organic matter. Crop straw return affects soil physical and chemical properties, which may further change the community and diversity of soil fauna. For example, crop residues increased soil microarthropod abundance but had no significant effect on biodiversity in loamy sand; another study on soil meso- and microarthropods in tidal soil reported that straw returned to wheat–maize fields reduced the number of soil faunal groups. However, in another study, the return of crop residues with synthetic fertilizer, nitrogen, phosphorus and potassium, caused a significant decrease in the number of nematodes and an increase in the number of earthworms and ground beetles in sod-podzolic gley mid-loamy soil. Straw return provided richer living conditions, and the dominant groups, including Oribatida and Actinedida, accounted for 77.1% of the meso- and microfauna population in black soil, while the dominant groups in fluvo-aquic soils were Collembola and Acari, which accounted for nearly 80%. Through bottom-up and top-down processes, organic fertilizers, including straw residues, have a strong impact on a wide range of biological communities and enhance the processes of decomposition and carbon, nitrogen and phosphorus cycling in the soil food webs. Therefore, fertilizer application generally has positive, negative, or no significant effects on soil faunal abundance (earthworms, Collembola, Acari, and nematodes, respectively) and diversity. The impact of straw

1Key Laboratory of Mountain Surface Processes and Ecological Regulation, Institute of Mountain Hazards and Environment, Chinese Academy of Sciences, Chengdu 610041, China. 2Institute of Mountain Hazards and Environment, Chinese Academy of Sciences, No. 9, Section 4, South Renmin Road, Chengdu 610041, Sichuan, China. 3University of Chinese Academy of Sciences, Beijing 100049, China. 4Jilin Agricultural University, Changchun 130118, China. *email: zhxdong@imde.ac.cn
Soil erosion and degradation, straw return is recommended for application to agricultural fields to improve soil NPK were significantly higher than those under N fertilization alone (\( P < 0.05 \)). The effects of different fertilization regimes on the contents of soil pH, TK, DOC, NH4+ were significantly higher compared with the treatment without phosphate (N fertilization alone). The COI coding of soil arthropods was an effective biological monitoring method. Morise et al. explored the soil nematode community in nonmanaged flower soil and indicated that the COI gene technique provided a detailed structure (23 COI gene-derived operational taxonomic units, or OTUs) of the nematode community. Drummond et al. studied soil fauna in tropical secondary forests and terrestrial arthropods, respectively, and concluded that COI coding of soil arthropods was an effective biological monitoring method. Watts et al. and Kvist stated that the current COI metbarcoding method was not ideal because the COI sequence databases (NCBI and BOLD) were devoid of data for many animal groups, and few OTUs could be confidently identified at the genus level. As stated above, the COI gene coding technique needs to be validated in determining the taxonomic identity of the soil fauna. Soil nematodes are one of the most numerous of all multicellular organisms, occupying many key nodes of the soil food webs. Here, we simultaneously observed the diversity and composition of soil nematodes using a traditional morphological method and compared it with a molecular method based on the COI gene.

Therefore, the main objectives of this study were to explore (1) the differences in the identification of soil nematodes between the COI genetic technique and traditional morphological method; (2) the effects of straw return combined with different synthetic fertilizers on the composition and diversity of the purple soil fauna community; and (3) the relationship between soil fauna abundance, diversity and soil properties under different fertilization treatments.

Results

The impact of different fertilization on soil properties. Different fertilization regimes significantly influenced the soil AP, NO3-N, TDN, TP, TN, SOM and WC (Table 1). The SOM and TN of the purple soil under the straw return treatments (RSDNP, RSDN and RSDNPK) were significantly higher than those in the absence of straw return (NPK and N) (\( P < 0.05 \)). Additionally, the soil TDN, NO3-N and WC in RSDNP, RSDN and RSDNPK were significantly higher than those under N fertilization alone (\( P < 0.05 \)). The application of phosphate fertilizer (NPK, RSDNP and RSDNPK) improved soil TP compared with the treatment without phosphate (N and RSDN). The effects of different fertilization regimes on the contents of soil pH, TK, DOC, NH4+ and AK were not significant (\( P > 0.05 \)). Purple soil is rich in potassium, and there was no significant difference in the soil characteristics between the RSDNP and RSDNPK fertilization treatments.

The soil nematode community identified by traditional morphological and molecular biological methods. In this study, both traditional morphological and molecular biological methods were used to identify soil nematodes. A total of 508 nematodes were isolated by the traditional morphological method and belonged to three classes, eight orders, twenty families and thirty-eight genera (Supplementary Table S1). At the genus level, Acrobeloides and Mesorhabditis were the dominant genera (relative abundance > 5%), and the common groups (5% > relative abundance > 1%) were Epidorylaimus and Plectus.

Based on the mitochondrial COI gene (Supplementary Table S2), we observed that 4225 OTUs of the nematode community belonged to two classes, three orders, seven families and nine genera. Cylcosesthenus was the most dominant genus, and Schistonchus, Fergusobia, Xiphinema, and Thelandros were the common genera. Some parasitic nematodes (Heterakidae, Ancylostomatidae) were also detected by the molecular biological method.

Community composition of soil fauna. Based on a COI gene sequence similarity cutoff value of 97%, 357,634–463,460 OTUs were observed in the 15 soil samples. Soil fauna were classified by species annotation and belonged to 16–18 phyla, 37–42 classes, 111–125 orders, 315–349 families and 290–509 genera across all samples.
Quadrulella addition. Results verified that the soil faunal community was relatively sensitive to straw return and phosphate fertilizer application of straw and phosphate fertilizer (RSDNPK) increased the relative abundance of Coryne, and Corynidae were increased by phosphate fertilizer addition (NPK). In addition, the simultaneous application of crop straw return (RSDN) and the common taxa were Clitellata (8.99%), and Insecta (3.54%), Ptecticus and the common taxa were Araneae (4.10%) and Rotifera (3.80%). In the RSDNPK treatment, the dominant taxa were Araneae (4.10%), and Insecta (3.54%), Coenobita (3.16%), Lepidoptera (3.13%) and Allothrombium (2.95%) were the common taxa in the RSDNPK treatment.

To further understand the effects of five different kinds of fertilization treatments on the soil faunal taxa, LEfSe analysis (Fig. 2) was used to identify biomarkers in different fertilization treatments. Significantly different common taxa in the RSDNP treatment.

