Effects of straw mulching practices on soil nematode communities under walnut plantation

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Agricultural management techniques such as mulching with crop straw can impact soil properties and may in turn change the structure and function of the soil food web. We investigated different straw mulching types and straw mulching coverage levels on soil nematodes community structure in walnut orchards. We set up a randomized experimental design with three straw mulch types, and three straw mulch distance treatments in a walnut plantation. The results indicated that the number of soil nematodes after straw mulching was lower than that found in the control (CK). However, the metabolic and structure footprints of the omnivore-predator nematodes showed higher values as compared to CK. The abundances of plant parasite and omnivore-predator nematodes were negatively correlated with ammonium nitrogen (NH4+-N) and dissolved organic nitrogen (DON), whereas soil moisture content (SM) had a negative correlation with the abundance of total nematodes. High structure index (SI), maturity index (MI) and low enrichment index (EI) values revealed a structured soil food web, medium soil enrichment, and fungal decomposition channel under the mix straw mulching treatments. Soil nematodes should be used as an indicator of soil functional changes resulting from straw mulching.

Crop straw has become an effective way to supplement soil nutrients and increase crop yield in modern agriculture because it is rich in various nutrients and physiologically active substances1. Straw mulching has important ecological significance for maintaining farmland fertility, reducing the use of chemical fertilizers, improving the carbon sink capacity of terrestrial soil, promoting the soil nitrogen cycle2,3, and reducing or avoiding environmental pollution caused by burning4.

In recent years, most studies of straw mulching have mainly focused on the physical, chemical and biological effects of soil and the physiological and ecological responses of mulched tree species to yield5–7. However, there is little research on straw mulching technology, and it has generally been performed on areas with extensive tree cover or in gardens; fine straw mulching technology has not been studied. In addition, straw mulching is mainly concentrated on food crops, and there are few studies of straw mulching in orchards. For walnut orchards, traditional management practices such as clean tillage cause serious soil erosion and reduced soil fertility, resulting in slow growth of walnut trees and reduced yield8. Therefore, it is necessary to consider using straw mulching to improve the sustainable development of walnut orchards.

With the growth of young walnut trees, the canopy width increases each year, so it is reasonable to designate the canopy radius at the coverage distance, considering the effect of canopy shading. In addition, we have previously reported that suitable straw mulching materials can promote the growth of walnut trees and increase the

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Therefore, we hypothesized that different straw mulching treatments could increase the number of food for soil nematodes, thereby increasing soil nematode numbers and improving soil nematode community. Because straw is rich in nutrients and active substances, nutrients such as C and N can be released under straw mulching treatment affecting the structure of soil nematode communities, biodiversity and function. Because straw is rich in nutrients and active substances, nutrients such as C and N can be released under straw mulching treatment affecting the structure of soil nematode communities, biodiversity and function. We also hypothesized that mixed-straw mulching would increase nutrient availability and improve soil fertility compared with rice straw mulching and rapeseed straw mulching because it would have a more suitable C/N ratio and faster degradation rate. Mixed-straw mulching would increase nutrient availability and improve soil fertility compared with rice straw mulching and rapeseed straw mulching because it would have a more suitable C/N ratio and faster degradation rate. Potential yield. Therefore, it is an important step for the sustainable management of walnut orchards to determine suitable materials for soil mulch and location for straw mulch placement. Potential yield. Therefore, it is an important step for the sustainable management of walnut orchards to determine suitable materials for soil mulch and location for straw mulch placement.

Soil nematodes are one of the most abundant metazoans on the earth. They exist widely in various habitats and play an important role in maintaining the stability of soil ecosystems, promoting material circulation and energy flow. Nematodes are simple to extract and identify, and feed on diverse nutrient resources, making them very sensitive to agricultural management measures and environmental changes; thus, they can be used as indicators of soil quality and health. Previous experiments showed that the application of straw and other organic fertilizers could increase the number of beneficial soil nematodes and decrease the number of phytophagous soil nematodes. However, little information is available about whether and how the microenvironment soil conditions under straw mulching affect the structure of soil nematode communities, biodiversity and function in walnut orchards.

