Fungivorous Nematodes and Collembola Alleviate Disease Incidence Caused by Pathogen *Pythium Ultimum* in Tomato

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Abstract

Purpose

Soil fauna are potential biological regulators of plant pathogens and soil nutrient cycles; however, the direct evidence demonstrating faunal controls over pathogens and plant diseases are still limited and the interactions between diverse faunal groups, soil-borne pathogen and microbial community remain elusive.

Methods

We introduced the nematode *Aphelenchus avenae*, two collembolan species (*Hypogastrura perplexa* and *Sinella curviseta*) and their combinations to test their suppression effects on the soil-borne fungus *Pythium ultimum* in tomato seedlings, and impacts on the biomass and activities of soil microbes.

Results

The introduction of *A. avenae*, *H. perplexa* and combinations of three faunal species showed significant suppression on *P. ultimum* population and decreased tomato seedling damping-off incidence, and diverse soil faunal species had the greatest suppression. The suppression efficiency is significantly different among the faunal species, with *A. avenae* was the most efficient, followed by *H. perplexa*, and the suppression effects of *S. curviseta* were poor. This may be attributed to the non-specific feeding behavior of collembolan, different spatial niches and body size of three faunal species. Furthermore, the presence of *S. curviseta* and *H. perplexa* increased the microbial biomass carbon and nitrogen, and *A. avenae* increased the soil respiration in microcosms, suggesting that activities of soil fauna may promote microbial turnover and nutrient cycling to alleviate plant disease.

Conclusion

Effective management of soil fauna can facilitate the control of root pathogens and stimulate soil microbes in organic farming systems. Future sustainable agricultural management is recommended to examine the applications of soil biodiversity in maintaining plant health and soil fertility in the arable fields.

Introduction

Soil-borne plant pathogens can cause root and crown rots, wilts and damping-off, which will bring widespread damage to the yield and quality of many economically important crops (Bebber et al. 2014; Delgado-Baquerizo et al. 2020). Main agricultural soil-borne fungal diseases including *Fusarium* wilt, *Verticillium* wilt and damping-off diseases induced by *Rhizoctonia solani* and *Pythium* spp., has caused

losses up to 20-35% and will jeopardize soil health and food security worldwide (Lamichhane et al. 2017; Newbery et al. 2016; Qiu et al. 2019). Traditional control methods are based on pesticide application, which are highly polluting and provide partial even ineffective protection, calling to expand nature-based solutions via considering soil biodiversity and associated ecosystem functions (Guerra et al. 2021; Paul et al. 2018; Xu and Jeger 2021).

In recent decades, agricultural lands have shifted from high energy input agroecosystems towards more ecologically sustainable production systems, in which plant diseases management has to rely more on biologically based processes (Pal and McSpadden Gardener 2006; Paul et al. 2018; Raaijmakers et al. 2009). Therefore, understanding the complex species interactions between phytopathogenic microbes and other soil organisms is a premise of success to pathogen control in sustainable agriculture (Goncharov et al. 2020). Several major mechanisms have been applied to explain the low disease incidence and soil suppressiveness to soil-borne pathogens in organic systems, including competition for C and nutrients (Schroth and Hancock 1982), antibiotic response of co-existing microorganisms (Haas and Keel 2003; Pliego et al. 2011), presence of hyperparasites of pathogens, and induced resistance in plants by beneficial microbes (Pal and McSpadden Gardener 2006).

