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Biofumigation effects of brassicaceous cover crops on soil health in cucurbit agroecosystems in Hawaii, USA

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ABSTRACT

Brassicaceous cover crops, such as brown mustard (*Brassica juncea*) and oil radish (*Raphanus sativus*), are commonly used for biofumigation, a process that utilizes isothiocyanates (ITCs) generated from the hydrolysis of glucosinolates in *Brassica* plants to suppress soil-borne pathogens, including plant-parasitic nematodes. Given the biocidal nature of ITCs, limited information is available on the non-target effects of biofumigation on free-living nematodes, which are reliable soil health indicators. The objectives of this study were to determine if biofumigation methods effective against plant-parasitic nematodes would have non-target effects on free-living nematodes, and to examine the relationships between biofumigation indicators and nematode communities. Three field trials were conducted to examine whether different biofumigation methods would affect free-living nematodes. Tissue maceration of biofumigant crops, soil tillage, and black plastic mulching were adopted singly or in combination to generate different regimes of biofumigation efficacy. Termination of biofumigant crops by tissue maceration and soil tillage followed by black plastic mulching for one week was most effective in suppressing plant-parasitic nematodes and enhancing bacterial decomposition. However, these effects did not last through the subsequent zucchini (*Cucurbita pepo*) crop cycle. When comparing changes in soil glucose and sulfate concentrations as indicators of biofumigation efficacy, we found that soil sulfate was a better indicator of biofumigation efficacy than soil glucose, owing to the more stable state of sulfate in soil. Canonical correspondence analysis between soil sulfate as a biofumigation indicator and nematode soil health indicators revealed strong positive correlations of sulfate level with the abundances of bacterivorous and carnivorous nematodes, enrichment index, brown mustard biomass, and soil temperature. However, biofumigation did not affect the nematode community structure. This study demonstrated that biofumigation can suppress plant-parasitic nematodes without compromising soil health.

Key Words: brown mustard, free-living nematode, glucose, oil radish, plant-parasitic nematode, soil-borne pathogens, sulfate

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INTRODUCTION

Biofumigation is an agronomic practice that utilizes isothiocyanates (ITCs) generated from the breakdown of glucosinolates (GLs) present in brassicaceous crops to manage soil-borne pests and pathogens in agroecosystems (Kirkegaard *et al*., 1993). Glucosinolates are stored in the vacuoles of brassicaceous plant cells. Upon tissue maceration, GLs get in contact with the endogenous enzyme myrosinase in the cell wall or cytoplasm and undergo hydrolysis to release glucose and aglycone moieties. Depending on environmental conditions or the presence of specifier proteins in biofumigant crops (Kuchernig *et al*., 2012), the aglycone moiety is converted to volatile compounds, including ITCs, sulfur, nitriles, and thiocyanates (Uda *et al*., 1986). Some soil microorganisms release extracellular myrosinase to break down GLs in the rhizosphere exuded from roots or leaf washings (Ohtsuru *et al*., 1973; Tani *et al*., 1974). The biofumigation effects against a target pathogen depend on ITCs reacting with biological nucleophiles of the pathogen, targeting the thiol and amine groups of various enzymes (Avato *et al*., 2013).

Several approaches have been proposed to maximize ITC production to enhance biofumigation effects. These include pulverizing biofumigant crop tissues to activate the hydrolysis of GLs (Morra and Kirkegaard, 2002; Matthiessen *et al*., 2004), irrigating the pulverized tissues to maximize hydrolysis (Matthiessen *et al*., 2004; Matthiessen and Kirkegaard, 2006), and incorporating the pulverized tissues into the soil followed by sealing the soil with an impermeable plastic film or immediately compacting the soil to minimize volatilization loss of ITCs (Kirkegaard and Matthiessen, 2004; Riga, 2011).

However, limited information is available on the non-target effects of biofumigation on free-living nematodes. Free-living nematodes can be classified into functional groups based on their morphology and are reliable soil health indicators because they are ubiquitous, sensitive to subtle

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variations in land management, correlated strongly with soil functions, and inexpensive to quantify (Yeates *et al*., 1993; Doran and Parkin, 1994). Many researchers have shown that analysis of nematode community can tell whether the soil is undergoing bacterial or fungal decomposition, the soil is enriched with nutrients or stressed, and the soil food web is disturbed or in stable conditions (Yeates and Bird, 1994; Ferris *et al*., 2001; Wang and McSorley, 2005).

As ITCs from *Brassica* crops are chemically similar to metam sodium (methyl ITC), it is logical to question whether biofumigation could compromise soil health. Several studies have suggested that biofumigation has no adverse effects on free-living nematodes if not increases their abundance (Stirling and Stirling, 2003). However, many of these studies showed variable effects on plant-parasitic nematodes. For example, incorporating brown mustard (*Brassica juncea*) into the soil neither affected free-living nematodes nor reduced stubby root (*Trichodorus* spp.) and stunt (*Tylenchorhynchus* spp.) nematodes (Vervoort *et al*., 2014). Arugula (*Eruca sativa*) suppressed Columbia root-knot nematode (*Meloidogyne chitwoodi*) in a greenhouse when the nematodes were allowed to undergo only one generation (Riga, 2011). However, the author found that when the nematodes underwent multiple generations per season in the field, the cover crop neither suppressed *Meloidogyne chitwoodi* nor affected free-living nematodes following the incorporation of cover crop in soil and roller crimping. In another study, incorporation of rapeseed (*Brassica napus*) and turnip (*Brassica campestris*) in soil increased the abundance of free-living nematodes but did not suppress the abundance of *Meloidogyne javanica* (Stirling and Stirling, 2003). Oil radish (*Raphanus sativus*) and rapeseed increased the abundances of bacterivorous and fungivorous nematodes but did not suppress plant-parasitic nematodes when winterkilled or incorporated in soil by disking and cultipacking (Gruver *et al*., 2010). The effectiveness of biofumigation is dependent on the life-cycle duration of nematode species. Hence, it must be used accordingly.

The abovementioned studies on the effect of biofumigation on soil health were based on methods ranging from mere soil incorporation, winterkill, disking, and cultipacking to roller crimping. Their results were inconclusive because none of the termination methods suppressed plant-parasitic nematodes, while simultaneously increasing or reducing free-living nematodes. This highlights the need to evaluate the effects of biofumigation on soil health. Recently, Waisen *et al*. (2020) compared different biofumigant crop termination methods with or without tissue maceration, with or without tillage, and with or without black plastic mulching. The current study is an extension of the same line of work by Waisen *et al*. (2020), but examined whether different biofumigant crop termination methods would negatively affect free-living nematodes. To further generate different regimes of biofumigation, brown mustard, a high-GL cover crop, was compared to oil radish, a low-GL cover crop (Gimsing and Kirkegaard, 2006), in this study.