The major objectives of this study were to explore whether and how straw mulching affects the soil nematode community. Because straw is rich in nutrients and active substances, nutrients such as C and N can be released into the soil by a degradation pathway after straw mulching a walnut orchard, which provides a rich source of food for soil nematodes, thereby increasing soil nematode numbers and improving soil nematode community structure. Therefore, we hypothesize that different straw mulching treatments could increase the number of nematodes and improve the community structure of soil nematodes. We also hypothesize that mixed-straw mulching would increase nutrient availability and improve soil fertility compared with rice straw mulching and rapeseed straw mulching because it would have a more suitable C/N ratio and faster degradation rate.

**Results**

**Soil environmental conditions.** Mix straw mulching treatments significantly correlated dissolved organic carbon (DOC) ($p < 0.05$) and NO$_3$–N content ($p < 0.05$) (Table 1). In general, mix straw mulching and a cover distance of n (Mix-n) had a higher content of DOC and NO$_3$–N than the other treatments (i.e., single-straw mulching). Meanwhile, the soil DOC content of the Mix-n treatment was significantly higher than that of the CK treatment. Though the content of NO$_3$–N was higher in the Mix-n treatment than in the CK treatment, this difference was not significant. However, the soil pH, SMC and NH$_4$–N responses to different straw mulching treatments were not significant ($p > 0.05$).

**Soil nematode communities.** The number of nematode genera in Rice-n, Rice-1.5n, Rice-all n, Rape-n, Rape-1.5n, Rape-all n, Mix-n, Mix-1.5n, Mix-all n and CK were 34, 37, 31, 30, 26, 31, 30 and 29, respectively (Appendix 1). Compared with those in other straw mulching treatments (rice straw, rapeseed straw, and mix straw), the total nematode genera were found to be significantly ($p < 0.05$) more numerous in the control treatment (CK) (Table 2). The control treatment (CK) and mix-all n treatment had significantly ($p < 0.05$) higher abundances of fungivores than the Rapeseed-n and Rapeseed-1.5 n treatments. Two Rice straw treatments (those with cover distances of n and all n) and one Rapeseed straw treatment (cover distance of 1.5 n) had significantly ($p < 0.05$) lower abundances of plant parasites than the CK treatment. However, there were no significant differences among the different treatments in the abundance of omnivore-predator nematodes (Table 2).

| Treatment | DOC mg kg$^{-1}$ | DON mg kg$^{-1}$ | NH$_4$–N mg kg$^{-1}$ | NO$_3$–N mg kg$^{-1}$ | pH | SM (%) |
|-----------|----------------|-----------------|---------------------|--------------------|----|--------|
| Rice-n    | 73.84 ± 0.72ab| 11.15 ± 0.52a   | 2.67 ± 0.23b        | 51.25 ± 5.46de     | 6.02 ± 0.27ab | 24 ± 1.15a |
| Rice-1.5n | 70.31 ± 1.04b | 9.40 ± 0.60a    | 3.63 ± 1.11ab       | 38.35 ± 1.79ec     | 5.97 ± 0.15bc | 21.33 ± 0.33b |
| Rice-all n| 75.12 ± 1.36ab| 10.79 ± 1.02a   | 4.2 ± 0.30abc       | 66.69 ± 11.15abcd  | 6.47 ± 0.35abc | 21.33 ± 0.33b |
| Rape-n    | 74.44 ± 0.59ab| 10.44 ± 0.83a   | 4.58 ± 0.57ab       | 90.31 ± 9.77ab     | 6.49 ± 0.05abc | 22.66 ± 1.2ab |
| Rape-1.5n | 70.31 ± 0.39b | 10.00 ± 0.60a   | 4.78 ± 0.50a        | 86.34 ± 11.59abc   | 6.3 ± 0.12abc  | 21.33 ± 0.33b |
| Rape-all n| 71.48 ± 1.72b | 10.24 ± 1.02a   | 4.16 ± 0.54ab       | 61.22 ± 1.52cde    | 6.58 ± 0.14abc | 21.33 ± 0.33b |
| Mix-n     | 79.94 ± 4.57a | 11.39 ± 0.97a   | 3.1 ± 0.25ab        | 93.56 ± 6.14a      | 6.54 ± 0.23abc | 22.33 ± 0.66ab |
| Mix-1.5n  | 70.79 ± 1.67b | 9.68 ± 0.09a    | 3.26 ± 0.60ab       | 64.97 ± 9.67bcd    | 6.79 ± 0.15a   | 21.66 ± 0.88ab |
| Mix-all n | 75.79 ± 2.07ab| 11.69 ± 0.41a   | 3.61 ± 0.68ab       | 73.84 ± 9.91abcd   | 6.46 ± 0.12abc | 22 ± 0.57ab |
| CK        | 71.12 ± 0.75b | 9.93 ± 1.04a    | 3.74 ± 0.20abc      | 81.68 ± 8.35abc    | 5.83 ± 0.38bc | 20.33 ± 0.88b |