Predation of fungal pathogens by soil fauna such as collembolans and nematodes may be a potential mechanism for disease suppression in organic soils (Friberg et al. 2005; Goncharov et al. 2020; Meyer-Wolfarth et al. 2017). Soil biodiversity including abundant soil micro- and mesofauna can play critical roles in improving soil health, food supply and other ecosystem services (Briones 2018; Mommer et al. 2018; Wall, Nielsen and Six 2015). The virulence of many soil-borne pathogens is suppressed in C-rich soils (e.g., organic soil or soils with manure additions), although pathogen growth or populations were not necessarily suppressed (Cohen et al. 2005; Martin 2003; Xu and Jeger 2021). Mycophagous collembolans or nematodes also have been successfully used to regulate the population dynamics and activities of pathogenic fungi, such as *Phytophthora* (Lussenhop 1992), *Gaeumannomyces graminis* var. *tritici* and *Fusarium culmorum* (Curl and Lartey 1996; Sabatini and Innocenti 2000), and *R. solani* (Lagerlöf et al. 2011) in microcosm or mesocosm experiments. A few studies reported the suppression effects of nematodes and collembolans on pathogens simultaneously, while their conclusions are controversial and the interactions between phytopathogenic fungi and other soil organisms are still poorly known (Lootsma and Scholte 1997a; Meyer-Wolfarth et al. 2017; Wolfarth et al. 2013).

Both collembolans and nematodes are important soil fauna in influencing composition, biomass and activity of the microbial community, either directly by selectively grazing bacteria or fungi, or indirectly by alternations of nutrient availability via excretions, and the disseminations of microbial propagules (Moore et al. 1988; Neher 2010). Consequently, soil nutrient cycling and plant growth are regulated by collembolans and nematodes (Briones 2018; Crowther et al. 2019). The contributions of collembolans and nematodes in suppressing soil-borne pathogen and regulating the microbial community may depend on their body size, feeding preference, life form and population density (Coulibaly et al. 2019; Crowther and A'Bear 2012; Potapov et al. 2021). Microconsumers, such as nematodes, often have fast turnover and control the microbial loop, thus functionally represent a fast energy channel and drive nutrient release in

soil food webs (Moore et al. 2005; Neher 2010). While mesofauna, dominated by collembolan and mites, show a weaker trophic interaction with basal resources (e.g., bacteria and fungi) compared with small-sized nematodes (Digel et al. 2011; Potapov et al. 2021). However, to our knowledge, the impacts of nematodes and collembolans on specific soil-borne pathogens, as well as the biomass and activity of microbial community have not been simultaneously tested, although that the capacity of disease suppression and nutrient turnover both are key in evaluating soil health and agricultural sustainability (El Mujtar et al. 2019; Pliego et al. 2011). Therefore, the interactions between plant pathogen, soil fauna and microbial community need further studies.

In our study, we conducted three separate but complimentary experiments to examine whether and how nematodes and collembola may contribute to *P. ultimum* suppressiveness and soil microbial community, using tomato-*P. ultimum* as a plant-pathogen model system. The specific objectives were to 1) determine and quantify one nematode, two collembolan species and their combinations impacts on *P. ultimum* population, biomass and pathogenicity; 2) examine effects of nematodes and collembola on soil microbial biomass and activities. Considering the fungal feeding habits of the three faunal species (Lartey 2006; Okada and Kadota 2003), we hypothesized that a combined inoculation of three faunal species would have a greatest suppressive impact on *P. ultimum* than inoculation of nematode or collembolan alone.

Materials And Methods

Soil preparation

Soil used in this study was collected from an organically managed field (35°43'N, 78°40'W) for over 20 years in Chapel Hill, NC, USA. The soil was a clay loam characterized by 3.67% total C, 0.23% total N and 151 mg kg⁻¹ soil extractable N. The sampling depth of soil is 0-20 cm and about 80 kg soils were collected. Sampled soil was cleared of plant residues and roots by hand, sieved (mesh size 4 mm) and pre-incubated in darkness under room temperature at 50% water holding capacity (WHC) for 8 weeks. Prior to use, soils were subject to heat by oven at 65 °C for 3 hours twice within 48 hours interval for defaunation (Wright et al. 1989). The defaunated soils were then re-adjusted to 70% WHC.

