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Litter additions reduce the side effects of biocides on soil nematode communities in *Illicium verum* forest

Y. LÜ[1,*,#], W. F. XUE[2,*,#], P. P. YAN[1]

[1]China Certification & Inspection Group LiaoNing Co.Ltd., Dalian116039, Liaoning, China,*E-mail: *jzlvying@163.com*;
[2]Technique Center of Dalian Customs, Dallian, 116000, Liaoning China, *E-mail: *xwf526@163.com*

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Summary

The application of biocides may create unintended consequences on soil biota and ecosystem stability. The inputs of organic matter can increase biocides adsorption and reduction of non-target organisms influence. A field experiment was conducted to study the changes of soil abiotic and nematode communities resulting from biocides application in non-litter-added and litter-added soils in *Illicium verum* forest. Our results showed that litter addition could change the responses of soil nematodes to biocides. The influence of fungicide was evident mainly in litter-added plots in which it increased nematode abundance. Insecticide and its interaction with fungicide significantly decreased the diversity index and the abundance of omnivores-predators and herbivores in non-litter-added plots. While, insecticide had little effect on nematode diversity and abundance in litter-added plots. Litter addition may help to maintain the structure and stability of soil food web and result in bacteria dominant decomposition pathway. Our results suggest that litter addition may be a critical factor for maintaining soil ecosystem stability when biocides are applied in *Illicium verum* forest.
Keywords: Soil nematodes; soil food web; insecticide; fungicide; litter

Introduction

Biocides are employed in many parts of the world for protection of crops from pests and diseases. However, biocides may exert toxicity to organisms other than their intended targets (Komárek *et al.*, 2010; Rahmat *et al.*, 2019; Ju *et al.*, 2017). Intensive and repeated biocides inputs can threaten endangered species, reduce biodiversity, inhibited bacterial and fungal growth, and impact on soil quality (Fernández-Calviño *et al.*, 2021). Previous studies have found that biocides adsorption positively correlated with contents of clay and organic matter (Komárek *et al.*, 2010; Chelinho *et al.*, 2011). Although organic matter plays a crucial role in decreasing adverse effects of biocides (Agegnehu *et al.*, 2017), the insights into the degree and consequence of organic matter influence on biocides have not been well studied. Previous studies advocated the use of soil nematode parameters to assess ecological risk (Yang *et al.*, 2017) and to monitor environmental safety (Wilson 2009), because they can rapidly reflect changes in soil, and provide accurate data to indicate soil quality (Chagnon *et al.*, 2018). Therefore, soil nemaotdes can be used as potential indicators to estimate the influence of organic matter on the effects of biocides on soil ecosystem.

Star anise (fruit of *Illicium verum Hook. f.*) is an important traditional medicine as well as a commonly used spice in China (Itoigawa *et al.*, 2004). It is also the industrial source of shikimic acid, a major component of drug for the bird flu H5N1 strain of virus (Itoigawa *et al.*, 2004; Wang *et al.*, 2011). Nearly 90 % of star anise production in China is from Guangxi province (Liu 2012). With the devel-

* – corresponding author
# – authors contributed equally to this paper

opment of anise production, the plant diseases and insect pests have attracted more and more attention, and biocides have been applied as an effective method to reduce the pest and disease damage as well as maintain the anise productivity (Liu & Nong 2005). However, our knowledge about the influence of biocides on non-target organisms was limited, especially when they combined with litter additions. The objectives of the present study are to evaluate changes in the soil nematode communities influencing by fungicide and insecticide with and without litter additions, to estimate the litter addition as an alternative management to resist the side effects of chemical biocides.

Materials and Methods

Experimental design

The experiment took place in Nanning, Guangxi province, China (107°45'-108°51'E, 22°13'-23°32' N). The annual mean temperature and precipitation of this region are 21.6 ˚C and 1304 mm, respectively. The soil is classified as red soil (Udic Ferrosols) according to the Chinese Soil Taxonomy Classification (Gong, 1994). This experiment was arranged as a split-plot with different biocides treatments as main plots, and non-litter added and litter added treatments as subplots. Each plot has three replications. Within each plot, 4 treatments including control, insecticide treatment, fungicide treatment and insecticide × fungicide treatment are randomly distributed. Each treatment was divided into two subplots with or without litter addition. Each subplot (2 m × 2 m) was placed 2 m apart in order to minimize the risk of contamination.