Table 1. Overview of main effect of straw mulching quality and distance on environmental factors based on ANOVA. Data are the means of three replicates ± SD. Within each column, the values with the same lower case letter are not significantly different. Different letters indicate statistically significant differences between treatments, according to the Duncan's multiple range test ($p < 0.05$). CK is no straw mulching treatment.
Table 2. The abundances of total soil nematodes and trophic groups (means ± standard errors, n = 3) as affected by straw mulching treatments. Different letters indicate statistically significant differences between treatments, according to the Duncan’s multiple range test (p < 0.05).

| Treatment       | Total nematode number/100 g dry soil | Bacterivore number/100 g dry soil | Fungivore number/100 g dry soil | Plant parasites number/100 g dry soil | Predator and omnivore footprint/100 g dry soil |
|-----------------|-------------------------------------|-----------------------------------|--------------------------------|--------------------------------------|-----------------------------------------------|
| Rice-n          | 178.1 ± 24.8de                      | 69.1 ± 1.9b                       | 48.8 ± 11.6abc                 | 19.0 ± 0.9b                          | 41.0 ± 12.2a                                  |
| Rice-1.5n       | 292.2 ± 18.8bc                      | 99.5 ± 9.3b                       | 89.7 ± 43.5abc                 | 61.0 ± 25.9ab                         | 41.8 ± 17.0a                                  |
| Rice-all n      | 199.9 ± 7.4de                       | 54.2 ± 17.7b                      | 73.9 ± 3.1abc                  | 16.3 ± 3.9b                          | 55.4 ± 10.7a                                  |
| Rape-n          | 168.8 ± 11.2e                       | 71.4 ± 13.4b                      | 18.6 ± 12.3b                   | 43.1 ± 6.6ab                          | 35.5 ± 9.0a                                   |
| Rape-1.5n       | 167.7 ± 17.6e                       | 83.2 ± 10.0b                      | 26.4 ± 11.2b                   | 19.2 ± 7.1b                          | 38.8 ± 6.6a                                   |
| Rape-all n      | 286.9 ± 41.6bc                      | 88.2 ± 32.3b                      | 26.4 ± 11.2b                   | 19.2 ± 7.1b                          | 38.8 ± 6.6a                                   |
| Mix-n           | 256.6 ± 32.2bcd                     | 96.1 ± 24.9b                      | 55.9 ± 28.6abc                 | 52.7 ± 9.8ab                          | 51.7 ± 9.4a                                   |
| Mix-1.5n        | 210.2 ± 38.3cde                     | 60.4 ± 7.1b                       | 51.1 ± 32.7abc                 | 41.5 ± 8.7ab                          | 57.0 ± 7.0a                                   |
| Mix-all n       | 300.3 ± 31.4b                       | 81.7 ± 28.3b                      | 115.4 ± 9.8a                   | 40.2 ± 5.3ab                          | 62.9 ± 17.3a                                  |
| CK              | 410.7 ± 8.1a                        | 174.1 ± 13.3a                     | 74.3 ± 35.5a                   | 43.2 ± 4.4a                          |                                               |