Source and preparation of fungal isolates *P. ultimum* and soil animals

The isolate of kanamycin-tolerant *P. ultimum*, obtained from Dr. Greg Rothrock at University of Arkansas, was cultured on corn meal agar (CMA) for 5 days, and then was transferred into V₈-rice medium at 28°C for 10 days. Mycelial rice kernels were used for the inoculum. A preliminary experiment was conducted to test the pathogenicity of the inoculum using the experimental soil and tomato seeds (Taylor 2010). Soil animals used in this study were one mycophagous nematode species (*Aphelenchus avenae*) and two collembolan species (*Hypogastrura perplexa* and *Sinella curviseta*). *S. curviseta* is larger than *H. perplexa* in size (Fig. 1). Prior to experiment, the nematodes were cultivated on potato dextrose agar (PDA) with a

non-pathogenic binucleate *Rhizoctonia* fungus. Collembolans were maintained in a covered jar containing a mixture of 7:3 activated charcoal:plaster saturated with deionized water in the bottom at room temperature. After 48 hours starvation, the collembolans were applied to the microcosm pots.

Experimental design

A total of 24 microcosm pots were used as experimental units. Each pot was filled with 500 g dry weight equivalent defaunated soil and added a beaker for soil respiration measurements. Pathogen *P. ultimum* was inoculated using 8 infected rice kernels for each pot. The inoculation amount of *P. ultimum* was the lowest level to provide sufficient disease pressure on the host tomato plant according to the preliminary experiment. Then the soil animals were introduced into 20 pots by twice. Four remaining pots were kept as control without soil fauna (CK). There were five different faunal treatments: single nematode *A. avenae* (Nem, 2000 individuals per pot each time); single collembolan *H. perplexa* (Col_H, 140 individuals per pot each time); single collembolan *S. curviseta* (Col_S, 140 individuals per pot each time); both collembola species (Col_HS, 70 individuals per species per pot each time), and mixed treatment with nematode and both collembola (Nem+Col, 2000 nematodes and 70 individuals of each collembola species per pot each time). Each treatment included 4 replicates. The total inoculated individuals of soil fauna in each treatment were shown in Table 1. Overall, the total inoculated soil animals were about one order of magnitude higher to their mean densities in the field (Meyer-Wolfarth et al. 2017). After 20 days of pathogen and soil animal inoculation, 30 tomato seeds of cultivar “Big Boy” (Wyatt-Quarles Seed Company, Garner, USA) were added to each pot about 0.5 cm below soil surface. Seeds were evenly placed by three rows beside the beaker. According to our preliminary experiment, the targeted pathogen *P. ultimum* was not isolated from soils and seedlings grown in non-inoculated *P. ultimum* microcosms for any of the replications. Thus, neither the seeds nor the soil are supposed to infest with *P. ultimum* which can impose a detectable disease pressure on the host plant. Then all experimental pots were incubated for 20 days under room temperature in the same environments and the soil was kept 70% WHC during the incubation.

Table 1

Individual numbers of nematode and collembola in microcosms at the end of experiment compared to the initial inoculated individual numbers.

Treatment	Inoculated individuals (number kg ⁻¹ soil)			Individuals at the end of experiment (number kg ⁻¹ soil)		
	<i>Aphelenchus avenae</i>	<i>Hypogastrura perplexa</i>	<i>Sinella curviseta</i>	<i>Aphelenchus avenae</i>	<i>Hypogastrura perplexa</i>	<i>Sinella curviseta</i>
CK	0	0	0	0	0	0
Nem	4000	0	0	2236 (514)	0	0
Col_H	0	280	0	0	823 (225)	0
Col_S	0	0	280	0	0	125 (49)
Col_HS	0	140	140	0	1116 (150)	60 (9)
Nem+Col	4000	140	140	1456 (195)	1240 (148)	27 (3)
CK: control without soil animals; Nem: treatment inoculated with <i>Aphelenchus avenae</i> ; Col_H: treatment inoculated with <i>Hypogastrura perplexa</i> ; Col_S: treatment inoculated with <i>Sinella curviseta</i> ; Col_HS: treatment inoculated with <i>H. perplexa</i> and <i>S. curviseta</i> ; Nem+Col: treatment inoculated with nematode and both collembolans. Data are means with 1SE indicated in parentheses (n=4).						