Carbosulfan and benomyl-hymexazol are broad-spectrum biocides, which are often applied to forest pest control. For the insecticide treatment, carbosulfan (granular formulation and 5 % active ingredient) was applied at rate of 250 g/m^2 in July, it was mixed with soil (non-litter-added subplots) or litter (litter-added subplots) and spread under the crown geometry. For the fungicide treatment, benomyl-hymexazol (6 % of benomyl and 24 % of hymexazol) was applied at rate of 90 g/m^2 (3 times as recommended dose and diluted 300 times with water when in use). Because the fungicide is liquid, it could not be intercepted by litter layer (about 2 – 3 cm in depth), the fungicide was applied twice in each subplot on the litter layer (litter-added subplots) or bare soil (non-litter-added subplots), in July and August. Control plots received an equivalent volume of water. Insecticide × fungicide treatment applied both insecticide and fungicide as described above. *I. verum* forests can produce a large amount of litter every year. Shao *et al*. (2015)

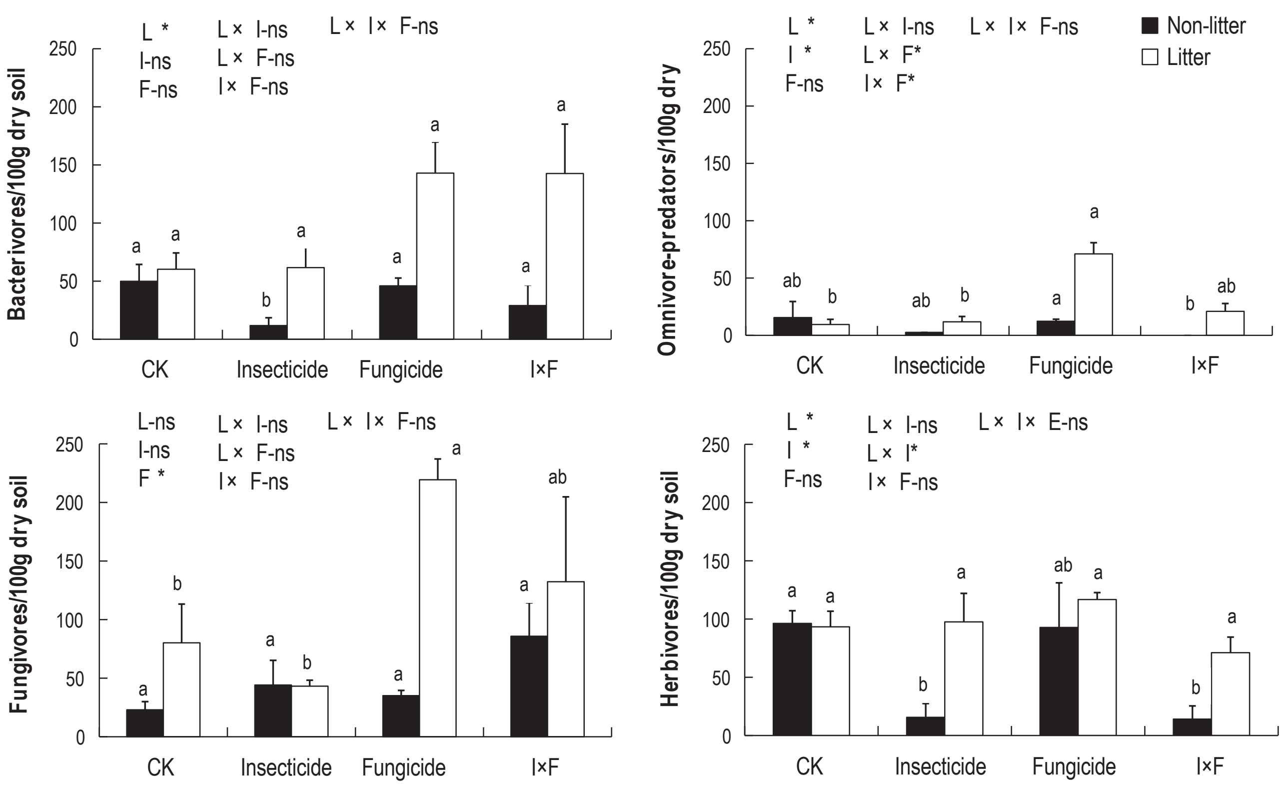

Fig.1. Changes of nematode abundance in different treatments (n=3, means + 1SE). Results of Three way-ANOVAs with significant effects of factors (litter, insecticide and fungicide) and their interactions are shown: L, litter addition; I, insecticide treatment; F, fungicide treatment; L×I, interaction between litter addition and insecticide; L×F, interaction between litter addition and fungicide; I×F, interaction between insecticide and fungicide; L×I×F, interaction between litter addition, insecticide and fungicide.*, $P < 0.05$; **, $P < 0.01$, ns, non-significant. Different letters (a,b etc.) show significant differences among different treatments with and without litter addition, respectively, as determined by Tukey's honestly significant difference test, $P<0.05$.

found litter provided basal resources for soil microorganisms and connects with soil organism closely in *I. verum* forest, so it was chosen as a remediator for soil ecosystem disturbance. Litter of *I. verum* was collected from the previous growth season. Upon collection, litter was oven dried to constant weight at 60 °C and then mechanically chopped to smaller particles (length=2 cm). Litter was completely mixed before adding to each treatment with pH 5.50, total C 483.0 g/kg and total N 15.9 g/kg. In litter-added subplot, 2000 g/m^2 dry litter was covered uniformly on the soil surface under the crown geometry, a layer of fine soil was sprinkled in order to prevent the litter from being blown away.

Soil sampling and analysis