Table 3. Soil nematode metabolic footprints (μg C kg⁻¹ soil) (means ± SE). Different letters indicate statistically significant differences between treatments, according to the Duncan’s multiple range test (p < 0.05).

| Treatment       | tfootprint | sfootprint | PP footprint | FF footprint | BF footprint | OP footprint |
|-----------------|------------|------------|--------------|--------------|--------------|--------------|
| Rice-n          | 4.93 ± 1.83a | 10.82 ± 3.05c | 3.63 ± 1.40a | 2.21 ± 0.13ab | 7.38 ± 2.11ab | 12.95 ± 4.03c |
| Rice-1.5n       | 5.72 ± 1.56a | 18.29 ± 4.17abc | 4.93 ± 1.40a | 2.62 ± 1.04ab | 7.55 ± 1.72ab | 23.89 ± 7.58bc |
| Rice-all n      | 4.43 ± 0.94a | 21.89 ± 7.11abc | 5.02 ± 3.18a | 3.12 ± 0.34ab | 5.15 ± 0.89b | 33.7 ± 15.1abc |
| Rape-n          | 5.29 ± 0.70a | 18.20 ± 1.30abc | 6.36 ± 1.21a | 1.07 ± 0.42b | 9.29 ± 3.22ab | 28.15 ± 0.35abc |
| Rape-1.5n       | 8.75 ± 5.21a | 30.48 ± 6.44abc | 5.83 ± 4.17a | 1.68 ± 0.55abc | 13.74 ± 5.44a | 48.92 ± 11.55abc |
| Rape-all n      | 4.00 ± 0.44a | 21.15 ± 1.34abc | 5.36 ± 3.73a | 3.57 ± 0.66a | 4.11 ± 0.88b | 34.09 ± 4.34abc |
| Mix-n           | 2.85 ± 1.30a | 20.09 ± 5.92abc | 6.28 ± 2.09a | 1.83 ± 0.50ab | 7.40 ± 1.88ab | 26.63 ± 7.94abc |
| Mix-1.5n        | 2.80 ± 1.73a | 34.14 ± 6.07a  | 7.82 ± 4.26a  | 2.14 ± 1.08ab | 5.49 ± 0.67b | 55.42 ± 11.30a |
| Mix-all n       | 5.54 ± 1.37a | 10.57 ± 3.10abc | 7.05 ± 3.15a  | 3.43 ± 0.49a | 6.10 ± 1.99ab | 11.11 ± 3.47c |
| CK              | 7.17 ± 2.70a | 16.67 ± 4.31abc | 3.52 ± 1.73a  | 2.51 ± 0.75ab | 9.09 ± 1.28ab | 26.80 ± 8.82abc |
| p value         | 0.79        | 0.048       | 0.986        | 0.245        | 0.279        | 0.037        |
| F               | 0.59        | 2.421       | 0.231        | 1.419        | 1.339        | 2.59         |

The Rapeased-1.5n and Mix-1.5n treatments had significantly higher omnivore-predator footprints and structure footprints than the Rice-n and Mix-all n treatments (p < 0.05) (Table 3). The metabolic footprints of fungivores were higher under Rapeased-all n and Mix-all n than under the other treatments, while the metabolic footprint of bacterivores had greater values in the Rapeased-1.5n treatment than in other treatments. There was no obvious change in the plant parasite footprint or the enrichment footprint among different treatments.

Soil nematode faunal profile. The soil food webs of the straw mulching treatments were plotted along their respective SI and EI trajectories in Fig. 1. A discernible pattern was found in the nematode faunal profile of different straw mulching treatments. The nematode fauna analysis showed that the all different coverage distance of the rice straw treatment and the coverage distances were n and 1.5n of the rapeseed straw treatment was distributed in the B quadrant (Fig. 1A,B). While, when the coverage distance was increased to cover the whole plot (all n) of the rapeseed straw treatment, it was distributed in the C quadrant. For the mix straw treatment, all coverages distances were distributed in the C quadrant. The variation in SI value increasing coverage distance is not obvious. EI value tends to decrease with increasing coverage distance, and the distribution of the straw mulching treatments gradually approaches the C quadrant level from the B quadrants (Fig. 1).