### Table 1. Soil properties under different fertilizer regimes.

| Soil properties | N       | NPK     | RSDN    | RSDNP   | RSDNPK  |
|-----------------|---------|---------|---------|---------|---------|
| WC (mL g⁻¹)     | 18.15 ± 0.31b | 19.67 ± 0.51a | 22.21 ± 0.24a | 22.59 ± 1.52a | 22.30 ± 0.74a |
| pH              | 8.33 ± 0.09a  | 8.24 ± 0.09a  | 8.30 ± 0.09a  | 8.18 ± 0.09a  | 8.20 ± 0.09a  |
| SOM (g kg⁻¹)    | 8.12 ± 0.17b  | 9.91 ± 0.50b  | 14.51 ± 0.89a | 16.46 ± 1.20a | 16.52 ± 1.48a |
| TN (g kg⁻¹)     | 0.68 ± 0.32c  | 0.86 ± 0.28b  | 1.15 ± 0.54a  | 1.26 ± 0.83a  | 1.20 ± 0.60a  |
| TP (g kg⁻¹)     | 0.71 ± 0.03b  | 1.11 ± 0.09a  | 0.68 ± 0.05b  | 1.30 ± 0.10a  | 1.10 ± 0.18a  |
| TK (g kg⁻¹)     | 20.02 ± 0.67a | 18.94 ± 0.82a | 20.21 ± 1.69a | 19.84 ± 1.03a | 20.61 ± 0.98a |
| TDN (mg kg⁻¹)   | 10.97 ± 0.88b | 16.63 ± 3.01a | 24.02 ± 1.70a | 23.18 ± 2.31a | 25.30 ± 7.48a |
| NO₃⁻N (mg kg⁻¹) | 2.33 ± 0.64b  | 4.68 ± 2.16b  | 7.98 ± 1.51a  | 7.38 ± 1.62a  | 6.34 ± 3.38a  |
| NH₄⁺N (mg kg⁻¹) | 0.65 ± 0.16a  | 0.82 ± 0.04a  | 1.19 ± 0.03a  | 1.22 ± 0.11a  | 3.32 ± 2.26a  |
| AP (mg kg⁻¹)    | 3.30 ± 0.1c   | 20.79 ± 1.46b | 5.21 ± 0.28b  | 31.14 ± 6.13a | 11.48 ± 5.59ab |
| AK (mg kg⁻¹)    | 100.98 ± 2.08a | 121.20 ± 4.03a | 166.36 ± 15.25a | 139.51 ± 8.98a | 170.09 ± 39.71a |
| DOC (mg kg⁻¹)   | 44.67 ± 1.50a | 47.13 ± 1.99a | 66.98 ± 4.09a | 51.82 ± 14.43a | 63.93 ± 4.82a |

The difference in the total soil faunal community among the fertilization treatments was significant (Adonis test, $R^2 = 0.43, P < 0.01$). The heatmap (Fig. 1) showed the top 40 abundant soil faunal taxa. Cluster analysis (Fig. 1) showed that the RSDNP, RSDNPK, and NPK treatments and the RSDN and N treatments were clustered into two categories (without phosphate fertilizer: RSDN, N and with phosphate fertilizer: RSDNP, RSDNPK, NPK), which indicated that the application of phosphate fertilizer significantly changed the composition of the soil faunal community.

The dominant taxa in the N treatment were Araneae (5.34%) and Insecta (8.42%), and the common taxa were Quadrulaella (4.2%), Rotifera (3.03%) and Lepidoptera (2.80%). The highest abundance of taxa in the RSDN treatment was detected in Insecta (15.31%), followed by Coryne (6.55%), Alcyonacea (6.27%), Ypthima (3.54%), Microcionidae (2.75%), Lepidoptera (2.65%), Araneae (1.76%) and Arachnida (1.43%).

The dominant taxa in the NPK treatment were Coryne (10.57%), Insecta (8.12%), and Lepidoptera (7.84%), and the common taxa were Araneae (4.10%) and Rotifera (3.80%). In the RSDNPK treatment, the dominant taxa were Clitellata (8.99%), Pecticus (17.29%), and Insecta (9.16%), and the common taxa were Monochamus (4.12%) and Lepidoptera (3.47%). In addition, the highest abundance of taxa in RSDNP was Entomobryidae (11.03%), and Insecta (3.54%), Coenobita (3.16%), Lepidoptera (3.13%) and Allothrombium (2.95%) were the common taxa in the RSDNPK treatment.

To further understand the effects of five different kinds of fertilization treatments on the soil faunal taxa, LEfSe analysis (Fig. 2) was used to identify biomarkers in different fertilization treatments. Significantly different abundant taxa were identified in N, including Quadrulaella, Emesis, Roidinidae, Acrolochiidae and Dysderidae; Anthothecata, Coryne, and Corynidae presented the highest LDA score in NPK; Glyptoclastes, Ancylus, Lymnaeidae, Tetragnathidae and Pyralidulidae were present in high proportions in RSDN; and Diplotoda was the biomarker of RSDNPK.

Compared with the N treatment, the relative abundances of Glyptoclastes, Ancylus, Tetragnathidae, Lymnaeidae, and Pyralidulidae were increased by crop straw return (RSDN). The relative abundances of Anthothecata, Coryne, and Corynidae were increased by phosphate fertilizer addition (NPK). In addition, the simultaneous application of straw and phosphate fertilizer (RSDNPK) increased the relative abundance of Diplotoda. The results verified that the soil faunal community was relatively sensitive to straw return and phosphate fertilizer addition.

### The impact of straw return and phosphate addition on soil faunal diversity.

Two-way analysis of variance demonstrated that the species number (S) (Fig. 3b; $F = 6.66, P = 0.027$) and Margalef richness index (D) (Fig. 3c; $F = 9.88; P = 0.01$) were significantly positively affected by straw return, while there was no significant difference in the Shannon–Wiener index (H’) (Fig. 3a; $F = 0.594; P = 0.457$) and Pielou evenness index (J) (Fig. 3d; $F = 0.997; P = 0.339$) between the fertilization regimes. In addition, phosphorus fertilizer had no significant effect on the four indices (Fig. 3a–d; $P > 0.05$).

The relationship between soil properties and soil faunal diversity was analyzed by Pearson correlation coefficients (Fig. 4). The Margalef richness index (D) was positively correlated with soil NO₃⁻N, TDN, AP, AK, and TP ($P < 0.05$) and extremely positively correlated with soil WC, SOM and TN ($P < 0.01$), whereas it had a significantly negative correlation with soil pH ($P < 0.05$). Species number (S) had a positive correlation with soil TDN, AK, TP, TN, and SOM ($P < 0.05$) and a negative correlation with soil pH ($P < 0.01$). The Shannon–Wiener index (H’) had a negative correlation only with soil WC ($P < 0.05$), and the Pielou evenness index (J) had a negative
correlation with soil WC and SOM ($P < 0.05$). These results showed that soil factors such as soil NO$_3$–N, TP, AP, TDN, TN and SOM had a close relationship with the soil faunal diversity.

**Effect of soil properties on the main soil faunal taxa.** RDA (Fig. 5) was performed to correlate the relative abundance of the soil fauna with the fertilizer treatments to define the major environmental variables that impacted the soil faunal community. The Monte Carlo test illustrated that all of the axis explanatory variables were significant ($F = 1.5, P = 0.004$). On the basis of the forward-selection option, all of the environmental variables accounted for 55.9% of the variation in the soil faunal community among the samples. The first canonical axis was mainly determined by soil TP and AP and explained 19.00% of the total variation. The second canonical axis included soil SOM and TK and explained 10.00% of the variation. Furthermore, soil TP ($P = 0.002$) was the major soil environmental factor that influenced the soil faunal community. The abundances of Culicidae, Agriolimacidae, Insecta, Crabronidae, Alcyonacea, Microcionidae, Diptera, Ypthima, Clubiona, Cyanea, and Coenobita presented negative correlations with soil NO$_3$–N, DOC, SOM, TN, AP and TP, and they had strong positive correlations with soil TK. The Arthropoda was positively correlated with soil factors (NO$_3$–N, DOC, SOM, TN, AP and TP). As decomposers, the major soil fauna exhibited sensitive responses to changes in soil fertility.