The suppressions of nematodes and collembola on *P. ultimum* in microcosms

The suppressions of soil fauna on *P. ultimum* pathogenicity were tested by post-damping off and pre-damping of tomato seedlings in microcosms, i.e. tomato seedling percentage. At the end of the experiment, healthy tomato seedlings in each pot were counted and adjusted by germination rate (0.83) to calculate seedling percentage in each treatment. Soil animals were extracted from 200 g soil per pot. Nematodes were extracted by Baermann funnel method (Baermann 1917). The two collembola species were extracted using MacFadyen high-gradient apparatus (Macfadyen 1961). The exacted soil animals including *A. avenae*, *H. perplexa* and *S. curviseta* were counted under a stereoscope (American Optical Co., Massachusetts, USA), separately.

The isolation and detection of *P. ultimum* population in soil samples were achieved using serial dilution approach (Jeffers and Martin 1986). At the end of experiment, all soil in the pot was collected and mixed. Five grams (dry weight equivalent) fresh soil subsamples were mixed with 50 mL 0.3% water agar (WA) by shaking for 20 min under 200 rpm. Then 2 mL above solutions were taken from the soil-WA mixture and diluted in 8 mL 0.3% WA. Isolation of *P. ultimum* was carried out on corn meal agar (CMA) amended with 5 µg/mL pimarinic acid, 250 µg/mL ampicillin, 10 µg/mL rifampicin and 100 µg/mL PCNB (P₅ARP; Jeffers and Martin 1986). Kanamycin (1500 mg/L) was added (i.e. CMA-P₅ARPK) to suppress other *Pythium* colonies for direct detection of kanamycin-tolerant *P. ultimum*. Above diluted solutions were

taken and spread on five CMA-P₅ARPK plates per sample. Then all plates were incubated at 22°C for 36 hours in darkness and colonies on each plate were counted.

The impacts of nematodes and collembolans on soil microbial biomass and activities

Soil microbial biomass C (MBC) and N (MBN) were measured using the fumigation-extraction method (Vance et al. 1987). Two replicates of 20 g dry weight equivalent soil subsamples were weighted; and one of them was fumigated for 48 h with 0.5 mL ethanol-free chloroform. Both fumigated and non-fumigated soil were extracted by shaking with 50 mL 0.5 M K₂SO₄ for 30 min. Soil extractable organic C was determined using a TOC analyzer (Shimadzu TOC-5050A, Shimadzu Co., Kyoto, Japan). Soluble inorganic N was determined on the Lachat flow injection analyzer (Lachat Instruments, Milwaukee, WI, USA) after alkaline persulfate oxidation (Cabrera and Beare 1993). MBC and MBN were estimated by the differences of extractable organic C and inorganic N between fumigated and non-fumigated soil extracts, respectively. Soil extractable inorganic N referred to the sum of NH₄⁺ and NO₃⁻ in non-fumigated soil extracts (Hart et al. 1994).

Soil heterotrophic respiration was determined on Jan-30th, Feb-7th, 14th, 20th and 28th using an incubation-alkaline method (Coleman et al. 1977). Briefly, each pot was placed in a Mason jar on each sampling day and then incubated at 25°C in the darkness for 5 hours. Respired CO₂ was trapped in 5 mL 0.5 M NaOH contained in the beaker of each pot. The CO₂ captured in the NaOH solution was titrated with 0.5 M HCl to determine the amount of CO₂ evolved from the soil. Soil microbial respiration rate was expressed as mg CO₂ kg⁻¹ soil h⁻¹ by averaging the data across 5-hours incubations.