Specific objectives of this research were to determine: i) if biofumigation methods effective against plant-parasitic nematodes could also have non-target effects on free-living nematodes as indicators of soil health and ii) the relationships between biofumigation indicators and nematode soil health indicators.

MATERIALS AND METHODS

Trial I

A field trial was initiated on November 17, 2016, at the Poamoho Experiment Station, Waialua, Hawaii, USA (21°32′14.7″ N, 158°5′20.2″ W; 166–215 m above sea level). The soil at the experimental site was a well-drained silty clay Oxisol (Wahiawa series, very fine, kaolinitic, isohyperthermic, Rhodic Haplustox), with a pH of 6–7 and organic matter content of 9 g kg^{-1}. The soil was naturally infested with reniform (*Rotylenchulus reniformis*) and root-knot (*Meloidogyne incognita* and *Meloidogyne javanica*) nematodes. Oil radish Sodbuster (Petcher Seeds, USA) and brown mustard Caliente 199 (Siegers Seed Co., USA) were used as biofumigant crops. The field was plowed to a depth of 10 cm and 28 treatment plots of 1.2 m × 5.5 m were prepared prior to seeding the biofumigant crops. The spacing between plots was 1.5 m and that between rows was 0.6 m. Each biofumigant crop was seeded at 11.2 kg seeds ha^{-1}. Six weeks later, both brown mustard and oil radish were subjected to three termination (biofumigation) methods or treatments, each with distinct regimes of biofumigation efficacy. The first was no-till (NT), where the aboveground plant tissues were clipped off at the soil line using a sickle, spread evenly over the plot, and covered with a woven weed mat. The second was maceration plus tillage (MT), where the aboveground plant tissues were macerated with a line trimmer, followed by tillage to 10-cm deep using a hand-held rototiller. The third was MT combined with impermeable black plastic mulching (MTBP), where the aboveground plant tissues were subjected to the same termination method as in MT but being covered with impermeable black plastic mulch. A no-biofumigation bare ground control was included, and the treatments were arranged in a randomized complete block design with four replicates. Black plastic mulch or weed mats were uncovered one week after the termination of the biofumigant crops, and in all plots two-week-old seedlings of zucchini (*Cucurbita pepo* cv. Felix) (Harris™ Seeds, USA) were transplanted at 1-m spacing or five plants per plot. The plants were drip irrigated. Chlorophyll content from three newly matured leaves per plant from three randomly selected plants per plot was estimated monthly using a

chlorophyll meter (SPAD-502 Plus, Konica Minolta Sensing Inc., Japan). Upon maturity, zucchini fruit was harvested weekly, and the total fruit weights were recorded. At the end of the experiment, each zucchini plant was uprooted with minimal root disturbance, weighed, and rated for root-gall index (RGI) based on a 0–10 scale, where 0 = healthy root system with no galls and 10 = roots are severely galled and non-functional (Bridge and Page, 1980).

Trial II

A second field trial was initiated on July 20, 2017, using only brown mustard Caliente 199 as a biofumigant crop. The field was plowed and 28 field plots of 1.2 m × 5.5 m were prepared. The brown mustard was seeded at the same seeding rate as in Trial I. Five weeks after planting, brown mustard was subjected to six termination methods, each with distinct regimes of biofumigation efficacy. These included: i) NT; ii) NT but the aboveground plant tissues were macerated using a line trimmer (MNT); iii) MNT but shoots were covered with impermeable black plastic mulch (NTBP); iv) direct tillage without prior shoot maceration to 10-cm deep using a hand-held rototiller (T); v) MT; and vi) MTBP. Bare ground was included as no-biofumigation control, and the experiment was arranged in a randomized complete block design with four replicates. One week after the biofumigant crop termination, black plastic mulch or weed mats were uncovered, and two-week-old zucchini seedlings were transplanted as in Trial I. Zucchini chlorophyll content was measured monthly, fruit was harvested weekly, severity of *Meloidogyne*-induced root galls was rated, and root weight was recorded as described in Trial I.

Trial III

This trial was initiated on December 7, 2017 and considered a technical repeat of Trial II with slight modifications. Since brown mustard grew slower in the winter, biofumigant crop termination was delayed until seven weeks after planting instead of five weeks. All treatments and data collection methods were the same as those in Trial II.

Nematode assay

Soil samples were collected before biofumigant crop planting, immediately before biofumigant crop termination, one week after biofumigant crop termination, and at monthly intervals thereafter for three months during the growth of zucchini bioassay crop in each trial. At each sampling time, six soil cores were taken from a depth of 10 cm per plot using a Ground Shark shovel (Forestry Suppliers Inc., USA) and composited in a sampling bag. The soil was then sieved to < 2 mm and homogenized. A subsample of 250 cm^3 soil was used for nematode extraction using the elutriation and centrifugal flotation method (Jenkins, 1964; Byrd *et al*., 1976). Nematodes were identified to the genus level using a Leica™ inverted microscope (Leica Microsystems Co., Germany) with reference to Goodey (1963) and Smart and Nguyen (1988), except for the members of Rhabditidae which were identified to the family level.

Nematode community analysis

All nematodes were categorized into one of six trophic groups: algaevores, bacterivores, fungivores, herbivores, omnivores, and carnivores (*i.e*., predators) according to Yeates *et al*. (1993), and the abundance of each trophic group was determined. *Filenchus* and *Tylenchus* genera were categorized as members of the fungivore group according to McSorley and Frederick (1999). Richness was calculated as the total number of taxa recorded per sample. Simpson's index of dominance (λ) was calculated as (Simpson, 1949):

$$\lambda = \sum_{i=1}^{n} (p_i)^2 \tag{1}$$

where p_i is the proportion of the ith genus present.

Simpson's index of diversity was calculated as $1/\lambda$. The abundance ratio of fungivores to fungivores plus bacterivores, *i.e*., F/(F + B), was calculated to characterize the decomposition pathway (Freckman and Ettema, 1993). The maturity index (MI) of free-living nematodes was calculated as:

$$\text{MI} = \sum_{i=1}^{n} (p_i c_i) \tag{2}$$

where c_i is the colonizer-persister (c-p) rating of the ith genus of free-living nematodes present according to the 1–5 c-p scale (Bongers and Bongers, 1998).