Nematode diversity. Significant differences were observed in the basal index (BI), channel index (CI), and maturity index (MI) between the different straw mulching treatments. MI and CI were significantly (p < 0.05) higher in the mix straw mulching treatment than in the CK treatment, especially in the mixed treatment with straw mulching distance at 1.5n (Fig. 2a–c). For all straw treatments, BI and CI were higher for the whole plot mulching (all n) than for the other mulching distances (n, 1.5n). Moreover, there were no fluctuations among different treatments in terms of the Shannon–Weaver index (H), Species richness index (SR), Trophic diversity index (TD) or Pielou’s evenness index (J) (Fig. 2d–g).
Environmental factors affecting soil nematode community variability. Under different straw mulching treatments, DON had a significant ($p < 0.05$) negative correlation with plant parasite nematodes (Table 4). NH$_4^+$–N was significantly ($p < 0.05$) negatively correlated with omnivore-predator nematodes. SM had a significant ($p < 0.05$) negative correlation with the total nematode abundance. However, DOC, NO$_3^-$–N, and pH had no significant correlations with the soil nematode communities (Table 4).

**Discussion**

**Soil environmental conditions.** It is clear that the Mix-n treatment had higher DOC and NO$_3^-$–N than the other treatments under all soil environmental conditions. Due to the different C/N ratios of the different straw types, N degradation and mineralization were also different. The change in soil nutrients caused by straw mulching is mainly due to the role of soil organisms. Therefore, we can explain the difference in soil nutrients by the soil biological composition of different straw mulching treatments. In general, the specific genus of soil nematode in the mix treatment can characterize the particular soil nutrient status. Previous studies have shown that some nematodes are found more often in areas with similar environmental variables and that nematode genera within the same trophic group responded differently to environmental variables. We found that the higher abundances of *Prismatolaimus*, *Cephalobus* and *Eucephalobus* corresponded to the higher soil NO$_3^-$–N (Appendix 1). Our results are consistent with the observations of Song et al. Moreover, the Mix-n treatment had a higher density of *Mesodorylaimus*, *Aphelenchoides* and *Thonus* where the DOC was higher. This result is in agreement with the findings of Olatunji et al., in which *Thonus*, *Aporcelaimus*, *Mesodorylaimus*, *Aphelenchoides*, *Criconemoides*, *Tylenchus*, and *Rhabditidae* were positively associated with DOC.

**Figure 1.** Distribution map of soil nematode flora under different straw mulching treatments (A representing rice straw pattern; B representing rape straw pattern; C representing mixed straw pattern).
Figure 2. Changes of soil nematode ecological index under different straw. (a) Effect of straw mulching on Basal index, (b) channel index, (c) maturity index, (d) nematode channel ratio, (e) Shannon–Weaver index, (f) Pielou's evenness, (g) species richness index, (h) trophic diversity index; means ± S. (p < 0.05).
control. Blankinship et al. used a meta-analysis method to study the response of soil nematodes to tempera-
any straw mulch treatment; that is, the number of soil nematodes after straw mulching was lower than that in
the total number of soil nematodes and a higher abundance of soil nematodes in different nutritional groups than
metabolic activity and abundance of omnivore-predator nematodes; omnivore-predator nematodes consume