Soil samples were collected in October. Four random cores (10 cm × 10 cm × 10 cm) were combined to form one composite sample for each treatment. Each composite sample was placed in an individual plastic bag and sealed. All samples were kept at 4 °C for biological and chemical analysis.

Soil organic carbon (SOC) was measured by the dichromate oxidation, and soil total nitrogen (TN) was determined with an ultraviolet spectrophotometer after Kjeldahl digestion (Bao 2000). Total carbon and total nitrogen of plant litter were measured using a Vario MACRO cube Elementary Analyzer (Elementar Analysensysteme Vario MACRO cube, Germany).Microbial biomass carbon (MBC) microbial biomass nitrogen (MBN) were determined using the fumigation extraction method (Wu *et al*., 2006).

Soil nematode extraction, identification and analysis

Nematodes were extracted from 100 g soil sample (fresh weight) by a modified cotton-wool filter method (Liang *et al*., 2009). The 100 individuals (or the total number in samples containing less than 100 individuals) of each sample were identified to genus level. The nematodes were assigned to the following trophic groups characterized by feeding habits (1) bacterivores; (2) fungivores; (3) omnivore-predators and (4) herbivores (Yeates *et al*., 1993).

The following ecological indices were calculated:

(1) Dominance index (λ), $\lambda = \Sigma p_i^2$, where *pi* is the proportion of individuals in the i-th taxon (Simpson 1949);

(2) Shannon index (H'), $H' = -\Sigma p_i(\ln p_i)$, where p_i is the proportion of individuals in the *i*-th taxon (Shannon & Weaver 1949);

(3) Generic richness $GR = (S - 1)/\ln(N)$, where *S* is the number of taxa and *N* is the number of total nematodes (Yeates & Bongers 1999);

(4) Nematode channel ratio (NCR), $NCR= B/(B+F)$, where *B* is the number of bacterivores and *F* is the number of fungivores (Yeates 2003);

(5) Structure index $SI = 100 \times (\sum k_s n_s/(\sum k_s n_s + \sum k_b n_b))$;

(6) Enrichment index $EI = 100 \times (\sum k_e n_e/(\sum k_e n_e + \sum k_b n_b))$,

where k_b is the weight assigned to guilds BF_2 and FF_2 and n_b is the abundance of nematodes in guilds BF_2 and FF_2, which indicate basal characteristics of the food web; k_s is the weight assigned to guilds BF_3-BF_5, FF_3-FF_5, and OP_2-OP_5, n_s is the abundance of nematodes in these guilds, which represent the structure condition of the food web; k_e the weight assigned to guilds BF_1 and FF_2, and n_e is the abundance of nematodes in these guilds, which represent an enriched condition of the food web (Ferris *et al*., 2001). BF_x, FF_x, OP_x, (where x = 1-5) represent the functional guilds of

Table 1. Nematode taxonomic diversity indices and channel ratio in different treatments (means ± SE).