The nematode fauna was also analyzed by a weighting system of the nematode functional guilds in relation to the enrichment and structure of the soil food web (Ferris *et al*., 2001). The enrichment index (EI, %) assesses soil food web responses to nutrient resources, and the structure index (SI, %) reflects the degree of trophic connectance in soil food webs of increasing complexity as the system matures or of progressive simplicity as the system degrades. These indices were calculated as follows.

$$\text{EI} = \frac{e}{e+b} \times 100 \tag{3}$$

where e is the abundance of nematodes in guilds representing enrichment, *i.e*., guild of bacterivores with a c-p rating of 1 (Ba1) and guild of fungivores with a c-p rating of 2 (Fu2), and b is the abundance of nematodes in guilds representing basal food web components, *i.e*., guild of bacterivores with

a c-p rating of 2 (Ba2) and guild of fungivores with a c-p rating of 2 (Fu2).

$$\mathrm{SI} = \frac{s}{s+b} \times 100 \tag{4}$$

where s is the abundance of nematodes in guilds representing structure, *i.e.*, guild of bacterivores with a c-p rating of 3–5 (Ba3–Ba5), guild of fungivores with a c-p rating of 3–5 (Fu3–Fu5), guild of omnivores with a c-p rating of 3–5 (Om3–Om5), and guild of carnivores with a c-p rating of 2–5 (Ca2–Ca5).

The channel index (CI, %), representing the decomposition pathway in the soil food web, was calculated as (Ferris *et al.*, 2001):

$$\mathrm{CI} = \frac{0.8\mathrm{Fu2}}{3.2\mathrm{Ba1} + 0.8\mathrm{Fu2}} \times 100 \tag{5}$$

Analyses of biofumigation indicators

Soil glucose analysis. Since ITCs are highly volatile and it is impractical to accurately quantify them in the field, glucose released from GL hydrolysis was measured as suggested by Al-Turki and Dick (2003) with some modifications. Soil samples were collected immediately before and one week after biofumigation for glucose determination to estimate biofumigation efficacy. Soil subsamples (20–30 cm^3) from Trials I and III were placed on dry ice whereas those from Trial II were added with 2 mL DriSolv® toluene (methylbenzene, MiliporeSigma, Germany) to deactivate soil microbial activities before transportation to the laboratory. In the laboratory, all soil subsamples were stored at -80 °C before use. One gram of soil (dry weight equivalent) was subjected to glucose analysis using a glucose assay kit (Sigma-Aldrich Chemical Co., USA) according to Al-Turki and Dick (2003). The percent change in soil glucose concentration (ΔGlu, %) was calculated using the following formula:

$$\Delta\mathrm{Glu} = \frac{\mathrm{Glu_f} - \mathrm{Glu_i}}{\mathrm{Glu_i}} \times 100 \tag{6}$$

where $\mathrm{Glu_f}$ is the final soil glucose concentration (g kg^{-1}), *i.e.*, glucose concentration one week after biofumigant crop termination, and $\mathrm{Glu_i}$ is the initial soil glucose concentration (g kg^{-1}), *i.e.*, glucose concentration before biofumigant crop termination.

Soil sulfate analysis. Due to microbial degradation of glucose which complicated glucose analysis in Trial III, soil sulfate, an alternative parameter for biofumigation efficacy estimation, was assayed as sulfate can be generated from the Lossen rearrangement of aglycone during GL hydrolysis or biofumigation (Borek *et al.*, 1996). A 5-g soil subsample (dry weight equivalent) was collected per plot at the termination of cover crop and one week after biofumigation. Each soil subsample was suspended in 25 mL deionized water in a sterile 50-mL Falcon™ conical centrifuge tube (Thermo Fisher Scientific, USA) and homogenized using a Wrist Action® shaker (Burrell Scientific LLC, USA) for 30 min. The suspension was filtered using a 2.5-μm Whatman® 42 filter paper (Sigma-Aldrich Chemical Co., USA). Extractable sulfate in the filtrate was quantified using an inductively coupled plasma-optical emission spectrometer (Thermo Fisher Scientific, USA) at the Agricultural Diagnostic Services Center, University of Hawaii at Manoa, Honolulu, USA.

Statistical and canonical correspondence analyses

Nematode data from each field trial were checked for normality using Proc Univariate in SAS version 9.4 (SAS Institute Inc., USA). Nematode abundance data were normalized using $\log_{10}(x+1)$, and nematode index data were transformed using square root prior to analysis of variance (ANOVA). The data in each trial were subjected to repeated-measures ANOVA using Proc GLM in SAS. If no significant interaction between treatment and sampling date was detected, data across sampling dates were pooled and analyzed; if the interaction was significant, the data were analyzed by date. Means were separated using the Waller-Duncan k-ratio ($k = 100$) t-test, and only true means were presented.

Nematode abundance assemblage, nematode indices, biofumigation indicators, environmental variables, zucchini growth and yield, and biofumigant crop biomass data were subjected to canonical correspondence analysis (CCA) using CANOCO™ 4.5 for Windows (Microcomputer Power, USA). The nematode abundance assemblage variables included nematode richness (Rich) and abundances of bacterivores (Ba), fungivores (Fu), herbivores (He), omnivores (Om), carnivores (Ca), root-knot nematodes (Rk), and reniform nematodes (Re) in soil. Since the root-knot and reniform nematodes were the targets of control in this study, they were separated from other herbivores. Nematode indices included EI, F/(F + B) ratio, CI, MI, and SI. Environmental variables included soil nitrate (NO_3^-), soil temperature (Temp), zucchini fruit weight (Frtwt), chlorophyll content (Chl), RGI, and cover crop dry shoot biomass (Bmass). The relationships of all these variables with the biofumigation indicators, *i.e.*, soil glucose (Glu) and soil sulfate (Sul), were investigated using CCA.

RESULTS

The nematode genera present in all three field trials are presented in Table I, and the abundance of each nematode genus in every trial is presented in Tables SI–SVI (see Supplementary Material for Tables SI–SVI).

Effects of biofumigation methods on nematode abundance

As no interaction between treatment and sampling date

TABLE I

Trophic groups, taxa, and functional guilds based on colonizer-persister values of soil nematodes present in all three field trials conducted in 2016–2018 at the study site at the Poamoho Experiment Station, Waialua, Hawaii, USA

Trophic group	Taxon		Functional guild
	Family	Genus	
Algaevore (Al)	Achromadoridae	*Achromadora*	Al3
Bacterivores (Ba)	Alaimidae	*Alaimus*	Ba4
	Cephalobidae	*Acrobeles*	Ba2
		Acrobeloides	Ba2
		Cephalobus	Ba2
		Cervidellus	Ba2
		Eucephalobus	Ba2
		Pseudoacrobeles	Ba2
		Pseudocephalobus	Ba2
		Zeldia	Ba2
	Diplogasteroididae	*Diplogasteroides*	Ba1
	Diploscapteridae	*Diploscapter*	Ba1
	Monhysteridae	*Monhystera*	Ba2
	Osstellidae	*Drilocephalobus*	Ba2
	Panagrolaimidae	*Anguilluloides*	Ba1
		Panagrellus	Ba1
		Panagrolaimus	Ba1
	Plectidae	*Plectus*	Ba2
		Tylocephalus	Ba2
		Wilsonema	Ba2
	Prismatolaimidae	*Prismatolaimus*	Ba3
	Rhabditidae	Miscellaneous	Ba1
	Teratocephalidae	*Teratocephalus*	Ba3
Fungivores (Fu)	Anguinidae	*Nothotylenchus*	Fu2
	Aphelenchidae	*Aphelenchus*	Fu2
	Aphelenchoididae	*Aphelenchoides*	Fu2
	Tylenchidae	*Filenchus*	Fu2
		Tylenchus	Fu2
Herbivores (He)	Hoplolaimidae	*Helicotylenchus*	He3
		Rotylenchulus	He3
	Heteroderidae	*Meloidogyne*	He3
	Trichodoridae	*Paratrichodorus*	He4
		Trichodorus	He4
Omnivores (Om)	Aporcelaimidae	*Aporcelaimellus*	Om5
		Aporcelaimus	Om5
	Dorylaimidae	*Dorylaimus*	Om4
		Eudorylaimus	Om4
		Mesodorylaimus	Om4
	Nordiidae	*Pungentus*	Om4
Carnivores (Ca)	Mononchidae	*Mononchus*	Ca4
	Qudsianematidae	*Discolaimus*	Ca5

