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Original article

Soil nematode assemblages indicate the potential for biological regulation of pest species

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ABSTRACT

In concept, regulation or suppression of target nematode pest species should be enhanced when an abundance of predator species is supported by ample availability of bacterial- fungal- and non-damaging plant-feeding prey species. We selected soils from natural and managed environments that represented different levels of resource availability and disturbance. In microcosm chambers of each soil, in its natural state or after heat defaunation, we introduced test prey species not already resident in the soils (*Meloidogyne incognita* and *Steinernema feltiae*). Survival of the test prey was determined after a 5-day bioassay exposure. Across the soils tested, predator abundance and biomass were greater in undisturbed soils with plentiful resources and lower in soils from agricultural sites. Suppressiveness to the two introduced species increased with both numerical abundance and metabolic footprint of the predator assemblages. The magnitude of the increase in suppressiveness was greater at low numbers of predators then dampened to an asymptotic level at greater predator abundance, possibly determined by temporal and spatial aspects of the bioassay system and/or satiation of the predators. The more resource-limited the predators were and the higher the metabolic predator footprint, the greater the suppressiveness. The applied implications of this study are that soil suppressiveness to pest species may be enhanced by increasing resources to predators, removing chemical and physical constraints to their survival and increase, and altering management practices so that predators and target prey are co-located in time and space.

1. Introduction

Soil health and ecosystem functioning are important topics in current ecological and agricultural research. Doran et al. (1996) defined soil health as “the continued capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain biological productivity, maintain the quality of air and water environments, and promote plant, animal and human health”. Soil suppressiveness, either general or specific, is an important function of a healthy soil (van Bruggen and Semenov, 2000) to which physical, chemical and biological factors might contribute (Janvier et al., 2007). Most of the research on indicators of soil suppressiveness focusses on microbial communities and is often performed with an experimental design in which few factors are varied (Postma et al., 2008). Many soil mesofauna, including nematodes in several trophic levels, are one or two steps higher in the food chain than microbes. Their generation time (weeks to months) is longer than that of the metabolically-active microbes (hours to days), making them more temporally stable rather than fluctuating with ephemeral nutrient flushes (Nannipieri et al., 1990; Neher, 2001). Moreover, nematodes have been used extensively as indicators of soil biodiversity and functioning (Ferris and Tuomisto, 2015; Neher, 2001) and as indicators of environmental disturbances (Bongers and Ferris, 1999; Ferris et al., 2001; Yeates, 2003).

Soil ecosystem services are benefits derived from ecosystems that are necessary to maintain soil health and productivity; they are delivered by the ecosystem functions of soil organisms (Brussaard, 2012). Guilds of soil biota are closely associated with different ecosystem functions, for example, Carrascosa et al. (2014) reported a positive and significant relationship between soil suppressiveness, soil food web structure and nematode diversity. Suppression of pest and disease organisms is an ecosystem service that is the outcome of the ecosystem function of biological population

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regulation (Brussaard, 2012). Suppression might be induced by adding biocontrol agents (e.g. Jaffee, 2000) but in the absence of specific predator-prey associations, higher trophic levels might generally enhance suppression by predation on plant-feeding species (Sánchez-Moreno and Ferris, 2007). In that case, target species could be regulated by controlled increases in bacterial-, fungal- and non-damaging plant-feeding nematodes, which will provide resources to enhance predator abundance and promote important and useful population interactions. System level regulation of soil pest species might be obtained by providing additional resources to predators through a trophic web of carbon and energy exchange. For this system to work, some criteria need to be fulfilled. First, the carbon and energy flow must be available to the relevant soil organisms and second, chemical and physical constraints to the survival and population growth of the specialist or generalist predators must be alleviated. Most studies on natural regulation by predator nematodes have focused on the direct top-down effect on the prey (Bilgrami et al., 2005; Khan and Kim, 2005) or on the impact of resources on predator and consequently also on prey guilds (Ferris et al., 2012a, 2012b). In those studies, additional resources (e.g., those provided by cover crops and organic amendments) not only affected organisms at the entry level of the food web (prey guilds) but were also transferred to higher trophic levels (predator guilds) which consequently increased top-down pressure on herbivore nematodes (Ferris et al., 2012b).