Figure 1. Heatmap of the top 40 abundant soil faunal taxa based on genus level under different fertilization regimes. N synthetic N fertilizer, NPK synthetic fertilizer: nitrogen, phosphorus and potassium, RSDN crop residues returned with nitrogen, RSDNP crop residues returned with nitrogen and phosphorus, RSDNPK crop residues returned with nitrogen, phosphorus and potassium. When the genus was unclassified, the corresponding higher-level annotation was adopted and denoted by f—, o—, c—, or p—.
Figure 2. LEfSe analysis of soil faunal taxa in different fertilization treatments. The LDA score represents the different sizes among fertilizer treatments with a threshold value of 2. N synthetic N fertilizer, NPK synthetic fertilizer: nitrogen, phosphorus and potassium, RSDN crop residues returned with nitrogen, RSDNPK crop residues returned with nitrogen, phosphorus and potassium.

Figure 3. Effects of straw return (RSD) and phosphate fertilizer (P) on the Shannon–Wiener index ($H'$) (a), species number (S) (b), Margalef richness index ($\bar{D}$) (c) and Pielou evenness index ($J$) (d) of the soil faunal community (mean ± standard error). The species number (S) was transformed using log (x). Treatments with different letters (a and b) are significantly different (Two-way ANOVA, $P < 0.05; n = 3$). RSD+ (the treatments with crop straw return): RSDN, RSDNP and RSDNPK; RSD− (the treatments without crop straw return): N and NPK; P+ (the treatments with phosphorus fertilizer): NPK, RSDNP and RSDNPK; P− (the treatments without phosphorus fertilizer): N and RSDN.
To further explore the influence of fertility regimes on soil faunal diversity, a structural equation model was established (Fig. 6, $\chi^2 = 9$, $\text{CMIN/DF} = 0.976$, $P = 0.458$, $\text{GFI} = 0.840$, $\text{CFI} = 1.000$, $\text{RMSEA} = 0.000$). The model indicated that the effects of straw return and phosphate fertilizer on soil faunal diversity were significant; the application of phosphate fertilizer to purple soil increased the content of available phosphorus (0.69, $P < 0.001$) and then directly affected the Margalef richness index of the soil fauna (0.74, $P < 0.001$). Straw return increased the soil organic matter content (0.76, $P < 0.001$) and enhanced the soil faunal Margalef richness index (0.74, $P < 0.001$) and species number (0.61, $P < 0.01$).

- Figure 4. Pearson correlation coefficients between soil faunal diversity and soil factors. The species number (S) was transformed using log (x). H Shannon–Wiener index, D Margalef richness index, S species number, J Pielou evenness index, SOM soil organic matter, TN total nitrogen, TP total phosphorus, TK total potassium, AP available phosphorus, AK available potassium, TDN dissolved total nitrogen, DOC dissolved organic carbon, NH ammonium nitrogen, NN nitrate nitrogen, WC water content. Red represents a positive correlation; blue represents a negative correlation; the depth of color indicates the strength of the correlation. *$P<0.05$, **$P<0.01$.

- Figure 5. Redundancy analysis of the top 40 abundant taxa of soil fauna with selected soil physical and chemical properties. Arthropd: p_Arthropoda; Insecta: c_Insecta; Alcyonac: o_Alcyonacea; Agriolim: f_Agriolimacidae; Culicina: f_Culicidae; Microcic: f_Microcinidae; Coenobit: g_Coenobita; Clubiona: g_Clubiona; Diptera: g_Diptera; Cyanea: g_Cyanea; Ypthima: g_Ypthima; Homo: g_Homo. N synthetic N fertilizer, NPK synthetic fertilizer: nitrogen, phosphorus and potassium, RSDN crop residues returned with nitrogen, RSDNP crop residues returned with nitrogen and phosphorus, RSDNPK crop residues returned with nitrogen, phosphorus and potassium, SOM soil organic matter, TN total nitrogen, TP total phosphorus, TK total potassium, AP available phosphorus, DOC dissolved organic carbon, NN nitrate nitrogen.
Discussion

Comparison of molecular and morphological techniques in identifying soil nematodes. By exploring the differences between traditional morphological and molecular biological methods in identifying the soil nematode community, we found that the traditional morphological method generated a relatively low number of nematodes. It could be partly explained by identification methods and the artificial bias. Nematodes need water films to survive and move around in the soil. The number of living nematode decreased when the soil samples were collected at a situation of less rainfall, but the genetic information of soil nematode could be detected through the molecular technique. And the COI genetic method generated a larger population of nematode taxa could not be identified to the family or genus level by the molecular method. However, large proportions of nematode taxa could not be identified to the family or genus level by the molecular technique. And some COI sequences acquired from soil eDNA were microbial (fungi, plants, etc.), a result consistent with Yang et al. and Horton et al. The reasons for this may range from DNA extraction to a bias in PCR amplification. The accuracy of sequencing, incomplete databases or primers selection also restricts species identification. In practical analysis, genus-level annotation information is not available for all feature sequences because of incomplete soil faunal databases. Therefore, there is a need to add new and validated reference sequences to the nematode databases for metabarcoding studies. When more taxa are morphologically consistent with the corresponding reference sequences, the molecular biological method for identifying the soil nematode community will be improved. The selected primers determine the accuracy and taxonomic breadth of acquired datasets. It is likely necessary to develop degenerate primers targeting the COI gene to include a broader range of soil nematodes. Despite both the morphological and molecular methods had limitations in characterizing the soil nematode community, the results indicated that the two methods could complement each other to identify soil nematode community.

Effects of different fertilization on the soil faunal community and diversity. Fertilization affects the soil faunal community, and organic fertilizer promotes soil faunal community diversity. The results of our study confirmed that different fertilizer caused changes in the soil faunal community (Adonis test, \( R^2 = 0.43, P = 0.011 \)). Straw return significantly increased soil TN, SOM, TDN and NO\(_3\) N (Table 1), and these altered soil factors exhibited a significant correlation with the soil faunal diversity (Fig. 4). Culicidae, Agriolimacidae, Insecta, Crabronidae, Alyconacea, Microcionidae, Diptera, Ypthima, Chibiona, Cyanea, and Coecnobia were the dominant taxa in the purple soil under the straw return treatments (Fig. 3). In addition, the return of straw to the field increased the relative abundances of Glyptocistes, Ancylus, Tetragnathidae, Lymnaeidae, and Pyramidulidae (Fig. 2), which showed that these soil faunal taxa were relatively sensitive to straw addition. Significant effect of straw return on the abundances of earthworms and Collembola and the Shannon–Wiener’s index of the soil invertebrates was reported previously. The return of straw to the field increased the contents of nitrogen, phosphorus, potassium and other elements in the soil, providing nutrients for microbes and soil fauna. In addition, straw return can further improve the soil water content, reduce the soil bulk density, increase soil permeability, and provide a variety of habitats. In summary, straw return provided a beneficial environment for the survival of soil fauna.