Suppression tests of *P. ultimum* growth via soil faunal grazing on petri dishes

Suppression effects of nematodes and collembolans grazing on *P. ultimum* hyphal growth were tested on 1/2 strength PDA. An autoclaved, weighted cellophane membrane (Midwest Scientific, USA) was placed on the medium in each petri dish. The kanamycin-tolerant *P. ultimum* was inoculated at the center of each membrane. Then soil animals were introduced to the petri dishes (Taylor 2010) including 5 treatments: single nematode *A. avenae* (Nem, 700 individuals per plate); single collembolan *H. perplexa* (Col_H, 50 individuals per plate); single collembolan *S. curviseta* (Col_S, 50 individuals per plate); both collembola species (Col_HS, 25 individuals per species per plate), and mixed treatment with nematode and both collembolans (Nem+Col, 700 nematodes and 25 individuals of each collembola species per plate). Nematodes were introduced with 0.5 mL solutions. Each treatment included 5 replicated plates. Another 10 plates were prepared without soil fauna as collembola control (CK1, 5 plates) and nematode control (CK2, 5 plates with 0.5 mL water). Then all plates were incubated for 3 days at room temperature. In the end of tests, cellophane membranes with *P. ultimum* hyphae were weighed.

Statistical analyses

The differences of tomato seedling percentage, *P. ultimum* populations, soil extractable nitrogen, microbial biomass carbon and nitrogen in microcosms between treatments (i.e., CK, Col_H, Col_S, Col_HS, Nem and Nem+Col) were analyzed by one-way ANOVA. Two separate one-way ANOVA were used to test the suppression effects of collembolans and nematodes on the growth of *P. ultimum* on petri dishes, respectively. Tukey HSD tests were performed for pairwise comparisons when the treatment had a significant effect. Repeated measures ANOVA was used to test the effects of nematode and collembolan inoculations on soil respiration rate in microcosms. Differences were regarded as statistically significant when $P < 0.05$. All statistical analyses were performed using Statistica 8.0 (StatSoft).

Results

Disease and *P. ultimum* growth suppression by nematodes and collembolans

The suppression effects of nematode *A. avena* and two collembolans *S. curviseta* and *H. perplexa* on the disease caused by *P. ultimum* were indicated by the tomato seedling percentages in microcosms. No matter considering the germination rate adjustment or not, our results showed a significant suppression in all faunal treatments except Col_S (real seedling percentage: $F_{5,18} = 12.14$, $P < 0.001$; seedling percentage divided by germination rate: $F_{5,18} = 14.33$, $P < 0.001$). There were more than 85% healthy tomato seedlings in Nem or Nem_Col treatment, which had the highest suppression effects on the disease incidence compared with control (33%; Fig. 2b). The introductions of collembolans and nematodes reduced the biomass of *P. ultimum* on petri dishes (collembolans: $F_{3,12} = 15.63$, $P < 0.001$; nematodes: $F_{2,9} = 19.65$; $P < 0.001$; Fig. 3). The treatment with three faunal species (Nem+Col: 59%) had a greater suppression effect on the growth of *P. ultimum* than that only with introduction of nematodes (Nem: 17%; Fig. 3).

Collembolans, nematodes and *P. ultimum* population densities in microcosms

At the end of microcosm experiment, population densities of *A. avena* and *S. curviseta* decreased in all inoculation treatments, but *H. perplexa* populations increased by more than 4 times of the initial number at the end of the 20-days tests (Table 1). The inoculations of *A. avena* and *H. perplexa* significantly decreased the densities of *P. ultimum* populations in microcosms in similar with results of suppression tests on petri dishes ($F_{5,18} = 3.06$, $P = 0.036$). However, the population density of *P. ultimum* in the microcosms of Col_S was much higher compared with other faunal treatments (Fig. 4).