Treatments		λ	H'	GR	NCR
Control	Non-Litter	0.15 ± 0.03	2.23 ± 0.15	3.36 ± 0.22	0.68 ± 0.08
	Litter	0.18 ± 0.01	2.15 ± 0.06	3.34 ± 0.07	0.47 ± 0.10
Insecticide (I)	Non-Litter	0.40 ± 0.17	1.29 ± 0.35	1.83 ± 0.35	0.30 ± 0.13
	Litter	0.20 ± 0.02	2.00 ± 0.12	2.84 ± 0.33	0.57 ± 0.04
Fungicide(F)	Non-Litter	0.14 ± 0.02	2.24 ± 0.10	2.91 ± 0.40	0.56 ± 0.16
	Litter	0.11 ± 0.01	2.42 ± 0.06	3.67 ± 0.23	0.39 ± 0.05
I×F	Non-Litter	0.55 ± 0.22	0.95 ± 0.47	1.14 ± 0.57	0.28 ± 0.17
	Litter	0.13 ± 0.01	2.38 ± 0.10	3.67 ± 0.17	0.55 ± 0.12
Litter (L)		ns	ns	**	ns
Insecticide (I)		**	**	**	ns
Fungicide (F)		ns	ns	ns	ns
L×I		*	**	*	**
L×F		ns	ns	*	ns
I×F		ns	ns	ns	ns
L×I×F		ns	ns	ns	ns

λ, dominance index; H', diversity index; GR, Generic richness; NCR, nematode channel ratio. *, $P < 0.05$; **, $P < 0.01$, ns, non-significant.

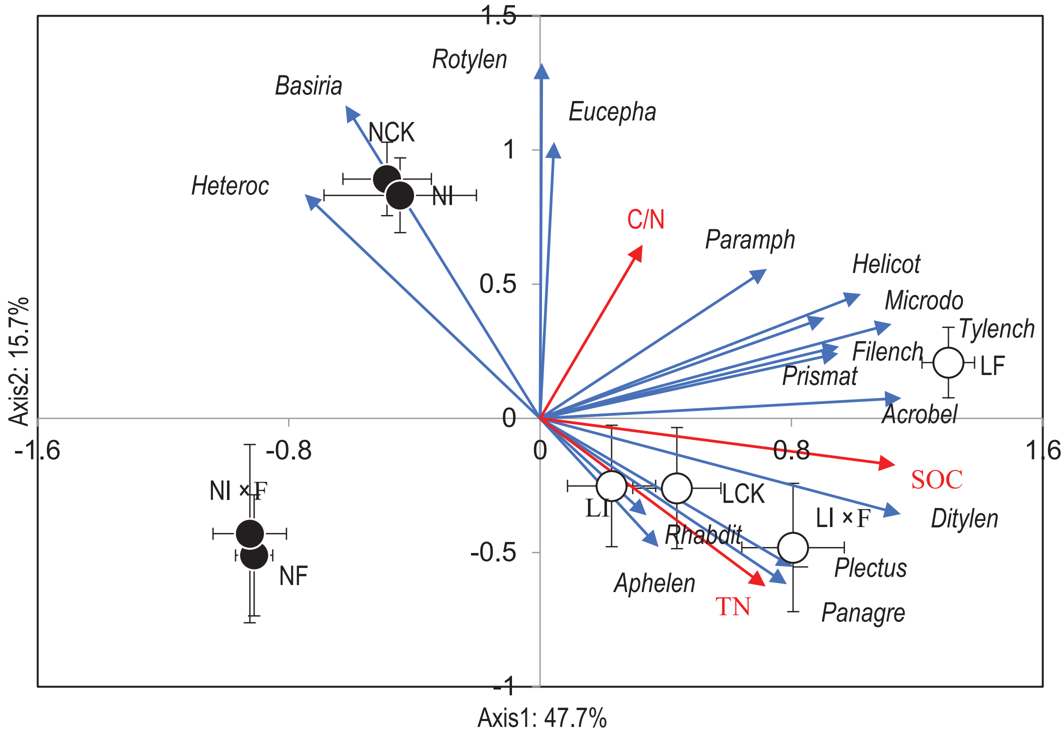

Fig.2. Redundancy analysis (RDA) of the soil nematode communities and soil abiotic variables. Species fit range more than 40% were presented. N, non-litter-added plots; L, litter-added plots. CK, control; I, insecticide application; F, fungicide application; I×F, interaction between insecticide and fungicide.

nematodes that are bacterivores, fungivores, predators and omnivores where the guilds have the characters indicated by x on the colonizer-persister (cp) scale (1-5) following Bongers and Bongers (1998).