Functional interactions between predators and prey can only occur if the organisms are in the same place at the same time and are thus highly affected by the patchiness of the component populations. Previous studies on this topic (e.g. Carrascosa et al., 2014; Ferris et al., 2012b; Min and Toyota, 2013; Sánchez-Moreno and Ferris, 2007), were based on composited and mixed bulk samples which essentially eliminates the spatial component of the above criteria. Therefore, we tested in intact soil cores the hypothesis that the numerical, biomass or functional abundance of predator nematodes, either specialists or generalists, are useful indicators of suppressiveness of opportunistic plant-feeding species. Differing abundance of resident prey and predator populations in each core led us to the hypothesis that soil patches with hungry (i.e., resource-restricted) predators are likely to be more suppressive than patches with abundant available prey and satiated predators. In that case, suppression is not only a function of predator abundance but also the availability of resident prey per predator. As a caveat to the experimental bioassay design and observations, we emphasize that besides their direct effects on the prey and their active involvement in succession within the soil food web, the predator nematodes are also indicators of the presence of predation and regulation by all organisms in the system that have similar life course characteristics and that participate in the ecosystem function (Stirling, 2014b; Yeates et al., 2009). Through their comparable function, response to resources and sensitivity to disturbances, many different types of organisms contribute to the same ecosystem services (Sánchez-Moreno et al., 2009). Consequently, our intact and undisturbed microcosm experiments potentially provide a proof of concept that can be translated to field scale application.

2. Materials and methods

Single soil patches, from a variety of soils and inhabited by a range (both in number and taxa) of naturally occurring predatory nematodes, were tested in 5-day bioassays inoculated with a constant number of introduced prey (*Steinernema feltiae* and *Meloidogyne incognita*).

2.1. Sampling methods and soil characteristics

To obtain a wide range of abundance and diversity of both predatory nematodes and prey, samples were collected from both natural and agriculturally-influenced sites in California, USA, representing a wide range of edaphic conditions and levels of disturbance (Fig. 1). The sites included seven natural and apparently undisturbed habitats: under a manzanita bush (*Arcostaphylos* sp.) (U-MAN) and a horse-chestnut tree (*Aesculus californica*) (U-HCT) at the Audubon Bobcat Ranch Reserve in western Yolo County at 38° 32.309′N, 122° 02.937′W and 38° 32.307′N, 122° 02.921′W, respectively, in the UC Davis Putah Creek Riparian Reserve (U-PCR1 and 2) at 38° 31.743′N, 121° 46.867′W and 38° 31.249′N, 121° 46.183′W, under a boxwood hedge (*Buxus* sp.) on the UC Davis campus near Hart Hall (U-HHH) at 38° 32.442′N,121° 45.072′ W, from undisturbed soil of the Field of Dreams (U-FOD) in which alfalfa (*Medicago sativa*) was the predominant plant (38° 31.743′ N, 121° 52.273′ W) at the UC Davis Russell Ranch Sustainable Agriculture Facility and from moist soil near a natural spring on Creekside Drive in Shingle Springs (U-SS) in Eldorado County at 38° 38.731 N, 120° 55.909′ W. To represent agriculturally-influenced and thus disturbed habitats, samples were collected from four locations: untreated and yard-waste-amended plots of an organic amendment experiment (no crop at time of sampling) at the Hansen Agricultural Research and Extension Center (D-HAR) (34° 19.575′ N, 119° 06.459′ W) near Santa Paula, Ventura County, from a wheat field (D-RR) (38° 31.743′ N, 121° 52.273′ W) in a long-term cropping systems project at the UC Davis Russell Ranch Sustainable Agriculture Facility, and from two grape vineyards, one in Lodi, San Joaquin County (D-GVL) (rootstock cv Freedom, 38° 10.693 N, 121° 13.800′ W) and one in Dunnigan, Yolo County (D-GVD) (rootstock cv 101-14, 38° 51.181′ N, 121° 55.452′ W).