Soil nematode communities. From the data in Table 2, it is apparent that the CK treatment had a higher
total number of soil nematodes and a higher abundance of soil nematodes in different nutritional groups than
any straw mulch treatment; that is, the number of soil nematodes after straw mulching was lower than that in the
control. Blankinship et al. used a meta-analysis method to study the response of soil nematodes to tempera-
ture increase under different ecosystem types. It was found that soil nematodes were mainly affected by annual
precipitation. When annual precipitation exceeded 626 mm, the increase in temperature had a positive effect
on the number of soil nematodes. In this study, the annual precipitation in this area (1033.9 mm) exceeded
626 mm, and straw mulching had a cooling effect during the growth period of young walnut trees. This could be
a possible reason of higher abundance of soil nematodes in the CK treatment than that in any straw mulching

treatment. Moreover, this finding is also contrary to our first hypothesis that different straw mulching treat-
ments would increase the number of soil nematodes. The reasons are as follows: on the one hand, phenolic acids
enter the soil through the secretions of walnut roots and the decomposition of a large amount of straw residues,
which results in an increase in phenolic acids in the soil and a decrease in the total number of soil nematodes
and other nematodes. On the other hand, straw mulching returns pathogenic bacteria and parasite eggs to the

In addition, a key finding was that fungal nematodes were more common than bacterial nematodes in the
treatments with complete mulch coverage than in the n and 1.5n coverage treatments. When rice straw, rapeseed straw and mix straw were applied at n and 1.5n distances, the decomposition pathway was a bacterial channel;
when the coverage distance increased to all n, the decomposition pathway gradually changed to decomposi-
tion equally distributed between bacterial and fungal decomposition pathways. In contrast, the CK treatment
was dominated by the number of bacterivorous nematodes, suggesting that the bacterial channel was the main
pathway of decomposition, which was consistent with the result of the distribution map of nematode fauna in
Fig. 1. At the same time, this result indicates that the coverage distance changed the dominant community of
nematode trophic groups.

The footprints of different nematode trophic groups are proxies for the carbon or energy flow entering the
soil food web through their respective channels. In our study, we found that the footprint and the carbon bio-
mass of the omnivore-predator nematodes and all structure metabolic footprints showed higher values under
all straw mulching treatments compared with those of the other soil nematode trophic groups (Table 3). This
observation may be explained by the predator–prey trophic cascade effect: straw mulching stimulates higher

Nematode diversity. The maturity index of nematodes is one of the key indices of soil health. In our study,
the MI values for rice straw and rapeseed straw treatment alone were not significantly higher than those for the
CK treatment (Fig. 2c). However, the MI values for the mix straw treatments were significantly higher than those
for the CK treatment, indicating that the structure of the nematode community is stable and that the complexity
of the soil food web could increase under the mix straw treatment.

Combined with the ecological indices BI, which is related to soil properties and decomposition pathways,
we found that higher CI value for the three straw mulching treatments appeared in the whole-plot coverage treat-
ments (all n). Our results contrast with those of other studies, which found that bacterial-dominated decomposi-
tion pathways were the most common pathways. This discrepancy could be explained mainly by the observed
variations in the abundances of bacterivores and fungivores among the different coverage distances. Specifically,
bacterivore nematodes predominate in different soil nematode trophic groups when the coverage distance is n,
while bacterivore nematodes and fungivore nematodes predominate in different soil nematode trophic groups
when the coverage distance is increased to all n (Table 2). In addition, soil nematode decomposition pathway
changed with the increase in coverage distance in the three straw mulching treatments, which may have been
caused by the increase in contact area between straw and soil. The specific mechanism needs to be further studied
in our next work.

| Factor | Total | BF | FF | PP | OP |
|--------|-------|----|----|----|----|
| DOC    | .058  | .087| -.073| -.010| .239|
| DON    | -.087| -.037| .083| -.417*| .124|
| NH4-N  | .005  | .104| .094| -.057| -.365*|
| NO3-N  | -.017| .074| -.126| -.018| .071|
| pH     | -.275| -.279| -.096| -.334| -.180|
| SM     | -.412*| -.237| -.337| -.238| -.024|

Table 4. Relationships between nematode abundances and environmental factors based on Pearson correlation. *p<0.05.
Soil nematode faunal profile. The SI is considered to indicate the structure of the soil food web response to disturbance and during remediation, while the EI reflects soil food web responses to available resources and the resource response to the primary decomposers17,25.