Plotting EI and SI provides a graphic representation of nematode faunal profile that indicate whether the soil community is basal, enriched, or structured and stable (Ferris *et al*., 2001). The faunal profile can be divided into four quadrants, quadrant A (SI<50, EI>50) shows that food web is affected by high disturbances and food web condition is disturbed; quadrant B (SI>50, EI>50) means that the disturbance level is low or moderate and food web condition is maturing; quadrant C (SI>50, EI<50) represents an undisturbed and structured food web; quadrant D (SI<50, EI<50) indicates a stressed and degraded food web (Ferris *et al*., 2001).

Statistical analysis

Data of nematode abundance was ln (x + 1) transformed prior to statistical analysis for normality of data. Three-way ANOVA was applied to assess the effects of litter addition, insecticide treatment, fungicide treatment and their interactions on soil nematodes communities. *P*-values < 0.05 were considered significant. The relationship between nematode genera and environmental factors was examined based on redundancy analysis (RDA) using the CANOCO software, version 5.0. A Monte Carlo permutation test (499 permutations) was used to test the significance of first and all canonical axes.

Results

Soil nematodes assemblage

In non-litter-added plots, the application of fungicide decreased the abundance of omnivores-predators in comparison with control (CK); while an opposite trend was observed in the treatments with litter additions (Fig.1). Similarly, the decrease in the abundance of herbivores in the insecticide treatments was only observed in the non-litter-added plots. In addition, more bacterivores observed in the treatments with litter addition. The abundance of fungivores only showed response to the fungicide treatments, with higher abundance of fungivores observed in the treatments with litter addition.

The interactive effects between insecticide and litter addition significantly influenced nematode ecological indices ($P < 0.05$). In non-litter-added plots, the application of insecticide decreased the indices of H', GR and NCR and increased λ. However, in litter-added plots, insecticide did not cause obvious variations in the values of λ and H' (Table 1). The interactive effects between fungicide and litter addition were only found in GR. In comparison with the control, the application of fungicide increased GR in litter-added plots, but decreased GR in non-litter-added plots.

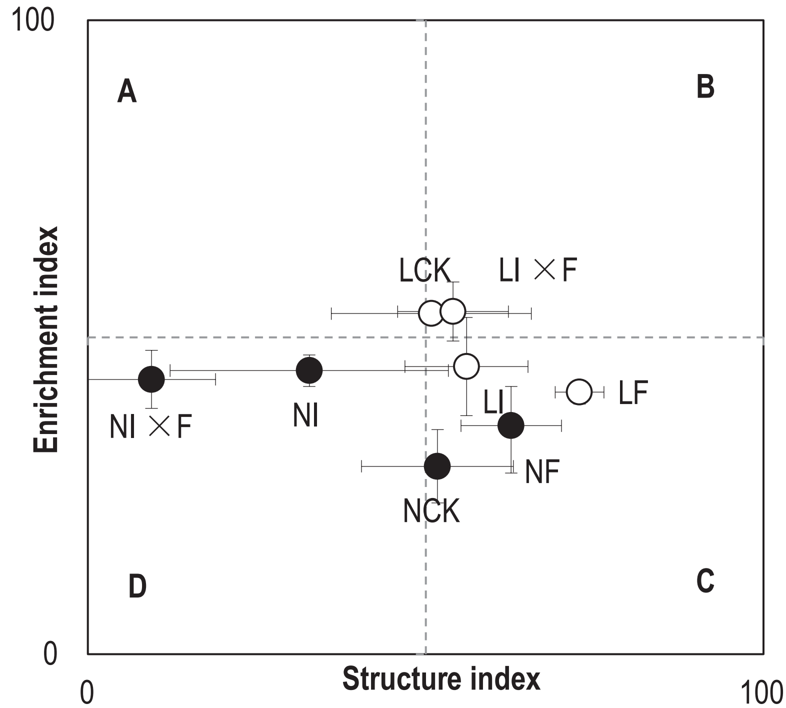

Fig.3. Changes in the structure and enrichment conditions of soil food web in different treatments (means ± 1SE). N, non-litter-added plots; L, litter-added plots. CK, control; I, insecticide application; F, fungicide application; I×F, interaction between insecticide and fungicide.