At each site, intact cores were collected in metal cylinders (depth 5 cm, diam. 5 cm, volume 98.2 cm^3) to ensure that patches of soil and organisms remained intact and undisturbed. Cylinders were pushed into the soil and then carefully excavated and covered top and bottom with plastic petri dishes to hold the soil in place during transportation. The rationale for this sampling strategy was to assemble microcosms with a diversity of predator/prey ratios by taking advantage of the patchy distribution of nematodes in soil. Between 3 and 15 pairs of cores were taken at each site. Each pair of cores was considered more likely to represent the same or similar soil patches than would be the case with individual cores positioned at random. In the laboratory, the soil in one core of each pair was heat-defaunated (DF), the other remained non-defaunated (NDF). The purpose of the DF cores was to obtain a measure of the suppressiveness of the physical and chemical component of the soil in the absence of resident biological activity while that of the NDF cores was to assess the additional suppressiveness of the biological component. Three additional samples from each site were used to check for natural occurrence of the test nematodes (i.e., *M. incognita* and *S. feltiae*), for abiotic soil measures (DOC, %N, % C, C/N, %silt, %sand, %clay, moisture %, pH), and microbial biomass carbon (MBC). All cores of soil were transported to the laboratory in insulated containers and stored at 4 °C until processed.

Dissolved organic carbon (DOC) was determined from unfumigated extracts and MBC was the difference between DOC in unfumigated and fumigated extracts (Brookes et al., 1985; Vance et al., 1987). Organic C in 0.5 M K_2SO extracts was measured after dilution (1:10) with a Phoenix 8000 UV enhanced-persulfate digestion TOC analyzer (Dohrmann [Tekmar-Dohrmann], Manson, OH) according to the method of Wu et al. (1990). Samples were air-dried, sieved (<2 mm) and ground in a mortar and pestle. Weighed subsamples of approximately 30 mg were analyzed for C and N content using an elemental combustion analyzer (Costech Analytical

Fig. 1. Samples were collected from seven natural and apparently undisturbed habitats: U-MAN, U-HCT, U-PCR1 and 2, U-HHH, U-FOD, U-SS, and from four agricultural habitats: D-HAR, D-RR, D-GVL, D-GVD. The habitats U-HCT + U-MAN and U-FOD + D-RR are represented by a single dot as the scale of the map did not allow to provide separate symbols.

Technologies, Inc., Valencia, CA) and 0.1–0.5 g of soil was used for particle-size distribution (PSD) analysis using a laser diffraction analyzer (Beckman–Coulter LS-230 with a 750 nm laser beam, International Equipment Trading, Ltd., Vernon Hills, Illinois, USA). Particle size categories for clay, silt and sand were 0.041–2.0 μm, 2–47.94 μm and 47.94–2000 μm respectively.

2.2. Bioassay

The suppressiveness bioassay was adapted from Sánchez-Moreno and Ferris (2007). In total, 72 paired cores, i.e., 21 from agricultural disturbed sites (10 from D-HAR,, 5 from D-RR, 3 from D-GVL, 3 from D-GVD) and 51 from natural undisturbed habitats (4 from U-MAN, 9 from U-HCT, 15 from U-PCR, 5 from U-HHH, 12 from U-FOD, 6 from U-SS) were used for the bioassay. To prevent loss of soil, the bottoms of the metal cylinders containing intact cores were covered with tissue paper affixed with a rubber band. Cylinders filled with sand (sand controls, 5 replicates in each test) were also inoculated with test nematodes and served as a biological quality control across the bioassays of soils from different sites. One cylinder of each pair was defaunated (3.5 h at 65 °C) while the other remained undisturbed. After cooling down, water was added to all cores (DF, NDF and sand controls) to bring them to field capacity (excess water drained overnight). An inoculum containing the same densities (125 ml^{-1}) of both *M. incognita* J2 and *S. feltiae* infective juveniles (IJ) was prepared from single species cultures on tomato roots and single species liquid cultures, respectively. To measure suppressiveness to introduced prey, 1 ml of test nematode suspension was injected into each of four locations in the core (depth ± 2.5 cm), a total of 500 individuals of each species, using a glass syringe with a metal canula. The cores were incubated at room temperature for 5 days in a plastic box containing wet tissue paper to minimize drying.