was detected for all nematode parameters in Trial I, the means of repeated measures are presented in Table II. Oil radish terminated by MTBP increased the abundance of bacterivorous nematodes, and brown mustard terminated by MT increased ($P < 0.05$) the abundances of both bacterivores and fungivores compared to the bare ground control (Table II). Omnivorous and carnivorous nematodes were not affected ($P > 0.05$) by the biofumigation methods compared to the control. The abundance of total herbivorous nematodes (*Helicotylenchus* spp., *Meloidogyne* spp., *Paratrichodorus* spp., *Rotylenchulus reniformis*, and *Trichodorus* spp.) was suppressed ($P < 0.05$) by both oil radish and brown mustard terminated by MTBP.

Similarly, as no interaction between treatment and sampling date was detected for all nematode parameters in Trial II, the means of repeated measures are presented in Table III. In contrast to Trial I, no significant difference was detected in the abundances of bacterivores, fungivores, and herbivores among the six biofumigation methods compared to the control in Trial II. However, brown mustard terminated by NT and MT slightly increased ($P < 0.05$) omnivore abundance compared to the control.

In Trial III, interactions between treatment and sampling date were observed for some nematode parameters (Table IV). The abundance of fungivorous nematodes was increased ($P < 0.05$) by brown mustard terminated by T, but omnivore and carnivore abundances were not affected ($P > 0.05$) by any of the termination methods compared to the control. The interaction effect of treatment and sampling date was significant ($P < 0.05$) for the abundances of bacterivorous and herbivorous nematodes; thus, these variables were analyzed by date. One week after biofumigation (February 1, 2018), the abundance of bacterivorous nematodes increased ($P < 0.05$) by brown mustard terminated by NTBP, T, MT, and MTBP (Table V). One month after biofumigation (February 22, 2018), the abundance of bacterivorous nematodes increased ($P < 0.05$) in all brown mustard treatments except in NTBP. However, this effect was dissipated by the end of the zucchini crop (March 29, 2018), as no difference was detected in the abundance of bacterivores. Similar to Trial II, the abundance of herbivorous nematodes was not affected ($P > 0.05$) by any of the biofumigation methods up to the middle of the trial period (February 1 and 22, 2018). However, brown mustard terminated by T suppressed ($P < 0.05$) the abundance of herbivores at the end of the zucchini crop (March 29, 2019) compared to the control.

Effects of biofumigation methods on nematode indices

In Trial I, oil radish terminated by MTBP increased ($P < 0.05$) nematode diversity and EI but reduced ($P < 0.05$) F/(F + B) ratio and CI compared to the control (Table II). Brown mustard terminated by MTBP increased ($P < 0.05$) nematode diversity compared to the control. However, in Trial II, none of the biofumigation methods affected ($P > 0.05$) nematode community indices compared to the control (Table III).

In Trial III, all biofumigation methods decreased ($P < 0.05$) CI, while all except NT reduced ($P < 0.05$) MI compared to the control (Table IV). Brown mustard NTBP was the only biofumigation method that increased ($P < 0.05$) richness in this trial. Since a significant interaction between treatment and sampling date was detected for EI (Table V), this variable was analyzed by date. All brown mustard biofumigation methods, regardless of soil, tissue, and cover treatments, increased ($P < 0.05$) EI one week after biofumigation (February 1, 2018), and this effect lasted until one month after biofumigant crop termination (February 22, 2018). However, this effect dissipated at the end of the zucchini cropping cycle.

TABLE II

Effects of oil radish (*Raphanus sativus* cv. Sodbuster) and brown mustard (*Brassica juncea* cv. Caliente 199) biofumigation methods[a)] on the abundances and related indices of soil nematode community in a field trial, Trial I initiated on November 17, 2016, at the Poamoho Experiment Station, Waialua, Hawaii, USA

Parameter[b)]	Control	Oil radish			Brown mustard		
		NT	MT	MTBP	NT	MT	MTBP
Abundance (cm^{-3} soil)							
Bacterivores	1.7 ± 0.3[c)]c[d)]	1.9 ± 0.7bc	2.0 ± 0.4bc	2.4 ± 0.3ab	1.3 ± 0.2c	3.1 ± 0.5a	1.8 ± 0.4c
Fungivores	1.0 ± 0.1bc	1.4 ± 0.3b	1.0 ± 0.2bc	1.0 ± 0.2c	1.0 ± 0.2c	2.1 ± 0.3a	0.7 ± 0.1c
Omnivores	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.1a	0.2 ± 0.1a	0.1 ± 0.0a
Carnivores	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
Herbivores	5.0 ± 0.8a	5.5 ± 0.9a	4.4 ± 0.5a	3.3 ± 0.5b	4.8 ± 0.7a	4.8 ± 0.8a	2.3 ± 0.4b
Richness	15 ± 4a	15 ± 4a	15 ± 4a	16 ± 4a	15 ± 4a	17 ± 4a	15 ± 4a
Diversity	5.0 ± 0.4cd	4.1 ± 0.5 d	5.6 ± 0.7bc	6.7 ± 0.8ab	5.0 ± 0.8cd	6.2 ± 0.5abc	7.6 ± 0.6a
EI (%)	58.6 ± 3.2b	58.9 ± 2.9ab	65.3 ± 2.9ab	70.4 ± 3.1a	55.3 ± 4.4b	64.7 ± 3.5ab	65.6 ± 3.1ab
F/(F + B) ratio	0.43 ± 0.05a	0.45 ± 0.03a	0.39 ± 0.05a	0.27 ± 0.04b	0.40 ± 0.05a	0.41 ± 0.04a	0.36 ± 0.04ab
SI (%)	21.5 ± 3.3a	24.3 ± 5.2a	20.0 ± 3.2a	28.7 ± 4.6a	30.9 ± 4.9a	18.0 ± 4.4a	28.8 ± 3.9a
MI	1.9 ± 0.0ab	2.0 ± 0.0ab	1.8 ± 0.0b	1.8 ± 0.1b	2.0 ± 0.1a	1.8 ± 0.1b	1.9 ± 0.1ab
CI (%)	40.5 ± 5.6a	42.8 ± 4.8a	32.4 ± 6.2ab	20.1 ± 3.8b	45.7 ± 6.9a	34.1 ± 4.4ab	29.6 ± 4.8ab