Phosphorus is a main limiting element in the studied purple soil. Exogenous phosphorus application can improve soil phosphorus availability and the soil nutrient balance to promote soil faunal community diversity. The responses of different soil fauna to phosphorus addition are highly heterogeneous in different ecosystems. In the process of litter decomposition in subtropical forests, the diversity and richness of soil arthropods increased through the coaddition of N and P. Phosphate fertilizer was also reported to increase the abundance of Collembola but decreased predatory nematodes in a phosphorus-limited woodland. In this study, phosphate fertilization significantly changed soil AP and TP (Table 1). Meanwhile, the relative abundances of Riodinidae, Dysderidae, Quadrupilla, Acroloxidae, and Emesidae decreased in the P addition treatments, while the relative abundances of Anthothecata, Coryne and Corynidae increased (Fig. 2). Pearson correlation analysis showed significant correlations between soil faunal diversity (Margalef diversity index and species number) and soil TP. The RDA (Fig. 5) further indicated that soil TP had a beneficial effect on soil Arthropoda. This was consistent with a study in forest regions.

![Structural equation model. Black arrows indicate a positive relationship; the number on the arrow is the normalized path coefficient. The \( R^2 \) value represents the ratio of variation to the other variables explained. * \( P < 0.05 \), ** \( P < 0.01 \), *** \( P < 0.001 \). SOM soil organic matter, AP available phosphorus.](image-url)
and boron deficiencies, shallow soil, and poor water retention and drought resistance50.

higher than 6%, approximately 10 g/kg soil organic matter, low nitrogen and phosphorus contents, serious zinc
follows: loose soil, good permeability, soil thickness of 20–60 cm, soil pH of 8.2–8.3, calcium carbonate content
(2002–2017, N 17%)/urea (since September 2017, N 46.3%), superphosphate (P2O5 12%), and potassium chlo-
gen: 200 kg N ha−1 y−1 plus crop straw returned: average 12.25 t ha−1 y−1, which provided 80 kg N ha−1 y−1), and RSDNP (synthetic fertilizer, nitrogen: 200 kg N ha−1 y−1, phosphorus: 180 kg P2O5 ha−1 y−1 plus crop straw returned: average 12.25 t ha−1 y−1, which provided 80 kg N ha−1 y−1) and treatments without straw, i.e., N (synthetic N fertilizer, nitrogen: 280 kg N ha−1 y−1), NPK (synthetic fertilizer, nitrogen: 280 kg N ha−1 y−1, phosphorus: 180 kg P2O5 ha−1 y−1, potassium: 72 kg K2O ha−1 y−1), and potassium: 72 kg K2O ha−1 y−1, phosphorus: 180 kg P2O5 ha−1 y−1, nitrogen: 200 kg N ha−1 y−1, phosphorus: 180 kg P2O5 ha−1 y−1, potassium: 72 kg K2O ha−1 y−1, plus crop straw returned: average 12.25 t ha−1 y−1, which provided 80 kg N ha−1 y−1) and treatments without straw, i.e., N (synthetic N fertilizer: 280 kg N ha−1 y−1), NPK (synthetic fertilizer, nitrogen: 280 kg N ha−1 y−1, phosphorus: 180 kg P2O5 ha−1 y−1, potassium: 72 kg K2O ha−1 y−1), and potassium: 72 kg K2O ha−1 y−1, phosphorus: 180 kg P2O5 ha−1 y−1, nitrogen: 280 kg N ha−1 y−1, phosphorus: 180 kg P2O5 ha−1 y−1, potassium: 72 kg K2O ha−1 y−1, and potassium: 72 kg K2O ha−1 y−1). The chemical N, P, and K fertilizers were ammonium bicarbonate (2002–2017, N 17%)/urea (since September 2017, N 46.3%), superphosphate (P2O5 12%), and potassium chloride (K2O 60%), respectively. The fertilizer application details were shown in Table 2.

In each plot, the cropping system was a wheat–maize rotation for all treatments each year during the whole
18-year experiment. Wheat–maize rotation is a typical intensive agricultural technique in the Sichuan Basin of
the upper Yangtze River, China, which is in accordance with the local farming system50. Winter wheat (Triticum
aestivum L.) was planted by direct sowing from late October to May of the following year, and summer maize
(Zea mays L.) was planted in planting holes from May to September51. The returned straws were harvested from
a large adjacent field, which was managed in accordance with NPK treatment and the nutrients content of straw
were measured. Maize straw [TN (total organic nitrogen) 0.8%; TOC (total organic carbon) 44%] was returned
to the soil before the wheat was planted, and wheat straw (TN 0.5%; TOC 43%) was returned to the soil before

Table 2. Details of the fertilizer application in the experiment. N synthetic N fertilizer, NPK synthetic fertilizer: nitrogen, phosphorus and potassium, RSDN crop residues returned with nitrogen, RSDNP crop residues returned with nitrogen and phosphorus and RSDNPK crop residues returned with nitrogen, phosphorus and potassium.

| Rotation system | Fertilizer type | Treatment | N | NPK | RSDN | RSDNP | RSDNPK |
|-----------------|----------------|-----------|---|-----|------|-------|--------|
| Wheat season (October to May) | Chemical N (kg ha−1) | 130 | 130 | 80 | 80 | 80 |
| | Maize straw N (kg ha−1) | 0 | 0 | 50 | 50 | 50 |
| | P2O5 (kg ha−1) | 0 | 90 | 0 | 90 | 90 |
| | K2O (kg ha−1) | 0 | 36 | 0 | 36 | 36 |
| Maize season (May to September) | Chemical N (kg ha−1) | 150 | 150 | 120 | 120 | 120 |
| | Wheat Straw N (kg ha−1) | 0 | 0 | 30 | 30 | 30 |
| | P2O5 (kg ha−1) | 0 | 90 | 0 | 90 | 90 |
| | K2O (kg ha−1) | 0 | 36 | 0 | 36 | 36 |
| Total amount of nitrogen applied per year (kg ha−1) | | 280 | 280 | 280 | 280 | 280 |

Conclusions
This study investigated the community and diversity of soil fauna in response to crop straw combined with dif-
ferent synthetic fertilizers in upland purple soil using the conserved COI gene fragment in eukaryotic cells as a
molecular marker. The results of our study showed that there were significant differences in the community
composition of the soil fauna under different fertilization treatments. Straw return significantly increased soil
SOM and TN. Soil SOM and AP were the main fertility factors affecting the soil faunal community and diversity.
The soil faunal Margalef richness index (D) and species number (S) increased as a result of increases in the soil
organic matter and available phosphorus contents. Although both the morphological and molecular methods
remained limited in the detection of soil nematodes, which required time to improve. The combination of them
was still useful to confirm the results of taxonomical identification. The results imply that crop straw return
improves the soil fertility and diversity of soil fauna in upland purple soil. The study provides an important
scientific basis and theoretical reference for maintaining agricultural sustainability.