Correlations between soil nematode communities and environmental factors

The RDA showed a correlation between soil nematodes and the abiotic factors (Fig. 2). The ordination of nematodes was strongly affected by litter addition and soil abiotic factors. *Acrobeloides*, *Ditylenchus*, *Plectus*, *Rhabditidae*, *Aphelenchus* and *Panagrellus*, associated with litter, SOC and TN positively, *Basiria*, *Heterocephalobus* and *Rotylenchus* associated with insecticide and CK without litter addition positively.

Soil food web structure and stability

Nematode faunal profiles showed that nematodes from CK and insecticide × fungicide treated soil in litter-added plots were located in quadrant B. Nematodes form single insecticide and fungicide treated soil in litter-added plots and from CK and single fungicide treated soil in non-litter-added plots were located in quadrant C. While nematodes impacted by insecticide and insecticide × fungicide without litter addition were located in quadrant D (Fig. 3).

Discussion

The effects of litter addition on responses of nematode assemblage to insecticide

Our results showed that non-target soil nematodes were affected adversely by insecticide without litter addition. Neher *et al*.(2014) also reported soils treated with conventional insecticide contained less complex and successionally mature nematode communities. Insecticide declined nematode diversity via increasing dominant genera abundance (λ) and decreasing rare genera abundance (H’) in non-litter-added plots (Table 1). Both herbivores and bacterivores were impaired by insecticide significantly, and omnivore-predators disappeared in insecticide × fungicide treatment without litter addition (Fig.1). The loss of this high trophic level of the soil micro-food web may cause loss of ecosystem functions (Van der Wurff *et al*., 2007). Thus, insecticide may decrease the risk of trees disease by killing harmful insect and herbivores, but simultaneously disturb the balance of soil ecosystem by impacting biodiversity.

Litter resources play an important role in shaping nematode community structure and function (Zhao *et al*., 2021). In our study, the increased soil SOC and TN content correlated with most nematode species positively with litter addition (Fig. 2), which indicated that litter could influence nematode communities by altering soil nutrient content. Litter with high SOC content influences insecticide behavior differently than soil (Puglisi *et al*., 2012). Singh and Srivastava (2009) suggested clay and organic matter could accelerate adsorption of Carbofuran. Moreover, microarthropods that feed directly on litter and microbes can fragment litter and disperse microbes in the process of feeding (Neher *et al*., 2012). The increase in microbial carbon and microbial nitrogen confirmed that nematodes could access to more food resource which is conducive to maintaining community stability with litter addition (Supplementary

Supplementary Table 1. Selected properties of soil abiotic factors, microbial carbon and microbial nitrogen in different treatments (means ± SE).

Treatment	SOC (g/kg)		TN (g/kg)		MBC (mg/kg)		MBN (mg/kg)	
	Non-Litter	Litter	Non-Litter	Litter	Non-Litter	Litter	Non-Litter	Litter
CK	13.8 ± 0.10	17.24 ± 0.26	1.54 ± 0.05	1.64 ± 0.04	132.69 ± 7.88	150.10 ± 8.81	20.77 ± 0.28	40.15 ± 14.56
Insecticide	13.03 ± 0.19	15.65 ± 0.70	1.37 ± 0.06	1.45 ± 0.10	106.96 ± 8.94	116.69 ± 18.83	23.68 ± 9.31	26.80 ± 7.31
Fungicide	12.3 ± 0.16	14.21 ± 0.41	1.29 ± 0.06	1.35 ± 0.09	80.09 ± 17.83	115.23 ± 27.83	17.27 ± 4.38	24.77 ± 7.38
I×F	13.18 ± 0.34	15.25 ± 0.17	1.33 ± 0.07	1.37 ± 0.01	83.02 ± 15.46	112.91 ± 31.82	24.40 ± 4.03	31.46 ± 10.03
Litter	***		ns		**		*	
Insecticide	ns		ns		ns		ns	
Fungicide	**		**		ns		ns	
L×I	ns		ns		ns		ns	
L×F	ns		ns		ns		ns	
I×F	**		*		ns		ns	
L×I×F	ns		ns		*		ns	

*, $P < 0.05$; **, $P < 0.01$, ns, non-significant.