2.3. Nematode analyses and interpretation

After a 5-day bioassay exposure, the soil was removed from the cores and nematodes were extracted using a combination of decanting and sieving and Baermann funnel methods (Barker, 1985). Samples were washed through a 0.25 mm aperture sieve to remove large particles and onto a 38 μm sieve to separate nematodes from excess water. Nematodes and residue from the 38 μm sieve were washed into beakers and placed on Baermann funnels for 48 h to allow active nematodes to separate from the residual debris. Nematodes were counted using a dissecting microscope, concentrated by centrifugation, fixed with 70 °C formaldehyde (4% with 1% glycerol) and mounted on mass slides (slide 75 mm × 50 mm, cover glass 48 mm × 60 mm). The first 150 nematodes other than the test species encountered in the sample were identified to genus or family. The number of surviving

introduced test prey in the nematode extracts was determined by assessing their abundance among at least half of the total number of nematodes in the samples. In the nematode extracts of the DF cores, occasionally, also few rhabditid juveniles were observed next to the introduced prey, indicating that defaunation was not always 100% efficient.

The nematode community was described using relative and absolute abundances, as appropriate for the metric, of nematode trophic groups, nematode taxa abundance and taxa richness. Five nematode trophic groups were used: bacterial feeders, fungal feeders, herbivores, predators *s.l* (= omnivores or generalists able to feed on a diversity of food sources) and predators *s.s.* (= specialist predators restricted primarily to nematodes as a food source) (adapted from Yeates et al. (1993)). Total biomass and trophic group metabolic footprints (Ferris, 2010a), were calculated using the online web application NINJA (Sieriebriennikov et al., 2014). In the faunal analysis, we observed that the Dorylaimidae in the U-PCR 2 cores were very small. Therefore, after measuring the length of 35 individuals, the body mass was manually adapted from 8.83 (Dorylaimidae family average generated by Ninja) to 1.04 (based on own measurements) for that site. This change resulted in a lower, yet more accurate, total biomasses and metabolic footprints for U-PCR2 cores compared the default settings in NINJA.

Soil suppressiveness was calculated as the absolute suppressiveness in the DF cores minus the absolute suppresiveness in the NDF cores divided by the average suppressiveness in the defaunated cores (AvDF) for that soil: (DF-NDF)/AvDF. The suppressive potential of the soil patches represented by each microcosm was calculated as the ratio of the numbers of predators and prey (i.e., all nematodes that are not predators are considered prey) (Sánchez-Moreno and Ferris, 2007). The resource availability to the predators was expressed as the quotient of resident prey biomass and predator metabolic footprint.

2.4. Statistical analyses

Differences in the abiotic factors between sample sites were analyzed using Principal Component Analysis (PCA) in Primer v6 (Clarke and Warwick, 2001). In order to test for and to visualize differences between nematode assemblages in the soil patches (taxa abundance data) all cores were compared using ANOSIM and non-metric MultiDimensional Scaling (nMDS) respectively, based on Bray–Curtis similarity matrices on square root transformed abundance data (individuals/100 cm^3) in Primer v6 (Clarke and Warwick, 2001). In Primer v6, Bray–Curtis was chosen as a similarity index for a number of ecologically relevant reasons (see Clarke et al., 2006). A mild transformation (square root) was performed on the abundance data in order to reduce the relative differences among taxa, allowing more species to play a role in the analysis, and at the same time reducing noise resulting from the fact that numerically dominant species also tend to have high variances. SIMPER analyses (Primer v6) was used to determine the taxa that account the most for the dissimilarities between different sites. Differences in suppressiveness between the sand controls of the different runs of the bioassay and the defaunated cores of the different soils and differences in taxa richness, relative predator abundance, survival of the introduced prey and suppressiveness potential between undisturbed and agricultural cores were tested using one way ANOVA in Statistica 7.0. (Statsoft, Tulsa, OK, USA) on square root transformed data for relative predator abundance and suppressiveness potential. Assumptions were tested using the Kolomogorov-Smirnov statistic for normality and Levene's test for homogeneity of variances. One way ANOVA's were followed by post hoc pair-wise comparisons using Tukey HSD. For the abundance of specialist predators assumptions could not be fulfilled and a non-parametric test (Mann–Whitney U test in Statistica 7.0, Statsoft, Tulsa, OK, USA) was used to test differences between agricultural and natural cores. The relationship between the predator metabolic footprint and suppressiveness was tested using the linear regression (REG) procedure with a log predictor in SAS v9.4 (SAS Institute Inc., Cary, NC, USA). Assumptions for linear regression were tested using Q–Q plots of the residuals. The relation between the predator metabolic footprint, resource availability and suppression of the introduced prey was tested using multiple regression. Product moment correlations between the enrichment footprint and the bacterievore and fungivore footprint and between the total predator footprint and resident prey biomass were calculated in Statistica 7.0 (Statsoft, Tulsa, OK, USA).