Materials and methods
Site description. The study site is located at the Yanting Agro-ecological Experimental Station of Purple
Soil (N 31°16′, E 105°28′) of the Chinese Academy of Sciences (CAS). It is located at an altitude of 400–600 m
in the middle of the Sichuan Basin, southwestern China. The climate at the site is a moderate subtropical mon-
soon climate, with a mean annual temperature of 16.3 °C and a mean annual precipitation of 756 mm (2002–
2019). The annual precipitation in this region is concentrated in the period from June to October when maize
is planted. The vegetation consists mainly of artificial alder (Alder cremastogyne), cypress (Cypressus funebris
mixed forest, and the main crops are rice (Oryza sativa), corn (Zea mays L.), wheat (Triticum aestivum L.), sweet
potato (Ipomoea, batatas Lam.), and rape (Brassica campestris L.). The soil used in this experiment is classified
as a Pup-Orthic-Entisol in the Chinese Soil Taxonomy, and has a clay loam texture49. The soil properties are as
follows: loose soil, good permeability, soil thickness of 20–60 cm, soil pH of 8.2–8.3, calcium carbonate content
higher than 6%, approximately 10 g/kg soil organic matter, low nitrogen and phosphorus contents, serious zinc
and boron deficiencies, shallow soil, and poor water retention and drought resistance50.

Experimental design. The experiment was initiated in 2002. The experiment followed a randomized
block design consisting of fifteen plots with five fertilization treatments. Each treatment consisted of three rep-
licate plots (24 m² each, slope 6.5°). The five fertilization treatments were as follows: RSDN (synthetic N nitro-
gen: 200 kg N ha−1 y−1 plus crop straw returned: average 12.25 t ha−1 y−1, which provided 80 kg N ha−1 y−1),
RSDNP (synthetic fertilizer, nitrogen: 200 kg N ha−1 y−1, phosphorus: 180 kg P2O5 ha−1 y−1 plus crop straw
returned: average 12.25 t ha−1 y−1, which provided 80 kg N ha−1 y−1) and RSDNPK (synthetic fertilizer, nitrogen:
200 kg N ha−1 y−1, phosphorus: 180 kg P2O5 ha−1 y−1, potassium: 72 kg K2O ha−1 y−1 plus crop straw returned:
average 12.25 t ha−1 y−1, which provided 80 kg N ha−1 y−1) and treatments without straw, i.e., N (synthetic N
fertilizer: 280 kg N ha−1 y−1), NPK (synthetic fertilizer, nitrogen: 280 kg N ha−1 y−1, phosphorus: 180 kg P2O5
ha−1 y−1, and potassium: 72 kg K2O ha−1 y−1). The chemical N, P, and K fertilizers were ammonium bicarbonate
(2002–2017, N 17%)/urea (since September 2017, N 46.3%), superphosphate (P2O5 12%), and potassium chloride
(K2O 60%), respectively. The fertilizer application details were shown in Table 2.

In each plot, the cropping system was a wheat–maize rotation for all treatments each year during the whole
18-year experiment. Wheat–maize rotation is a typical intensive agricultural technique in the Sichuan Basin of
the upper Yangtze River, China, which is in accordance with the local farming system50. Winter wheat (Triticum
aestivum L.) was planted by direct sowing from late October to May of the following year, and summer maize
(Zea mays L.) was planted in planting holes from May to September51. The returned straws were harvested from
a large adjacent field, which was managed in accordance with NPK treatment and the nutrients content of straw
were measured. Maize straw [TN (total organic nitrogen) 0.8%; TOC (total organic carbon) 44%] was returned
to the soil before the wheat was planted, and wheat straw (TN 0.5%; TOC 43%) was returned to the soil before
the maize was planted. The maize and wheat straws were chopped into small pieces (approximately 5 cm long) and then applied to the field according to the mulching pattern. All chemical fertilizers and organic materials were manually applied to the soil at a depth of 10 cm and once as basal fertilization on the day of sowing.

**Research involving plants.** Plant material and soil were collected from an experimental station, permissions were obtained from Yanting Agro-ecological Experimental Station of Purple Soil of the Chinese Academy of Sciences (CAS).

Experimental research on plants and soil, including the collection of plant material and soil comply with relevant institutional, national, and international guidelines and legislation.

**Soil sampling and analysis**

**Soil sampling.** Surface soil (0–15 cm, 1 kg) samples were collected with trowels for each treatment during the fallow period of the wheat–maize rotation in May 2019. Five soil cubes were randomly collected from each fertilization plot and mixed together to generate one composite sample (n = 15). All samples were immediately transported to the laboratory and passed through a 2 mm sieve after removing roots and stones. One subsample was air-dried for soil property analysis, another subsample was stored at 4 °C for nematode morphological identification, and the remaining subsample was immediately stored at ~80 °C for molecular analysis.

**Analyses of soil properties.** Dissolved total nitrogen (TDN), ammonium nitrogen (NH₄⁺-N), and nitrate nitrogen (NO₃⁻-N) were analyzed by an Auto Analyzer-AA3 (SEAL, Germany). Total nitrogen (TN) was measured with an elemental analyzer (Vario MICRO cube analyzer, Elementar, Germany). Total phosphorus (TP) was determined by the molybdenum blue colorimetric method following digestion with perchloric acid (HClO₄). Dissolved organic carbon (DOC) was extracted with 0.5 M K₂SO₄ and analyzed using a TOC-5000 analyzer (Shimadzu, Kyoto, Japan). Soil organic matter (SOM) was analyzed by the dichromate oxidation spectrophotometric method. Soil pH was measured in soil–water suspensions (1:2.5 w/v). The soil water content (WC) was measured after oven–drying for 24 h at 105 °C. Soil total potassium (TK) was determined by H₂SO₄/ H₂O₂ digestion and atomic absorption spectrophotometry. Available potassium (AK) was determined based on 1 mol/L ammonium acetate (NH₄OAc)-extractable K. Olsen-P (available phosphorus) was extracted with 0.5 mol/L sodium bicarbonate (NaHCO₃) at pH 8.5.

**Identification of soil nematodes by the morphological method.** Nematodes were extracted from 50 g of moist soil using the Baermann funnel method for 48 h. After being preserved in 4% formalin solution, the total number of soil nematodes was counted using an inverted composite microscope (Olympus SXZ16, Japan). Nematode abundance was expressed as the number of nematodes per 100 g of dry soil. After counting the total nematode abundance in each sample, one hundred individuals were randomly selected and identified to the genus level at 400× magnification.