[a)] Control = no-biofumigation bare ground; NT = no-till, where the aboveground plant tissues were clipped off at the soil line using a sickle, spread evenly over the plot, and covered with a woven weed mat; MT = maceration and tillage, where the aboveground plant tissues were macerated using a line trimmer, followed by tillage to 10-cm depth using a hand-held rototiller; MTBP = MT combined with impermeable black plastic mulching.
[b)] EI = enrichment index; F/(F + B) ratio = abundance ratio of fungivores to fungivores plus bacterivores; SI = structure index; MI = maturity index; CI = channel index.
[c)] Mean ± standard error ($n = 16$).
[d)] Values followed by the same letter(s) in a row are not significantly different based on the Waller-Duncan k-ratio ($k = 100$) t-test.

TABLE III

Effects of brown mustard (*Brassica juncea* cv. Caliente 199) biofumigation methods[a)] on the abundances and related indices of soil nematode community in a field trial, Trial II initiated on July 20, 2017, at the Poamoho Experiment Station, Waialua, Hawaii, USA

Parameter[b)]	Control	NT	MNT	NTBP	T	MT	MTBP
Abundance (cm^{-3} soil)							
Bacterivores	2.0 ± 0.4[c)]a[d)]	2.1 ± 0.4a	2.1 ± 0.5a	2.9 ± 0.6a	3.0 ± 0.4a	3.5 ± 0.6a	3.0 ± 0.6a
Fungivores	1.4 ± 0.3b	1.6 ± 0.4b	1.7 ± 0.5ab	1.4 ± 0.3b	2.6 ± 0.4a	2.4 ± 0.4ab	1.7 ± 0.4ab
Omnivores	0.0 ± 0.0bc	0.1 ± 0.0a	0.0 ± 0.0c	0.0 ± 0.0ab	0.0 ± 0.0abc	0.0 ± 0.0a	0.0 ± 0.0ab
Carnivores	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
Herbivores	1.7 ± 0.3ab	2.6 ± 0.3a	3.4 ± 1.6a	4.9 ± 2.7a	1.6 ± 0.4b	1.9 ± 0.4ab	1.3 ± 0.2b
Richness	11 ± 1a	14 ± 1a	12 ± 1a	13 ± 1a	13 ± 1a	13 ± 1a	12 ± 1a
Diversity	4.38 ± 0.37a	4.76 ± 0.30a	4.48 ± 0.56a	4.51 ± 0.51a	4.81 ± 0.20a	5.05 ± 0.35a	4.93 ± 0.29a
EI (%)	50.76 ± 3.83ab	55.80 ± 3.76ab	59.63 ± 3.16a	61.21 ± 4.53a	56.58 ± 2.97ab	60.78 ± 2.70a	47.92 ± 3.58b
F/(F + B) ratio	0.37 ± 0.04a	0.41 ± 0.05a	0.40 ± 0.04a	0.36 ± 0.05a	0.46 ± 0.03a	0.43 ± 0.04a	0.36 ± 0.05a
SI (%)	10.3 ± 2.5ab	14.8 ± 3.6a	8.0 ± 3.0ab	12.6 ± 3.7ab	12.1 ± 4.6ab	13.7 ± 3.8a	3.6 ± 0.9b
MI	1.9 ± 0.0a	1.9 ± 0.0a	1.9 ± 0.0a	1.9 ± 0.1a	1.9 0 ± 0.0a	1.9 ± 0.0a	1.9 ± 0.0a
CI (%)	52.0 ± 7.3a	48.1 ± 6.5a	39.6 ± 5.0a	48.4 ± 8.9a	45.9 ± 3.3a	57.5 ± 7.5a	37.3 ± 5.1a

[a)] Control = no-biofumigation bare ground; NT = no-till, where the aboveground plant tissues were clipped off at the soil line using a sickle, spread evenly over the plot, and covered with a woven weed mat; MNT = NT but the aboveground plant tissues were macerated using a line trimmer; NTBP = MNT but the aboveground plant tissues were covered with impermeable black plastic mulch; T = direct tillage using a hand-held rototiller without prior tissue maceration; MT = maceration and tillage, where the aboveground plant tissues were macerated using a line trimmer, followed by tillage to 10-cm depth using a hand-held rototiller; MTBP = MT combined with impermeable black plastic mulching.
[b)] EI = enrichment index; F/(F + B) ratio = abundance ratio of fungivores to fungivores plus bacterivores; SI = structure index; MI = maturity index; CI = channel index.
[c)] Mean ± standard error ($n = 16$).
[d)] Values followed by the same letter(s) in a row are not significantly different based on the Waller-Duncan k-ratio ($k = 100$) t-test.

Relationships between biofumigation indicators and response variables

Relationships between biofumigation indicators (soil glucose and sulfate) and response variables (nematode parameters, environmental variables, and plant growth and yield) for each trial are depicted in ordination diagrams (Figs. 1–3). In Fig. 1 for Trial I, the first two canonical axes in the ordination diagram explained up to 88.3% of the variance between the nematode abundance assemblage variables and other variables, including nematode indices, biofumigation indicators, environmental variables, zucchini growth and yield, and biofumigant crop biomass. Soil glucose was negatively correlated with the abundances of root-knot, omnivorous, and carnivorous nematodes, but it was positively related to EI and soil temperature.