In the present study, the rice straw mulching treatments and rapeseed straw mulching treatments with high EI and SI values at different straw mulching distances were in quadrant B, indicating that the structure of the soil food web was fairly mature, the N concentration was high, the C/N ratio was low, the decomposition pathways of fungi and bacteria was balanced, and the disturbance level of the soil environment was low to moderate. These conditions occurred is mainly because of the large amounts of dissolved organic carbon and dissolved organic nitrogen in the soil due to straw degradation and the straw mulching water retention effect making the soil moisture content higher than that found in the CK treatment (Table 1).

However, the mix straw mulching treatments with high SI and low EI values at different straw mulching distances were in quadrant C, which indicates a structured food web, medium soil enrichment, a moderately high C/N ratio, fungal decomposition channels, and no disturbance. Our previous research suggested that the mix straw mulching treatment had a moderate carbon nitrogen ratio (C:N) and that mix straw degrades more quickly than rice straw or rapeseed straw9. In addition, the mix straw may have provided stable moisture content and higher dissolved organic carbon and dissolved organic nitrogen than rice straw or rapeseed straw (Table 1), thus increasing nutrient availability and soil fertility levels. This result is supported by other agricultural management practices26,28,29. This evidence supported our hypothesis that the mix straw mulching treatment led to a more stable soil food web and higher soil fertility levels.

Environmental factors affecting soil nematode community variability. Straw mulching directly increases the mineral nitrogen and DON contents in the soil through decomposition, which significantly increases the content of nitrogen in the soil, thus increasing the amount of soil nutrients and soil organisms. Plant parasite and omnivore-predator nematode abundances were negatively correlated with NH4+–N and DON contents, but there was no significant correlation between the nematode community and soil DOC content. This finding indicates that nitrogen in the soil of the agroforestry ecosystem had a more significant impact on the nematode community than carbon. This result is also consistent with previous results28,29. Another possible explanation was that ammonium toxicity may occur when soil nematodes feed on root fluid, resulting in a negative correlation between the nematode community and soil DON contents. This result was unexpected and suggests that nitrogen in the soil of agroforestry ecosystems had a more significant impact than soil carbon on the nematode community and the soil DOC content. This finding was unexpected and suggests that nitrogen in the soil of agroforestry ecosystems had a more significant impact than soil carbon on the nematode community and the soil DOC content. Recommendations for sustainable walnut orchard management based on the complexity and stability of soil food webs should advocate the use of mix straw mulching (mix) covering the whole plot (all n) and thus promote the accumulation of soil dissolved organic nitrogen and carbon nutrients.

Materials and methods
Experimental site. The study was conducted in a large walnut orchard field in Langzhong (31° 57′ 82″ N, 105° 96′ 63″ E; 712.5 m above sea level), which is the hilly area of the central Sichuan Basin, southwestern China. The area has a humid mid-subtropical monsoon climate, with an average annual precipitation of 1033.9 mm and an annual temperature of 18.7 °C. This site has purple soil, classified as Pup-Orthic Entisol in the Chinese Soil Taxonomy (CST) and Entisol in the USDA Soil Taxonomy31. The specific soil in this study was a loam soil with the following nutrient profile (0–15 cm depth): total nitrogen (2.4 g kg−1), available phosphorus (0.96 g kg−1), available potassium (86.57 mg kg−1) and total carbon (5.95 g kg−1)32.

Experimental design. The walnut sapling (Juglans regia) plantation covered a 30 m × 90 m area, with a southerly slope of c. 2.5 degrees. The walnut saplings were planted in April 2010 and then grafted in May 2015.