**DNA extraction and COI gene amplicon sequencing.** Total genomic DNA samples were extracted using Fast DNA SPIN extraction kits (MP Biomedicals, Santa Ana, CA, USA) following the manufacturer’s instructions and were stored at ~20 °C prior to further analysis. The quantity and quality of extracted DNA were measured using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis, respectively. PCR amplification of the COI gene was performed using the forward primer COIintF (5′-GGGACCGGWTGAACWGTAWYCCYCC-3′) and the reverse primer COIjgHCO2198 (5′-TANACYTNGGRTGNNCR AAR AAYCA-3′). Sample-specific 7-bp barcodes were incorporated into the primers for multiplex sequencing. The PCR mixture contained 5 μl of Q5 reaction buffer (5×), 5 μl of Q5 High-Fidelity GC buffer (5×), 0.25 μl of Q5 High-Fidelity DNA Polymerase (5 U/μl), 2 μl (2.5 mM) of dNTPs, 1 μl (10 μM) of each forward and reverse primer, 2 μl of the DNA template, and 8.75 μl of ddH₂O. Thermal cycling consisted of initial denaturation at 98 °C for 2 min, followed by 25 cycles consisting of denaturation at 98 °C for 15 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, with a final extension of 5 min at 72 °C. PCR amplicons were purified with Agencourt AM Pure Beads (Beckman Coulter, Indianapolis, IN) and quantified using the PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). After the individual quantification step, amplicons were pooled in equal amounts, and paired-end 2 × 300 bp sequencing was performed using the Illumina MiSeq platform with MiSeq Reagent Kit v3 at Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China).

**Sequence analysis.** The Quantitative Insights into Microbial Ecology (QIIME, v1.8.0) pipeline was employed to process the sequencing data, as previously described. Briefly, raw sequencing reads with exact matches to the barcodes were assigned to respective samples and identified as valid sequences. Low-quality sequences were filtered through the following criteria: sequences that had a length < 150 bp, sequences that had average Phred scores < 20, sequences that contained ambiguous bases, and sequences that contained mononucleotide repeats > 8 bp. Paired-end reads were assembled using FLASH. After chimera detection, the remaining high-quality sequences were clustered into operational taxonomic units (OTUs) at 97% sequence identity by UCLUST. A representative sequence was selected from each OTU using default parameters. OTU taxonomic classification was conducted by BLAST searching the representative sequences against the NCBI NT Database using the best hit. An OTU table was further generated to record the abundance of each OTU in each sample and the taxonomy of these OTUs. OTUs containing less than 0.001% of total sequences across all samples were discarded. To minimize the difference in sequencing depth across samples, an averaged, rounded rarefied OTU
Statistical analysis and data visualization. One-way analysis of variance (ANOVA) was used to test the effects of different fertilization treatments on soil properties. ANOVA was also used to test the soil nematode abundance and diversity (identified by the morphological method) under different treatments with SPSS 22.0 software (SPSS IBM Inc., USA), followed by the least significant difference test (LSD, \(P<0.05\)). The difference in the total soil faunal community among the five fertilization treatments \((n=15)\) was tested by the Adonis test, which was verified by the R 4.0.2 ‘vegan’ package\(^{64}\). A heatmap \((n=15)\) was plotted using heatmap tools in the Genescloud platform (https://www.genescloud.cn). The tool was developed from the ‘pheatmap’ package \((V 1.0.8)\), which was slightly modified to improve the layout style. The top 40 abundant soil faunal taxa among the five fertilization treatments were normalized by z scores and transformed using log10. The clustering method was complete (default), and the list of distances was Euclidean (default). Biomarkers for soil faunal taxa between the five fertilization regimes were found by LEfSe analysis, which was conducted using a free online platform for data analysis (http://www.cloud.biomicroclass.com/CloudPlatform/home). The alpha value of the Kruskal–Wallis test was set to 0.05, and the LDA threshold was set to 2.

Two-way analysis of variance (ANOVA) was used to test the effects of crop straw return and P addition on soil faunal diversity. The characteristics of the soil faunal community were described using the following indices, which were calculated using Excel 2016 (Microsoft Inc., USA) and R 4.0.2: (1) Shannon–Wiener index \((H')^{69}\), \(H' = \sum_{i=1}^{S} P_i \ln P_i\), where \(P_i\) is the proportion of the community represented by OTUs and \(S\) is the number of OTUs; (2) Margalef richness index \((D)^{66}\), which is an indicator of species richness, \(D = (S − 1)/\ln N\), where \(N\) is the total number of individuals of all taxa observed and \(S\) is the number of OTUs. (3) Pielou evenness index \((J)'\), which represents the degree of uniformity, \(J = H'/\ln S\). The bar graph (Fig. 3) was drawn using Origin 2019 (Origin Lab Inc., USA). (4) species number \((S)\), which is the number of species\(^{68}\). The correlations between the soil faunal diversity \((D, J, S, H, H')\) and soil properties \((\text{NO}_3^-\text{N}, \text{NH}_4^+\text{N}, \text{TP}, \text{AR}, \text{TDN, TN, AK, TK, DOC, WC, pH and SOM})\) were analyzed by Pearson correlation coefficients. The species number \((S)\) was transformed using log \((x)\). The analysis was performed by the gene cloud tools, a free online platform for data analysis (https://www.genescloud.cn).

Redundancy analysis (RDA) was used to analyze the relationships between the soil fauna relative abundance and soil fertility variables. The 40 most abundant taxa were selected, and their relative abundance was transformed using log \((x + 1)\) and center transformation. Soil environmental variables were selected by a forward selection procedure to obtain the best explained variables of the soil faunal distribution and were used in the analysis. The statistical significance of the RDA was assessed by the Monte Carlo permutation test \((P<0.05)\) using CANOCO 5 (Microcomputer Power Inc., USA).

We constructed a structural equation model (SEM) with the specification of a conceptual model of hypothetical relationships, assuming that the SOM and P addition alter soil SOM, TP, AR, TN, TDN, WC, and NO\(_3^-\text{N}\), which, in turn, affect the soil faunal Margalef richness index \((D)\) and species number \((S)\). Amos 23 (SPSS, IBM Inc., USA) software was used for SEM analysis, and the data were fitted to the models using the ‘robust’ maximum likelihood estimation procedures. The model was assessed based on the \(x^2\) value \((P>0.05, \text{CMIN/df}<2)\), goodness of fit \((\text{GFI})\) \((\text{values}>0.8 \text{ and } <1)\), the comparative fit index \((\text{CFI})\) \((\text{values}>0.9)\), and the root square mean error of approximation \((\text{RMSEA})\) \((\text{values}<0.05)^{69}\). Then, by progressively removing nonsignificant relationships between the remaining observed variables, we inferred the final model (Fig. 6). The \(R^2\) values were obtained for each dependent matrix, representing the proportion of total variance explained by the model\(^{62}\). All significant differences were set at a level of \(P<0.05\).