Effects of soil animal inoculations on soil microbial biomass and activities in microcosms

Soil animal inoculation significantly enhanced soil respiration at harvest ($F_{5,18} = 3.70$, $P = 0.018$), and the effects were different in three faunal species treatments (Fig. 5). The soil respiration rate in the Nem or

Nem_Col was highest, followed by Col_H, and the soil respiration in Col_S and Col_HS were lower than other faunal treatments (Fig. 5). The microbial biomass carbon and nitrogen were also significantly different among the treatments and control (MBC: $F_{5,18} = 3.26$, $P = 0.034$; MBN: $F_{5,18} = 5.21$, $P = 0.006$). The microbial biomass carbon and nitrogen were highest in the Col_S, followed by Col_H and Col_HS (Fig. 6a&b). Nematode inoculation in the microcosms did not increase MBC and MBN (Fig. 6a&b). There was not significant difference in extractable N among the treatments and control ($P = 0.696$, Fig. 7).

Discussions

Our results showed that the fungivorous nematode *A. avenae* and collembola *H. perplexa* effectively suppress growth of *P. ultimum* and tomato seeding damping-off caused by *P. ultimum* (Fig. 2a&b; Fig. 4). Previous studies have shown that *Pythium* species can be controlled by other fungi and bacteria, but few efficient controls of *Pythium* and its disease symptoms by soil animals were reported (El Titi and Ulber 1991; Löbmann et al. 2016). Faunal grazing can exert a strong modulating impact on plant-fungal interactions, and the presence of particular groups of soil animals are associated with the suppression of plant diseases (Jorge-Escudero et al. 2021; Schrader et al. 2013; Xu and Jeger 2021). For example, the fungivorous collembola *Folsomia hidakana* successfully suppressed cabbage seedling damping-off caused by *R. solani* under greenhouse conditions (Shiraishi et al. 2003). Mycophagous nematodes of *Aphelenchoides*, *Aphelenchus*, *Ditylenchus* and *Neotylenchus* are abundant in many cropping systems and their effects in controlling plant pathogens have received great attentions (Bollen et al. 1990; Lootsma and Scholte 1997a). Wherein *A. avenae* is the most popular test species, and has been found to efficiently control fungal and accompanying disease suppression under a wide range of temperatures and soil moisture levels (Lootsma and Scholte 1997a, b). Fungivorous nematodes are equipped with a mouth stylet, with which they can ingest the cell contents in fungal cells to directly kill fungi (Nicholas 1984). The characteristics that some springtails prefer feeding on soil-borne pathogens rather than the antagonistic fungi such as *Laetisaria arvalis*, *Trichoderma harzianum*, and *Gliocladium virens* (Curl and Old 1988; Lartey et al. 1989) can also help to explain the suppression effects of *H. perplexa* in our results (Fig. 2a&b). While nematodes and collembolans pose a suppression effect on fungal pathogen when applied separately, whether integration of different faunal species could provide a better soil-borne pathogen control is controversial in researches seeking an integrated biocontrol solution of soil health management (Meyer-Wolfarth et al. 2017; Wall, Nielsen and Six 2015; Xu and Jeger 2021). It is a pity that we did not test the suppression impacts of all combinations among the three faunal species. The combination of *A. avenae* and *H. perplexa* can show a good control effect on *P. ultimum* remains uncertain. However, our study supports that a combination of nematode and two collembolan species can provide a superior disease control than either alone (Fig. 2-3, also see Wolfarth et al. 2013). However, Meyer-Wolfarth et al. (2017) did not find an additive effect of nematode *Aphelenchoides saprophilus* and collembola *Folsomia candida* in controlling *Fusarium culmorum* in wheat straw, probably due to the interference between various introduced soil animals. The specific mechanisms that synergistic effects of diverse biocontrol agents may relate to the infection level of pathogen and feeding preference of the tested animals on the targeted pathogen (Lartey 2006; Meyer-Wolfarth et al. 2017).