TABLE IV

Effects of brown mustard (*Brassica juncea* cv. Caliente 199) biofumigation methods[a)] on the abundances of soil nematode trophic groups and related indices of free-living nematodes in a field trial, Trial III initiated on December 7, 2017, at the Poamoho Experiment Station, Waialua, Hawaii, USA

Parameter[b)]	Control	NT	MNT	NTBP	T	MT	MTBP
Abundance (cm^{-3} soil)							
Fungivores	1.4 ± 0.5[c)]b[d)]	2.4 ± 0.9b	1.7 ± 0.5b	1.8 ± 0.7ab	2.3 ± 0.5a	2.3 ± 0.6ab	3.7 ± 1.6ab
Omnivores	0.1 ± 0.0ab	0.1 ± 0.0a	0.2 ± 0.1a	0.2 ± 0.1a	0.1 ± 0.0ab	0.0 ± 0.0b	0.1 ± 0.0ab
Carnivores	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
Richness	12 ± 1b	13 ± 1ab	15 ± 2ab	15 ± 1a	14 ± 1ab	13 ± 1b	13 ± 1ab
Diversity	4.1 ± 0.5a	5.1 ± 0.5a	4.8 ± 0.6a	4.7 ± 0.5a	4.1 ± 0.4a	4.2 ± 0.4a	4.9 ± 0.6a
F/(F + B) ratio	0.3 ± 0.1a	0.3 ± 0.1a	0.2 ± 0.1a	0.3 ± 0.1a	0.3 ± 0.0a	0.3 ± 0.0a	0.3 ± 0.0a
SI (%)	22.6 ± 5.7a	24.0 ± 6.8a	27.0 ± 6.3a	20.5 ± 4.9a	16.3 ± 3.5a	10.5 ± 2.3a	9.1 ± 2.1a
MI	1.8 ± 0.1a	1.7 ± 0.1ab	1.6 ± 0.1bc	1.6 ± 0.1b	1.4 ± 0.0c	1.4 ± 0.1c	1.4 ± 0.1c
CI (%)	44.8 ± 9.8a	19.6 ± 4.7b	12.5 ± 3.3b	16.2 ± 4.5b	11.1 ± 1.5b	13.8 ± 3.7b	12.4 ± 2.5b

[a)] Control = no-biofumigation bare ground; NT = no-till, where the aboveground plant tissues were clipped off at the soil line using a sickle, spread evenly over the plot, and covered with a woven weed mat; MNT = NT but the aboveground plant tissues were macerated using a line trimmer; NTBP = MNT but the aboveground plant tissues were covered with impermeable black plastic mulch; T = direct tillage using a hand-held rototiller without prior tissue maceration; MT = maceration and tillage, where the aboveground plant tissues were macerated using a line trimmer, followed by tillage to 10-cm depth using a hand-held rototiller; MTBP = MT combined with impermeable black plastic mulching.
[b)] F/(F + B) ratio = abundance ratio of fungivores to fungivores plus bacterivores; SI = structure index; MI = maturity index; CI = channel index.
[c)] Mean ± standard error ($n = 16$).
[d)] Values followed by the same letter(s) in a row are not significantly different based on the Waller-Duncan k-ratio ($k = 100$) t-test.

TABLE V

Effects of brown mustard (*Brassica juncea* cv. Caliente 199) biofumigation methods[a)] on the abundances and enrichment index (EI) values of soil bacterivorous and herbivorous nematodes one week (February 1, 2018) and one month (February 22, 2018) after biofumigation and at the end (March 29, 2018) of the subsequent zucchini (*Cucurbita pepo* cv. Felix) growth season in Trial III initiated on December 7, 2017, at the Poamoho Experiment Station, Waialua, Hawaii, USA

Date	Parameter	Control	NT	MNT	NTBP	T	MT	MTBP
February 1, 2018	Abundance (cm^{-3} soil)							
	Bacterivores	0.4 ± 0.1[b)]d[c)]	0.4 ± 0.2d	0.5 ± 0.2cd	1.3 ± 0.7bc	4.6 ± 1.5a	2.2 ± 0.6ab	1.4 ± 0.5b
	Herbivores	0.5 ± 0.2a	0.5 ± 0.1a	0.4 ± 0.2a	0.9 ± 0.3a	1.4 ± 0.5a	1.1 ± 0.7a	0.2 ± 0.1a
	EI (%)	35.8 ± 5.6d	51.9 ± 3.1c	66.9 ± 1.2b	72.9 ± 4.7ab	84.7 ± 2.5a	79.7 ± 9.2ab	80.3 ± 2.6ab
February 22, 2018	Abundance (cm^{-3} soil)							
	Bacterivores	2.2 ± 0.5b	5.4 ± 2.4a	4.5 ± 0.8a	3.2 ± 0.4ab	5.4 ± 0.7a	5.6 ± 1.5a	6.2 ± 1.1a
	Herbivores	1.3 ± 0.4a	1.9 ± 0.3a	1.5 ± 0.1a	2.0 ± 0.5a	1.8 ± 0.4a	0.9 ± 0.3a	1.1 ± 0.3a
	EI (%)	49.9 ± 5.3b	69.9 ± 6.2a	78.5 ± 1.9a	67.2 ± 7.3ab	75.5 ± 6.1a	76.6 ± 3.0a	74.6 ± 5.1a
March 29, 2018	Abundance (cm^{-3} soil)							
	Bacterivores	7.2 ± 2.5a	9.7 ± 1.0a	15.1 ± 3.0a	18.8 ± 6.0a	9.6 ± 2.8a	10.5 ± 3.8a	15.0 ± 5.0a
	Herbivores	6.4 ± 1.0a	3.4 ± 1.1ab	4.3 ± 1.1a	4.0 ± 0.8a	1.2 ± 0.5b	2.6 ± 0.8ab	2.7 ± 0.7ab
	EI (%)	86.4 ± 4.8a	89.1 ± 3.9a	94.0 ± 1.3a	90.9 ± 3.4a	91.4 ± 1.4a	89.2 ± 1.8a	89.5 ± 2.0a

[a)] Control = no-biofumigation bare ground; NT = no-till, where the aboveground plant tissues were clipped off at the soil line using a sickle, spread evenly over the plot, and covered with a woven weed mat; MNT = NT but the aboveground plant tissues were macerated using a line trimmer; NTBP = MNT but the aboveground plant tissues were covered with impermeable black plastic mulch; T = direct tillage using a hand-held rototiller without prior tissue maceration; MT = maceration and tillage, where the aboveground plant tissues were macerated using a line trimmer, followed by tillage to 10-cm depth using a hand-held rototiller; MTBP = MT combined with impermeable black plastic mulching.
[b)] Mean ± standard error ($n = 16$).
[c)] Values followed by the same letter(s) in a row are not significantly different based on the Waller-Duncan k-ratio ($k = 100$) t-test.

In Trial II, the first two canonical axes in the ordination diagram explained up to 95.7% of the variance between the nematode abundance assemblage variables and other variables (Fig. 2). Soil glucose was negatively related to the abundance of herbivores, SI, chlorophyll content, and zucchini fruit weight, but it was positively related to the abundances of bacterivores, fungivores, carnivores, and omnivores, CI, and diversity (Fig. 2).