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Received: 27 June 2022; Accepted: 7 November 2022
Published online: 11 November 2022

References

1. Lavelle, P. et al. Soil invertebrates and ecosystem services. *Eur. J. Soil Sci.* **42**, S3–S15 (2006).
2. Nielsen, U. N. et al. Response of belowground communities to short-term phosphorus addition in a phosphorus-limited woodland. *Plant Soil*** **391**, 321–331 (2015).
3. Nielsen, U. N., Ayres, E., Wall, D. H. & Bardgett, R. D. Soil biodiversity and carbon cycling: A review and synthesis of studies examining diversity function relationships. *Eur. J. Soil Sci.* **62**, 105–116 (2011).
4. Lu, P. et al. Composition and structure of soil fauna communities and their relationships with environmental factors in copper mine waste rock after re-vegetation. *Glob. Ecol. Conserv.* **32**, e01889 (2021).
5. Lin, D. et al. Soil fauna promote litter decomposition but do not alter the relationship between leaf economics spectrum and litter decomposability. *Soil Biol. Biochem.* **136**, 107519 (2019).
6. Shao, Y., Zhang, W., Liu, S., Wang, X. & Pu, S. Diversity and function of soil fauna. *Acta Ecol. Sin. (in Chinese)* **35**, 6614–6625 (2015).
7. Voronin, A. N. & Kotyak, P. A. Influence of different agricultural practices on the number of soil fauna and productivity of agricultural crops. *Taurida Herald Agrar. Sci.* **3**, 49–56 (2019).
8. Zhu, X. & Zhu, B. Effect of different fertilization regimes on the main groups of soil fauna in cropland of purple soil. *Sci. Agric. Sin. (in Chinese)* **45**, 911–920 (2015).
9. Islam, M. U., Guo, Z., Jiang, F. & Peng, X. Does straw return increase crop yield in the wheat-maize cropping system in China? A meta-analysis. *Field Crop Res.* **279**, 108447 (2022).
10. Cui, H. et al. Straw return strategies to improve soil properties and crop productivity in a winter wheat–summer maize cropping system. *Eur. J. Agron.* 133, 126436 (2022).
11. Wang, X. et al. Changes in soil characteristics and maize yield under straw returning system in dryland farming. *Field Crop Res.* 218, 11–17 (2018).
12. Gai, X. et al. Contrasting impacts of long-term application of manure and crop straw on residual nitrate-N along the soil profile in the North China Plain. *Sci. Total Environ.* 650, 2251–2259 (2019).
13. Wang, W. et al. Effects of different fertility-building practices on the meso-micro soil fauna communities in a black soil area. *Chin. J. Appl. Environ. Biol. (in Chinese)* 25, 1344–1351 (2019).
14. Kautz, T., López-Fando, C. & Ellmer, F. Abundance and biodiversity of soil microarthropods as influenced by different types of organic manure in a long-term field experiment in Central Spain. *Appl. Soil Ecol.* 33, 278–285 (2006).
15. Zhang, T. et al. Effects of straw returning on soil meso- and micro-arthropod community diversity in wheat–maize fields in North China. *Chin. J. Appl. Environ. Biol. (in Chinese)* 25, 70–75 (2019).
16. Yang, P., Wang, H. & Yue, J. Ecological distribution of middle-small-size soil faunas under conservation tillage and straw mulch conditions. *Res. Soil Water Conserv. (in Chinese)* 20, 145–150 (2013).
17. Zhu, Q., Zhu, A., Zhang, J., Zhang, H. & Zhang, C. Effect of conservation tillage on soil fauna in wheat field of Huang-huai-hai Plain. *J. Agro Environ. Sci. (in Chinese)* 28, 1766–1772 (2009).
18. Cao, Z. et al. Changes in the abundance and structure of a soil mite (Acari) community under long-term organic and chemical fertilizer treatments. *Appl. Soil Ecol.* 49, 131–138 (2011).
19. Li, Y., Xu, Z., Xu, H., Chen, Y. & Ruan, H. Review of the effect of fertilizer application on the soil fauna in soil ecosystems. *J. Nanjing For. Univ. Nat. Sci. Ed. (in Chinese)* 42, 179–184 (2018).
20. McGee, K. M. & Eaton, W. D. A comparison of the wet and dry season DNA-based soil invertebrate community characteristics in large patches of the bromeliad Bromelia punicea in a primary forest in Costa Rica. *Appl. Soil Ecol.* 87, 99–107 (2015).
21. Zhu, B., Wang, T., You, X. & Gao, M. Nutrient release from weathering of purplish rocks in the Sichuan Basin, China. *Pedosphere* 18, 257–264 (2008).
22. Zhu, B. et al. Measurements of nitrate leaching from a hillside cropland in the Central Sichuan Basin, China. *Soil Sci. Soc. Am. J.* 73, 1419–1426 (2009).
23. He, Y. Purple Soil of China Part II (Science Press, 2003).
24. Huang, R. et al. Responses of soil carbon pool and soil aggregates associated organic carbon to straw and straw-derived biochar addition in a dryland cropping mesocosm system. *Agric. Ecosyst. Environ.* 265, 576–586 (2018).
25. Zha, X., Dong, Z., Kuang, F. & Zhu, B. Effects of fertilization regimen on soil faunal communities in cropland of purple soil. *Acta Ecol. Sin. (in Chinese)* 33, 464–474 (2013).
26. Querner, P. & Bruckner, A. Combining pitfall traps and soil samples to collect Collembola for site scale biodiversity assessments. *Appl. Soil Ecol.* 45, 293–297 (2010).
27. Smith, M. A. et al. Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology, and collections. *PNAS* 105, 12359–12364 (2008).
28. Müller, C. A. et al. Meiofaunal diversity in the Atlantic Forest soil: A quest for nematodes in a native reserve using eukaryotic metabarcoding analysis. *For. Ecol. Manag.* 453, 117591 (2019).
29. Ding, J. et al. Effects of long-term fertilization on the associated microbiota of soil collombolan. *Soil Biol. Biochem.* 130, 141–149 (2019).
30. Oliverio, A. M., Gan, H., Wikings, K. & Fierer, N. A DNA metabarcoding approach to characterize soil arthropod communities. *Soil Biol. Biochem.* 125, 37–43 (2018).
31. McGee, K. M., Porter, T. M., Wright, M. & Hajibabaei, M. Drivers of tropical soil invertebrate community composition and richness across tropical secondary forests using DNA metasystematics. *Sci. Rep.* 10, 18429 (2020).
32. Porter, T. M. et al. Variations in terrestrial arthropod DNA metabarcoding methods recovers robust beta diversity but variable richness and site indicators. *Sci. Rep.* 9, 18218 (2019).
33. Morise, H., Miyazaki, E., Yoshimitsu, S. & Eki, T. Profiling nematode communities in unmanaged flowerbed and agricultural field soils in Japan by DNA barcode sequencing. *PLoS One* 7, e51785 (2012).
34. Drummond, A. J. et al. Evaluating a multigene environmental DNA approach for biodiversity assessment. *Gigascience* 4, 46 (2015).
35. Dopheide, A. et al. Estimating the biodiversity of terrestrial invertebrates on a forested island using DNA barcodes and metabarcoding data. *Ecol. Appl.* 29, e01877 (2019).
36. Watts, C. et al. DNA metabarcoding as a tool for invertebrate community monitoring: A case study comparison with conventional techniques. *Austral Entomol.* 58, 675–686 (2019).
37. Krist, S. Barcoding in the dark? A critical view of the sufficiency of zoological DNA barcoding databases and a plea for broader integration of taxonomic knowledge. *Mol. Phylogenet. Evol.* 69, 39–45 (2013).
38. Shao, Y. et al. Nematodes as indicators of soil recovery in tailings of a lead/zinc mine. *Soil Biol. Biochem.* 40, 2040–2046 (2008).
39. Neher, D. A., Wu, J., Barbercheck, M. E. & Anas, O. Ecosystem type affects interpretation of soil nematode community measures. *Appl. Soil Ecol.* 30, 47–64 (2005).
40. Yang, C., Ji, Y., Wang, X., Yang, C. & Yu, D. W. Testing three pipelines for 18S DNA-based metabarcoding of soil faunal diversity. *Sci. China Life Sci.* 56, 73–81 (2013).
41. Horton, D. J., Kershner, M. W. & Blackwood, C. B. Suitability of PCR primers for characterizing invertebrate communities from soil and leaf litter targeting metazoan 18S ribosomal or cytochrome oxidase I (COI) genes. *Eur. J. Soil Biol.* 80, 43–48 (2017).
42. Geisen, S., Laros, I., Vizzaccio, A., Bonkowski, M. & De Groot, G. A. Not all are free-living: High-throughput DNA metabarcoding reveals a diverse community of protists parasitizing soil metazoa. *Mol. Ecol.* 24, 4556–4569 (2015).
43. Clarke, I., J., Soubrier, J., Weyrich, L. S. & Cooper, A. Environmental metabarcodes for insects: In silico PCR reveals potential for taxonomic bias. *Mol. Ecol. Resour.* 14, 1160–1170 (2014).
44. Kitagami, Y. & Matsuda, Y. High-throughput sequencing covers greater nematode diversity than conventional morphotyping on natural cedar forests in Yakushima Island, Japan. *Eur. J. Soil Biol.* 112, 103432 (2022).
45. Juliet, W. K., Lisa, B. F., Lamers, J. P. A., Till, S. & Christian, B. Soil fertility and biodiversity on organic and conventional smallholder farms in Kenya. *Appl. Soil Ecol.* 134, 85–97 (2019).
46. Li, Q., Zhou, D. & Chen, X. The accumulation decomposition and ecological effects of above-ground litter in terrestrial ecosystem. *Acta Ecol. Sin. (in Chinese)* 34, 3807–3819 (2014).
47. Tie, L. et al. Phosphorus addition reverses the negative effect of nitrogen addition on soil arthropods during litter decomposition in a subtropical forest. *Sci. Total Environ.* 781, 146786 (2021).
48. Nottingham, A. T., Turner, B. L., Stott, A. W. & Tanner, E. V. J. Nitrogen and phosphorus constrain labile and stable carbon turnover in lowland tropical forest soils. *Soil Biol. Biochem.* 80, 26–33 (2015).
49. Xiao, Q. et al. Impact of soil thickness on productivity and nitrate leaching from sloping cropland in the upper Yangtze River Basin. *Agric. Ecosyst. Environ.* 311, 107266 (2021).
50. Zhu, X. & Zhu, B. Diversity and abundance of soil fauna as influenced by long-term fertilization in cropland of purple soil, China. *Soil Till. Res.* 146, 39–46 (2015).
51. Wei, K., Wang, J., Dong, Z., Tang, J. & Zhu, B. The combined application of organic materials and chemical fertilizer mitigates the deterioration of the trophic structure of nematode community by increasing soil N concentration. *J. Soil Sci. Plant Nutr.*, **21**, 2530–2537 (2021).