Table 1). Although litter addition could keep nematode away from adverse effects of biocides, it may limit the effectiveness of insecticide via increasing the abundance of herbivores. Litter addition may result in higher soil moisture retention and nutrient content, and then increase abundance of herbivores. Consequently, further studies were needed to evaluate the proper quantity of litter applied in order to match the needs for both keeping insecticide effectively and maintaining soil ecosystem functions.

Unexpectedly, fungivorous seem not to be impaired by insecticide (Fig. 1), one possible reason is that relatively higher microbial carbon content in insecticide treatment can provide more food resources for fungivorous (Supplementary Table 1). Another reason could be that most fungivorous correlated with soil SOC and TN closely (Fig. 2), and those nutrients were not impacted by insecticide (Supplementary Table 1). Additionally, fungivorous are dependent partly on root-derived resources (Kudrin *et al*., 2021), which help them resist environment stress. Chen *et al*. (2016) also found some specific microorganisms adapted or improved their tolerance to hexaconazole.

The effects of litter addition on responses of nematode assemblage to fungicide

The effects of fungicide on the nematode appeared not to be relevant in non-litter added plots, our results are in agreement with findings of Neher *et al*.(2019), in which they found that biocides had no effects on nematode trophic diversity, maturity indices and channel index, and assumed that crop rotation and tillage are main contributors to changes of nematode communities rather than biocides. Wang (2014) reported that soil nematodes showed a clear recovery trend 120 days after fungicide application. Fungicide was applied twice in July and August, and soil samples were collected in October. Therefore, we propose that spaced fungicide application gave nematodes time to recover and adapt to environment. Compensatory mechanisms such as the increase of tolerant nematode were also probably responsible for this phenomenon. For instance, *Aphelenchoides* which was most abundant in fungicide treatment with litter additions also showed tolerance to benomyl (Schmidt *et al.*, 2000) (Supplementary Table 2).

Liu *et al*. (2019) reported the accumulation of litter addition may benefit the nematode community. In our study, fungicide increased the abundance of nematode markedly in litter-added plots (Fig.3). Our results were partly in accordance with the findings of Ekelund (1999) who found that fenpropimorph stimulated fungivorous flagellates and ciliates with glucose addition and suggested that the fungicide effect on soil protozoa was not mediated via effects on fungal populations. Hence, the effect of fungicide on fungivores, obviously, may also have no relation to the reduction of their fungal food source in this study.

The effects of litter addition on food web structure condition

Nematode faunal profile derived from EI and SI can provide information about the status of the soil food web (Ferris *et al*., 2001). In this study, the samples from insecticide and its interaction with fungicide treatments were all located in quadrant D and separated obviously from single fungicide and the control treatments without litter addition (Fig. 3). Therefore, we propose that soil food web length and connections are significantly reduced in insecticide and its interaction with fungicide treatments without litter addition. Our result was in line with Bai *et al*. (2017) who revealed that soil food web with high level of pesticides addition was stressed and degraded. Neher *et al*. (2014) also found SI value in insecticide treatment was lower than in non-insecticide treatment, and suggested insecticide shifted ecological succession back to earlier stages. The contributor for this phenomenon is the reduced abundance of high nutrient level nematodes which have ability to regulate soil food web (Fig.1). The normal operation of soil functions depends on the overall cooperation of soil microorganisms (Álvarez-Martín *et al*., 2016). SI and EI placed nematode communities into quadrant C and quadrant B with litter addition. It can be assumed that litter addition may help to reduce the adverse effects of biocides and maintained the structure and stability of soil food web. Furthermore, lowest SI value was found in insecticide × fungicide treatment (Fig. 3), suggesting that insecticide and fungicide may accumulate their effects and create worse influence on soil food web than single insecticide or fungicide treatment.

The effects of litter addition on soil decomposition pathway

Liu *et al*. (2019) reported that litter was a limiting food source for the soil food web. Xu *et al.* (2013) found litter input is a crucial pathway for carbon and nutrient fluxes to the soil. In this study, litter addition changed soil biota community composition, which in turn may affect soil nutrient decomposition and mineralization. In non-litter-added plots, insecticide changed decomposition pathway from bacteria dominant (NCR > 0.5) to fungi dominant (NCR < 0.5) (Table 1). Because fungi have higher C assimilation efficiencies (Rousk & Bååth 2011), fungi dominant pathway could slow down soil nutrient cycling and decrease nutrient availability (Waring *et al*.,2013). Alternatively, fungal dominant channel maybe one of the defense mechanisms of soil food web that could promote resistance to environment change. Conversely, in litter-added plots, applications of insecticide resulted in bacterial based channels (NCR > 0.5). The present results are in line with the findings of Sauvadet *et al*. (2016), in which they show that the bacterial channel developed faster with leaf litter addition. Because bacterial based channels can accelerate decomposition rate, nutrient availability in litter-added plots was higher than in non-litter-added plots.