In Trial III, the first two canonical axes in the ordination diagram explained up to 89.0% of the variance between the nematode abundance assemblage variables and the other variables (Fig. 3). Glucose was negatively related to zucchini RGI, chlorophyll content, fruit weight, and soil nitrate concentration at the end of the experiment. In contrast, positive relationships were detected between soil glucose and the abundance of fungivores and nematode diversity. Apart from soil glucose, soil sulfate was included as an indicator of biofumigation in this trial. One week after biofumigant crop termination, soil sulfate concentration had strong negative relationships with the abundance of root-knot nematodes, total plant-parasitic nematodes, CI, and MI, and it had strong positive relationships with the abundance of bacterivores,

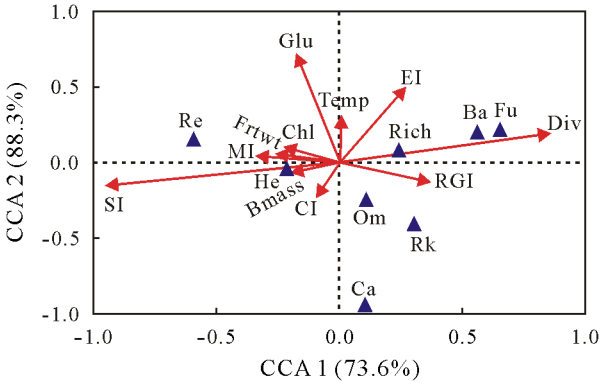

Fig. 1 Canonical correspondence analysis (CCA) ordination diagram showing relationships among soil glucose, a biofumigation indicator, and response variables (soil nematode parameters, environmental variables, and plant growth and yield) in the zucchini (*Cucurbita pepo* cv. Felix) cropping system following oil radish (*Raphanus sativus* cv. Sodbuster) and brown mustard (*Brassica juncea* cv. Caliente 199) biofumigation in a field trial, Trial I initiated on November 17, 2016, at the Poamoho Experiment Station, Waialua, Hawaii, USA. Blue triangles are nematode abundance assemblage variables for bacterivores (Ba), fungivores (Fu), herbivores (He), omnivores (Om), carnivores (Ca), reniform (Re) and root-knot nematodes (Rk), and richness (Rich). Red arrows are biofumigant crop dry biomass (Bmass), zucchini chlorophyll content (Chl), channel index (CI), diversity (Div), enrichment index (EI), zucchini fruit weight (Frtwt), maturity index (MI), soil glucose (Glu), root-gall index (RGI), structure index (SI), and soil temperature (Temp).

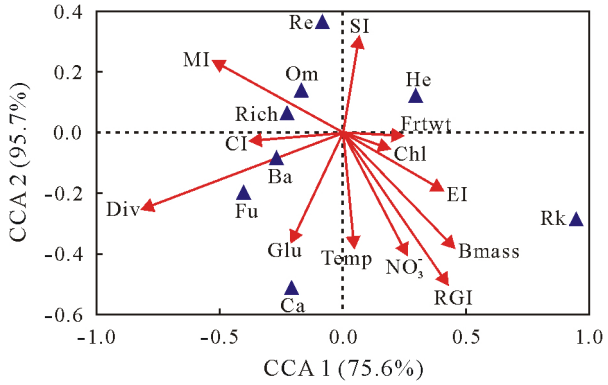

Fig. 2 Canonical correspondence analysis (CCA) ordination diagram showing relationships among soil glucose, a biofumigation indicator, and response variables (nematode parameters, environmental variables, and plant growth and yield) in the zucchini (*Cucurbita pepo* cv. Felix) cropping system following brown mustard (*Brassica juncea* cv. Caliente 199) biofumigation in a field trial, Trial II initiated on July 20, 2017, at the Poamoho Experiment Station, Waialua, Hawaii, USA. Blue triangles are nematode abundance assemblage variables for bacterivores (Ba), fungivores (Fu), herbivores (He), omnivores (Om), carnivores (Ca), reniform (Re) and root-knot nematodes (Rk), and richness (Rich). Red arrows are biofumigant crop dry biomass (Bmass), zucchini chlorophyll content (Chl), channel index (CI), diversity (Div), nutrient enrichment index (EI), zucchini fruit weight (Frtwt), maturity index (MI), soil glucose (Glu), soil nitrate (NO_3^-), root-gall index (RGI), structure index (SI), and soil temperature (Temp).

EI, soil temperature, and biomass of brown mustard.

DISCUSSION

Effects of oil radish and brown mustard biofumigation on nematode communities

Based on the nematode community analysis, oil radish was better for soil health improvement than brown mustard especially when it was terminated by MTBP. This termination method suppressed plant-parasitic nematodes, increased nematode diversity and bacterivore abundance, and reduced the F/(F + B) ratio and CI, indicating higher bacterial decomposition than fungal decomposition in the soil. In addition, oil radish terminated by MTBP increased EI, indicative of soil nutrient enrichment. All of these effects lasted for the entire zucchini cropping cycle. This observation was in line with previous findings that oil radish is a good green manure cover crop that can enhance soil nutrient enrichment (Gruver *et al.*, 2010). The efficacy of oil radish biofumigation in suppressing plant-parasitic nematodes has been well documented (Melakeberhan *et al.*, 2008; Ploeg, 2008). The present study further demonstrated that terminating oil radish using the MTBP method could improve soil health, enrich soil nutrients, and suppress plant-parasitic nematodes. Brown mustard Caliente 199 is a known biofumigant crop with a higher GL content than other *Brassica* crops (Rudolph *et al.*, 2015), and it was expected to be more effective in biofumigation. However, brown mustard terminated by MTBP,

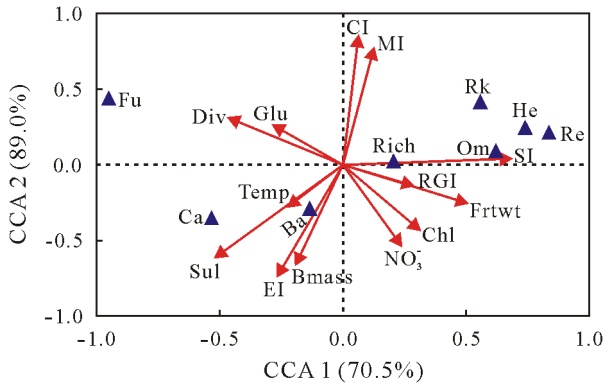

Fig. 3 Canonical correspondence analysis (CCA) ordination diagram showing relationships among biofumigation indicators (soil glucose and sulfate) and response variables (soil nematode parameters, environmental variables, and plant growth and yield) in zucchini (*Cucurbita pepo* cv. Felix) cropping system following brown mustard (*Brassica juncea* cv. Caliente 199) biofumigation in a field trial, Trial III initiated on December 7, 2017, at the Poamoho Experiment Station, Waialua, Hawaii, USA. Blue triangles are nematode abundance assemblage variables for bacterivores (Ba), fungivores (Fu), herbivores (He), omnivores (Om), carnivores (Ca), reniform (Re) and root-knot nematodes (Rk), and richness (Rich). Red arrows are biofumigant crop dry biomass (Bmass), zucchini chlorophyll content (Chl), soil nitrate (NO_3^-), channel index (CI), diversity (Div), nutrient enrichment index (EI), zucchini fruit weight (Frtwt), maturity index (MI), soil glucose (Glu), root-gall index (RGI), structure index (SI), soil sulfate (Sul), and soil temperature (Temp).

the biofumigation method with the highest suppression of plant-parasitic nematodes, had no negative effects on the free-living nematode counterparts. Only the abundance of bacterivores was lower in the brown mustard MTBP than in the oil radish MTBP. This confirmed that effective biofumi-

gation against plant-parasitic nematodes did not compromise soil health.