52. Kuo, S. Phosphorus. In *Methods of Soil Analysis* (ed. Sparks, D. L.) 869–919 (Soil Science Society of America, 1996).

53. Nelson, D. W. & Sommers, L. E. Total carbon, organic carbon and organic matter. In *Methods of Soil Analysis* (ed. Sparks, D. L.) 960–1010 (ASA and SSSA, 1996).

54. Lu, R. *Analysis of Soil Agro-Chemistry* (Chinese Agricultural Science and Technology Press, 2000).

55. Page, A. L., Miller, R. H. & Kenney, D. R. Chemical and microbiological properties. In *Methods of Soil Analysis* (ASA and SSSA, 1982).

56. Olsen, S. R., Cole, C. U., Watanabe, F. S. & Deen, L. A. Estimation of Available Phosphorus in Soil by Extracting with Sodium Bicarbonate (USDA Circular 939, 1954).

57. Townshend, J. L. A modification and evaluation of the apparatus for the Oostenbrink direct cottonwool filter extraction method. *Nematologica* **9**, 106–110 (1963).

58. Geller, J., Meyer, C., Parker, M. & Hawk, H. Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in a taxonomic biotic surveys. *Mol. Ecol. Resour.* **13**, 851–861 (2013).

59. Yang, T., Song, X., Xu, X., Zhou, C. & Shi, A. A comparative analysis of spider prey spectra analyzed through the next-generation sequencing of individual and mixed DNA samples. *Ecol. Evol.* **11**, 15444–15454 (2021).

60. Chen, H. & Jiang, W. Application of high-throughput sequencing in understanding human oral microbiome related with health and disease. *Front. Microbiol.* **5**, 508 (2014).

61. Magoc, T. & Salzberg, S. L. FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **27**, 2957–2963 (2011).

62. Edgar, R. C. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**, 2460–2461 (2010).

63. Altschul, S. F. et al. Gapped BLAST and PSI-BLAST a new generation of protein database search programs. *Nucleic Acids Res.* **25**, 3389–3402 (1997).

64. R Core Team. R: A language and environment for statistical computing. http://www.r-project.org (2020).

65. Shannon, C. E. A mathematical theory of communication. *Bell Syst. Tech. J.* **37**, 379–423 (1948).

66. Margalef, R. *Perspectives in Ecological Theory* (The University of Chicago Press, 1970).

67. Pielou, E. C. The measurement of diversity in different types of biological collections. *J. Theor. Biol.* **88**, 131–144 (1966).

68. Zhou, Y. et al. Species richness and phylogenetic diversity of seed plants across vegetation zones of Mount Kenya, East Africa. *Ecol. Evol.* **8**, 8930–8939 (2018).

69. Wang, H. et al. Nitrogen addition reduces soil bacterial richness, while phosphorus addition alters community composition in an old-growth N-rich tropical forest in southern China. *Soil Biol. Biochem.*, **127**, 22–30 (2018).

70. Yang, K. et al. Responses of soil ammonia-oxidizing bacteria and archaea diversity to N, P and NP fertilization: Relationships with soil environmental variables and plant community diversity. *Soil Biol. Biochem.* **145**, 107795 (2020).

71. Zhang, S., Li, Q., Lü, Y., Zhang, X. & Liang, W. Contributions of soil biota to C sequestration varied with aggregate fractions under different tillage systems. *Soil Biol. Biochem.* **62**, 147–156 (2013).

Acknowledgements

We gratefully acknowledge the support of the Biodiversity Monitoring and Research Network, CAS (Sino-BON). We thank Tao Chen for help with soil sampling. The study was financially supported by the National Natural Science Foundation of China (Grant No: U20A20107) and the National Key Research and Development Program (Grant No: 2017YFD0200105).

Author contributions

All authors contributed to the study conception and design. X.X.: Writing—Original draft preparation, reviewing and editing. X.W.: conceptualization, reviewing and editing. Z.D.: conceptualization, methodology, reviewing and editing. B.Z.: Funding acquisition and conceptualization.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-022-23883-6.

Correspondence and requests for materials should be addressed to Z.D.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2022