In July 2016, we established a straw mulching experiment with a randomized block design in a walnut plantation with 3 m × 3 m spacing to investigate the potential effect of straw mulching on nematode community abundance and diversity and the associated agroecosystem function. We selected three different straw mulch types, rice straw, rapeseed straw, and mix straw (of equal quality, mixed 1:1), as the main plot. Then, under different main plots, we set up three different straw mulching distances (covering the mean radius of the crown width (n), covering 1.5 times the mean radius of the crown width (1.5 n) and covering the whole experimental plot (all n)) as sub-plots. Plots with no straw mulching were used as the CK plots. The quantity of straw mulch in each treatment was 3 kg m−2, selected based on previous research results25,26. There were a total of 10 treatments, based on the three straw mulching types, the three different straw mulching distances and CK, and each treatment had three replicates. All treatments were subjected to random permutations.
Soil sampling and property analysis. Soil samples were collected on 19 October 2016. Five soil samples from the 0–20 cm soil layer were taken with a soil auger (ø = 2.5 cm) by using the five-spot method. The samples were combined to form one composite sample per plot location. Each composite sample was sieved (2 mm) and stored in individual plastic bags, immediately transferred to a cold room with a temperature of 4 °C, and then processed within a week. The samples used to analyze pH, SMC, NH₄⁺–N, NO₃⁻–N, DON and DOC were air-dried at room temperature.

Analysis of soil physicochemical properties. Soil moisture content was estimated gravimetrically by oven drying 20 g of each field composite soil sample at 105 °C for 24 h. Soil pH was determined with deionized water and an air-dried and fine-ground sample at a ratio of 1:2.5 (weight to volume, w/v) with an electronic pH meter. Soils were extracted with 2 M KCl, and the filtrate was analyzed for NH₄⁺–N and NO₃⁻–N content (with an Acquity Ultra-Performance Liquid Chromatograph, AA3, Bran + Luebbe, Germany). Dissolved organic carbon (DOC) and nitrogen (DON) were estimated using a TOC/TN analyzer (Multi N/C 2100(S), Analytik Jena AG, Germany).

Nematode extraction and identification. Soil nematodes were extracted from 50 g of fresh soil using a modified cotton-wool filter method. The extractions were used for identification (at least 100 nematodes) at the genus level using a microscope (OLYMPUS BX51) at 100× magnification (resolution: 0.25 μm) according to Ahmad et al. If fewer than 100 nematodes were observed in one sample, all specimens were identified. Nematode abundance was adjusted according to soil moisture and was expressed as the number of nematodes per 100 g dry soil. After identification (within one week), based on their feeding habits, nematodes were classified into four trophic groups: (1) bacterivores (Ba), (2) fungivores (Fu), (3) omnivores-predators (OP) and (4) plant parasites (PP). The assumed effects of straw mulching on soil nematodes were examined with the following variables: (1) total nematode abundance; (2) abundance of individual trophic groups including PP, Ba, Fu, OP; (3) Shannon–Weaver index (H'); (4) Pielou's evenness index (J); (5) maturity index (MI); (6) trophic diversity index (TD); (7) species richness index (SR); (8) and (9) basal index (BI).

The metabolic footprint approach uses existing data on nematode biovolumes and growth rates, and the weighting used in the enrichment index (EI), structure index (SI), and channel index (CI) calculations to estimate the C metabolism of the nematode community. The nematode metabolic footprints (NMF) was also divided into the enrichment footprint (efootprint), representing higher trophic levels (c-p 3–5), and the structure footprint (sfootprint), representing higher trophic levels (c-p 1–2). The above data were calculated using the online Nematode Indicator Joint Analysis (NINJA) tool.

Data analysis. The nematode abundances were ln (x + 1) transformed prior to statistical analysis for the normality of data. One-way ANOVA was used to test the effect of straw mulching on soil properties, nematode abundance, and nematode ecological index under each treatment. Correlation analyses between abiobiotic and biotic drivers, including pH, SMC, NH₄⁺–N, NO₃⁻–N, DON and DOC nematode community data, were conducted. Statistical significance was determined at p < 0.05. Differences between data means were analyzed with t-tests using SPSS v. 17.0 (SPSS Inc., Chicago, IL) statistical software. Least significant difference (LSD) was used to test for differences among treatment means.

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Author contributions

S.D. and K.P. designed the study including experimental design. S.D., A.T. and W.C. carried out field and laboratory work. S.D. and A.T. analysed the data and drafted the manuscript. K.P., Y.R., A.T., E.Z. and X.S contributed to revise the draft. A.Z. and W.C. contributed reagents, materials, analysis tools.

Competing interests

The authors declare no competing interests.

Additional information

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