3. Results

3.1. Abiotic variables and microbial biomass

Soil moisture content and pH were comparable across most sites but pH was rather high in U-HHH, U-PCR2, D- GVD, and D-HAR and low moisture was low in D-GVL and D-RR cores (Table 1). The C/N ratio was low in the D-HAR plots, MBC was high in the undisturbed U-FOD, U-MAN, U-HHH and U-PCR sites and D-GVD contained the largest proportion of clay particles. The first two axes of the PCA explained 66% of the variation in the variables measured (MBC, C/N, %C, %N, DOC, % Moisture, pH, % soil particles silt, clay and sand). The agricultural samples (D-HAR, D-RR, D-GVL and D-GVD) are separated from all undisturbed samples except for U-SS and U-FOD along the first axis based on sand, silt and clay percentages as well as %C (Fig. 2). The agricultural samples are separated in two groups, i.e. D-RR + D-GVL and D-GVD + D-HAR. The first axis of the PCA accounted for 46% of the variation. The second axis explained an additional 20% of the variation and here moisture and pH were the most important variables.

3.2. Resident nematode assemblages

The total numbers of nematodes in the microhabitats represented by the individual cores at each site differed considerably (See Appendix A), i.e., between 501 (U-MAN3) and 7232 (U-PCR1.10) nematodes per 100 cm^3. Based on the nematode community composition, cores collected at the same site are mostly similar (ANOSIM: all $p < 0.05$ except for FOD and HR cores) (Fig. 3). The SIMPER analyses showed that the ordination is mainly defined by the most common bacterial-feeding nematodes which had greatest contribution to the dissimilarity between the cores of the different sites, except for the dissimilarity between the U-PCR2 and the other sites where also the omnivorous Dorylaimida contributed to the dissimilarity. Not surprisingly, representatives of the prevalent bacterivore families, Cephalobidae and Rhabditidae, were recorded in all cores, while root-associate Tylenchidae were found in 70 out of 72 cores. *Plectus*, Panagrolaimidae, *Aphelenchus*, *Aphelenchoides*, *Tylenchorhynchus* and Qudsianematidae occurred in more than 50% of the cores, while *Bunonema*, *Mesocriconema*, *Rotylenchus*, *Tobrilia*, *Pristionchus*, *Xiphinema* and Neotylenchidae were rather rare (in less than 5 of the 72 cores). In general, taxa richness was significantly higher ($p < 0.001$) in the cores from undisturbed areas (on average 18 ± 2) compared to the agricultural cores (on average 14 ± 2). Although the differences in taxa richness are small, the taxonomic unit we used was either at family or genus level. A small difference at the level of these taxonomic units could include several species.

At a higher level of functional resolution, generalist (=omnivores) and specialist predators were represented by 14 taxa, five were specialist predators (i.e., *Discolaimus*, *Mylonchulus*,

Table 1
Abiotic variables (dissolved organic matter (DOC), Soil texture (% clay, silt and sand), %N, %C, C/N ratio and pH) and microbial biomass C (MBC) for all sites. Means ± SD based on three replications, except for * where only one replicate was available.