Effects of brown mustard biofumigation methods on the nematode communities

In Trials II and III, brown mustard terminated by T or MTBP suppressed herbivorous nematodes. Interestingly, none of the six termination methods, including the most plant-parasitic nematode-suppressive termination methods (T and MTBP), reduced the abundance of bacterivores, omnivores, or carnivores. Increase in EI and reduction in F/(F + B) ratio or CI by MTBP could be associated with a low carbon (C) to nitrogen (N) ratio (C:N of *ca*. 10) of oil radish and brown mustard tissues (Gruver *et al*., 2010). A low C:N ratio often stimulates higher bacterial decomposition than fungal decomposition (Gruver *et al*., 2010). In contrast, when the brown mustard growing period was extended to seven weeks, at which time the tissue became relatively fibrous, biofumigation by tillage only (T) but not MTBP increased the abundance of fungivorous nematodes in Trial III.

Overall, biofumigation using brown mustard terminated by MTBP increased EI rather consistently, and the effects lasted throughout the zucchini crop cycle in Trial I, or up to one month after biofumigant crop termination in Trial III. In Trial II, brown mustard biofumigation showed no nutrient enrichment, but termination by NT or MT increased the abundance of omnivorous nematodes, suggesting that the soil might have been in the later succession of nutrient cycling after the biofumigation. Nonetheless, in all three trials, biofumigation using brown mustard neither compromised soil nutrient enrichment nor disturbed the soil food web structure (no change in SI). Oil radish and brown mustard, similar to other green manures, when incorporated into the soil, added organic matter. This, in turn, increased the activities of microbes which then supported an increased abundance of bacterivorous nematodes in the soil food chain (Grabau *et al*., 2018).

Relationship between biofumigation indicators and other response variables

Canonical correspondence analysis of all three trials showed strong relationships among the variables measured, with the first two canonical axes in the ordination diagram explaining $> 85\%$ of the variance. In all three trials, soil glucose and sulfate levels were negatively correlated with the abundance of root-knot nematodes, RGI of zucchini, and total abundance of herbivores (plant-parasitic nematodes). The lack of relationships between glucose and the abundance of reniform nematodes in all three trials could be due to the insensitivity of these nematodes to biofumigation (Waisen *et al*., 2020).

However, it is worth noting that glucose was negatively correlated with root-knot nematodes only in Trials I and II but not in Trial III, where sulfate was negatively correlated with root-knot nematodes, reniform nematodes, and total herbivores (Fig. S1, see Supplementary Material for Fig. S1). These inconsistent trends suggest that soil glucose is a poorer indicator of biofumigation than soil sulfate. The glucose levels measured in Trial III might have been altered by microbial degradation of glucose in the soil during transportation from the field to the laboratory. Storage of the soil samples in dry ice did not arrest or minimize microbial degradation of glucose, as indicated by the lack of a relationship between glucose and herbivores. Glucose is a labile C that is more likely to be metabolized by soil microbes if precautionary measures are not taken (Rousk *et al*., 2014).

To address microbial degradation of glucose, in Trial II, toluene (methylbenzene) was immediately added to the soil samples after their collection and prior to their transport to the laboratory, because toluene has been shown to deactivate microbial degradation of glucose (Al-Turki and Dick, 2003). The use of toluene resulted in negative correlations of herbivores and root-knot nematodes with glucose and positive correlations between free-living nematode trophic groups (bacterivores, fungivores, omnivores, and carnivores) and glucose. This is consistent with previous findings that biofumigation increases the abundance of bacterivores (Valdes *et al*., 2012). In addition, glucose was positively related to CI and negatively related to EI and SI in Trial II. Soil health conditions in Trial II, as shown by ANOVA (Table II), suggest that the soil was in a later stage of decomposition, that is, undergoing mainly fungal decomposition. This was consistent with the CCA result, where a strong positive relationship between CI and glucose was observed in Trial II (Fig. 2).

The current study suggests that glucose can be used as a good indicator of biofumigation if soil preservation reagents, such as toluene, are used to arrest microbial degradation and respiration of glucose soon after soil sampling as was done in Trial II. However, handling soil with toluene can be harmful (Filley *et al*., 2004). Therefore, in Trial III, we examined soil sulfate as an alternative biofumigation indicator because sulfate is a by-product of GL hydrolysis (Borek *et al*., 1996). Knowing that soil sulfate can be derived from many other sources, we quantified its levels in different treatments (Fig. S2, see Supplementary Material for Fig. S2). Soil sulfate was negatively correlated with the abundances of total herbivorous, root-knot nematodes, and reniform nematodes, indicating that it was a good indicator of biofumigation. Strong positive relationships were detected between soil sulfate and the abundance of bacterivores and EI in Trial III, further confirming that biofumigation did not compromise soil nutrient enrichment.

Soil sulfate, as a stable biofumigation indicator, revealed positive relationships of biofumigation efficacy with biofumigant crop biomass and soil temperature. This verified that the biomass of biofumigant cover crops affected the efficacy of biofumigation, as reported by Ngala *et al*. (2015) and Vervoort *et al*. (2014). In Trials II and III, which involved only brown mustard, EI always increased with increasing biomass of brown mustard, indicating that biofumigation did not have a negative impact on soil nutrient enrichment. Ploeg (2008) and Fourie *et al*. (2016) highlighted a positive relationship between biofumigation efficacy and soil temperature. Zucchini is a short-term crop of only 2 months, which might not be long enough to observe an increase in SI associated with brown mustard cover cropping and biofumigation. However, the abundance of carnivorous nematodes had a strong positive relationship with soil sulfate concentration in Trial III. This is most likely due to a bottom-up regulation of the carnivorous nematodes, where the population densities of carnivorous nematodes are regulated by the abundance of bacterivorous nematodes, a scenario observed by Wang *et al*. (2004, 2011) and Cheng *et al*. (2018).

CONCLUSIONS

This study demonstrated that biofumigation can be effective against plant-parasitic nematodes without compromising soil health or changing the nematode community structure. Oil radish or brown mustard as a biofumigant crop, if terminated by the MTBP method for one week, suppressed the abundance of plant-parasitic nematodes. Bacterial decomposition was enhanced in the MTBP treatment, which led to nutrient enrichment. However, sometimes these effects did not last throughout the subsequent zucchini crop cycle. Soil sulfate was a better biofumigation indicator than soil glucose.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article can be found in the online version.

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