In our study, nematode inoculation achieved a greater suppression efficiency than the collembolans in suppressing tomato seedling damping-off caused by *P. ultimum*, and the largest body-sized collembola *S. curviseta* showed the weakest suppression (Fig. 2a&b). In general, smaller soil animals have greater influences on plant disease by selective grazing on pathogens directly, whereas the relatively larger animals have more diverse food sources and play a role mainly by indirect interactions (e.g., competing C and nutrients) or through non-trophic impacts such as dispersal and mixing soil (Friberg et al. 2005). The fungal-feeding habit of *A. avenae* is well-known and some common root-infecting fungi were highly favorable food source for *A. avenae* (Okada and Kadota 2003). By contrast, the food spectrum of collembola is more diverse and their preferential feeding habits on fungi probably depend on whether there was other available food (Lartey 2006). Our results found that the introduction of *S. curviseta* could feed on *P. ultimum* on petri dishes but poorly controlled *P. ultimum* growth in microcosms with a complete soil microbial community (Fig. 3-4), also verified this suggestion. In the microcosms, *S. curviseta* may not only fed on *P. ultimum*, but also ate some antagonistic microbes which may weaken the soil suppressiveness. Wiggins and Curl (1979) pointed out that the bristled bodies of collembolans are well suited for transport of fungal spores into the rhizosphere, which may also inhibit the suppression efficiency of mycophagous collembolans. Furthermore, the body size and spatial niche of some soil animals can affect the suppression efficiency via interfering the accessibility of animals to fungi and bacteria (Sabatini and Innocenti 2000). Collembola that feed on the microflora are not uniformly distributed in the soil but tend to congregate around living, dead or dying roots and organic matter where food sources are abundant. *S. curviseta* is a large-bodied epedaphic collembolan which often feed on food resources on the litterfall and soil surface, while *H. perplexa* is euedaphic which are deep-living and their foraging activities are often limited in the rhizosphere (Chauvat et al. 2014). Compared to *H. perplexa*, *S. curviseta* had a two-fold body size and longer legs (Fig. 1), which may also impede its contact with tiny fungal mycelium of *P. ultimum* in the rhizosphere.

Besides directly regulating pathogen growth, grazing activities of nematodes and collembolans may contribute to nutrient release and leaching, consequently altering soil nutrient availability and affecting plant health (Neher 2010; Coulibaly et al. 2019; Griffiths et al. 2021). Although we did not find an increase in soil extractable nitrogen (Fig. 7), presence of collembola increased microbial biomass (Fig. 6a&b), and nematode stimulated the microbial activity indicated by increased soil respiration (Fig. 5). These results suggest that the disease suppression in faunal inoculation treatments is partially attributed to enhanced microbial activity (Diab et al. 2003). Previous studies demonstrate that soil fungivores, including nematodes and collembolans, can trigger compensatory growth of fungi or limit the increase of microbial biomass via up-down effects (Coulibaly et al. 2019; Ingham et al. 1985), and the outcome of this interaction highly depend on the grazing intensity (Ingham et al. 1985). Mycogaphous collembola selectively graze on hyphae, and they can also selectively graze bacteria and even feed on nematodes (Thimm et al. 1998). Thus, collembola especially large-bodied *S. curviseta* is presumed to pose a relatively low grazing pressure on soil fungi, and at the same time, propagate fungal spores via foraging activities, thus promoting fungal growth and increasing the microbial biomass. Furthermore, differentiation of feeding preference between soil faunal groups can bring transformations in microbial

composition and associated energy channels in soil food webs (Coulibaly et al. 2019). We can speculate that the strong selective feeding activities of *A. avenae* on fungal mycelium may alter the microbial community tend to be more bacteria-dominated than that in the collembola treatments. The bacterial-dominated energy channel often retains less C in biomass per unit substrate consumed and has a higher turnover rate, representing a faster nutrient cycling than the fungal channel (Moore et al. 2005; Six et al. 2006). Therefore, a relatively higher dominance of bacteria in the *A. avenae* treatment may exert a greater impact on the microbial activity and enhance nutrient cycling than that in the collembola treatments.