Conclusion

Litter addition could reduce the damage caused by insecticide and its interaction with fungicide to soil nematodes. In non-litter-added plots, nematode abundance was reduced by insecticide applications. Contrarily, insecticide and its interaction with fungicide did not impact nematodes negatively in litter-added plots. Based on

Supplementary Table 2. Nematode genera identified in this study and their relative abundance (%) in the different treatments.

Genera	Non-litter added plots				Litter added plots			
	Control	Insecticide	Fungicide	I×F	Control	Insecticide	Fungicide	I×F
Bacterivores								
Prismatolaimus	1.5	3.7	1.1	0.7	4.3	2.4	5.5	11.3
Alaimus	0.5	1.6	0.0	0.7	2.2	2.0	1.0	0.7
Paramphidelus	1.0	0.5	0.0	1.3	0.0	0.4	1.4	1.2
Plectus	0.0	0.0	0.0	0.0	1.5	1.3	0.4	1.5
Drilocephalobus	3.0	2.1	0.0	0.0	0.4	0.0	4.2	1.5
Rhabditidae	0.5	1.0	1.3	0.8	3.3	2.1	0.3	1.5
Acrobeloides	3.0	2.1	1.3	0.8	7.7	13.6	9.9	13.4
Eucephalobus	11.6	9.7	4.9	15.3	4.0	6.1	1.0	2.7
Monhystera	0.0	0.0	0.0	0.0	0.0	0.0	2.0	0.0
Heterocephalobus	6.0	4.0	7.3	3.0	0.0	0.0	0.0	0.0
Panagrellus	0.0	0.0	0.0	0.0	1.4	0.9	0.4	5.0
Fungivores								
Tylencholaimus	1.1	3.7	0.0	0.0	1.4	1.3	9.8	7.6
Aphelenchoides	8.6	9.2	59.5	65.8	20.0	11.7	18.9	16.4
Ditylenchus	0.0	0.0	0.0	0.0	9.0	3.4	8.2	4.8
Aphelenchus	2.8	6.0	0.0	0.8	0.0	0.0	0.0	0.0
Filenchus	3.5	3.7	0.0	0.0	2.5	3.8	3.0	7.2
Herbivores								
Helicotylenchus	12.3	13.5	8.7	3.4	29.8	36.2	17.6	12.8
Rotylenchus	26.0	28.1	9.9	5.4	6.8	8.1	2.3	1.0
Dolichodoridae	0.0	0.0	0.0	0.0	1.5	0.8	0.0	1.2
Paratylenchus	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Macroposthonia	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
Basiria	6.1	4.6	0.0	2.2	0.0	0.0	0.0	0.0
Malenchus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rotylenchulus	2.5	0.0	2.4	0.0	0.0	0.0	0.0	0.0
Pratylenchus	0.0	0.0	0.0	0.0	0.4	0.4	0.9	3.6
Lelenchus	1.1	0.0	0.0	0.0	0.0	0.0	0.4	0.0
Dorylaimellus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5
Omnivore-predators								
Thonus	0.0	0.0	2.4	0.0	0.4	0.4	0.0	1.5
Dorydorella	1.0	1.0	0.0	0.0	0.0	0.4	0.0	0.0
Aporcelaimidae	0.6	1.0	0.0	0.0	0.0	0.0	0.0	0.0
Eudorylaimus	1.5	0.0	0.0	0.0	0.4	1.3	2.3	0.0
Mesodorylaimus	5.4	0.0	1.1	0.0	1.1	1.7	6.3	0.5
Microdorylaimus	0.0	4.6	0.0	0.0	2.1	1.7	3.0	1.0
Mylonchulus	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0
Tripyla	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.0
Seinura	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.7

I×F, insecticide × fungicide interaction treatment our findings, we suggest that litter addition may extenuate the negative effects of biocides, and maintaining an appropriate level of litter addition is crucial to the successful management of soil environment.

Conflict of Interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled "Litter additions reduce the side effects of biocides on soil nematode communities in *Illicium verum* forest".

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