In conclusion, introduction of nematode *A. avenae* and collembola *H. perplexa* showed great effects in suppressing tomato seedling damping-off caused by *P. ultimum* and enhancing nutrient cycling in artificial systems. The efficiency of faunal control over *P. ultimum* is may be attributed to the fungal grazing preference and increased microbial activity. It should be noted that only the *P. ultimum*-tomato system was tested in our study, whereas in field soils there are more phytopathogenic fungi that can damage crop health. Whether these biocontrol agents can play a general suppression role on field crops need further studies. Our results highlight the potential of soil animals in controlling soilborne pathogens and maintaining soil fertility in organic farming systems if properly managed, and future challenges will be the practice of these biocontrol agents in the arable fields for sustainable agriculture.

Declarations

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Author contributions

P. Z., W. Z. and S. H. conceived and designed the study. P. Z. analyzed the data and wrote the first draft of manuscript, all authors edited the manuscript and approved the final version to submit.

Statements and Declarations

The authors declare no competing interests.

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Figures

Figure 1

The collembola species *Sinella curviseta* and *Hypogastrura perplexa* with different body sizes used in the experiment.

Figure 2

(a) Damping-off disease suppression indicated by real tomato seedling percentage and **(b)** seedling percentage adjusted by germination rate in microcosms inoculated with nematode *A. avenae* (Nem), collembola *H. perplexa* (Col_H), *S. curviseta* (Col_S), both collembola species (Col_HS), nematode and both collembolans (Nem+Col) or without soil animal (CK). Values are means $\pm$ 1SE. Significant differences ($P \leq 0.05$) between treatments and control are denoted by different lowercase letters.

Figure 3

The growth suppression of *P. ultimum* by nematode *A. avenae* (Nem), collembola *H. perplexa* (Col_H), *S. curviseta* (Col_S), both collembola species (Col_HS), nematode and both collembolans (Nem+Col) on petri dishes. CK1 means control without soil animal on petri dishes; CK2 means control without soil animal but add 0.5 mL dH₂O. Values are means $\pm$ 1SE. Significant differences ($P \leq 0.05$) between treatments and control are denoted by different lowercase letters.

Figure 4

Population of *P. ultimum* in microcosms inoculated with nematode *A. avenae* (Nem), collembola *H. perplexa* (Col_H), *S. curviseta* (Col_S), both collembola species (Col_HS), nematode and both collembolans (Nem+Col) or without soil animal (CK). Values are means $\pm$ 1SE. Significant differences ($P \leq 0.05$) between treatments and control are denoted by different lowercase letters.

Figure 5

Microbial respiration rate in microcosms inoculated with nematode *A. avenae* (Nem), collembola *H. perplexa* (Col_H), *S. curviseta* (Col_S), both collembola species (Col_HS), nematode and both collembolans (Nem+Col) or without soil animal (CK) after 1, 2, 3, 4 and 5 weeks of *P. ultimum* inoculation. Values are means $\pm$ 1SE. Significant differences ($P \leq 0.05$) between treatments and control at harvest time are denoted by different lowercase letters.

Figure 6

(a) Microbial biomass carbon and (b) nitrogen in microcosms inoculated with nematode *A. avenae* (Nem), collembola *H. perplexa* (Col_H), *S. curviseta* (Col_S), both collembola species (Col_HS), nematode and both collembolans (Nem+Col) or without soil animal (CK). Values are means $\pm$ 1SE. Significant differences ($P \leq 0.05$) between treatments and control are denoted by different lowercase letters.

Figure 7

Soil extractable nitrogen in microcosms inoculated with nematode *A. avenae* (Nem), collembola *H. perplexa* (Col_H), *S. curviseta* (Col_S), both collembola species (Col_HS), nematode and both collembolans (Nem+Col) or without soil animal (CK). Values are means $\pm$ 1SE.