	Site	DOC	MBC	Soil texture			%N	%C	C/N	Moisture %	pH
		Ug C g^{-1} soil	Ug C g^{-1} soil	%Clay	%Silt	%Sand					
Undisturbed	**U-MAN**	341.4 ± 73.56	368.8 ± 73.56	7.3 ± 2.87	41.1 ± 4.61	51.6 ± 7.47	0.15 ± 0.073	3.82 ± 2.589	24.5 ± 4.3	11.8 ± 0.7	7.6 ± 0.06
	U-HCT	180.1 ± 175.56	121.9 ± 60.9	4.4 ± 0.14	36.9 ± 1.14	58.7 ± 1.28	0.26 ± 0.034	6.98 ± 1.007	26.5 ± 2.32	10.2 ± 0.6	7.8 ± 0.17
	U-PCR 1	180 ± 7.66	346.3 ± 82.62	4.4 ± 0.53	38.2 ± 4.19	57.3 ± 4.7	0.22 ± 0.035	3.51 ± 0.56	16.2 ± 0.13	20.5 ± 8.35	7.8 ± 0.15
	U-PCR 2	184.1 ± 58.44	248.4 ± 22.15	7.7 ± 0.33	56.1 ± 0.5	36.2 ± 0.8	0.23 ± 0.002	4.32 ± 0.098	18.8 ± 0.39	20.9 ± 1.53	8.2 ± 0.06
	U-HHH	259*	319.9*	3.7 ± 0.14	50.8 ± 2.44	45.5 ± 2.45	0.18 ± 0.014	3.49 ± 0.217	19.2 ± 0.4	33.7 ± 7.99	8.7 ± 0.12
	U-FOD	88.3 ± 77.68	434 ± 195.03	12.5 ± 0.58	48.2 ± 1.51	39.3 ± 1.95	0.16 ± 0.013	2.38 ± 0.248	14.9 ± 0.54	13.1 ± 1.16	7.9 ± 0.1
	U-SS	26.9 ± 22.58	218.9 ± 65.32	8.5 ± 0.39	46.4 ± 1.33	45.1 ± 1.71	0.1 ± 0.011	2.24 ± 0.309	21.6 ± 0.72	11.1 ± 0.53	7.9 ± 0.15
Disturbed	**D-GVD**	64.2 ± 26.84	112.8 ± 17.43	17.3 ± 0.03	48.2 ± 0.64	34.5 ± 0.55	0.08 ± 0.008	1.97 ± 0.746	23.3 ± 7.14	28.8 ± 1.09	8.8 ± 0.1
	D-GVL	42.5 ± 11.65	91.2 ± 10.88	5.8 ± 0.36	41.3 ± 2.13	52.9 ± 2.45	0.08 ± 0.009	1.6 ± 0.011	20.9 ± 2.7	5.2 ± 0.37	8.1 ± 0.23
	D-HAR	58.3 ± 5.66	37.4 ± 5.88	10.9 ± 0.45	60.2 ± 0.69	28.9 ± 0.93	0.11 ± 0.005	0.96 ± 0.028	8.4 ± 0.33	16.9 ± 1.35	8.7 ± 0.1
	D-RR	37.2 ± 9.73	103.4 ± 5.19	13.8 ± 0.14	49.4 ± 0.47	36.8 ± 0.62	0.09 ± 0.003	1.23 ± 0.033	13.2 ± 0.4	7 ± 0.6	7.5 ± 0.06

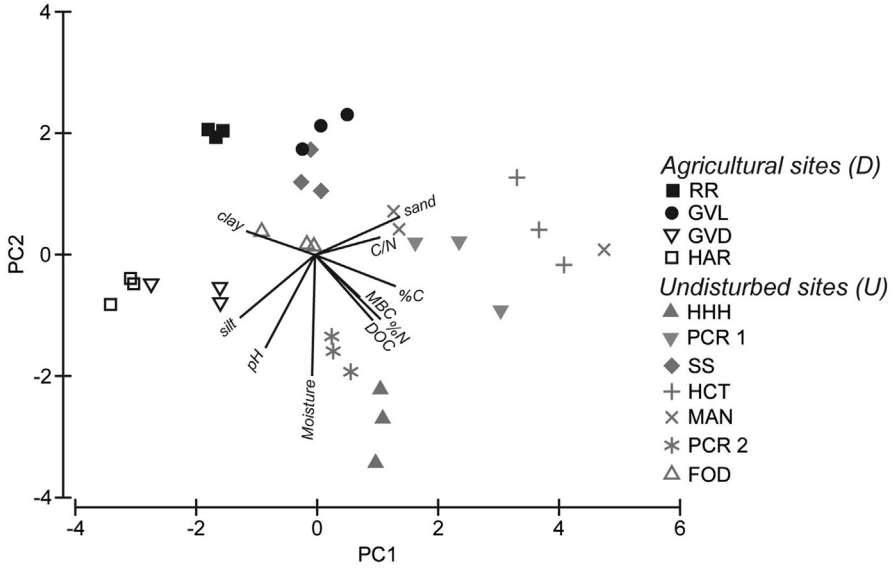

Fig. 2. Vector loading plot of all separate variables in a two-dimensional PCA ordination of abiotic soil variables (moisture %, pH, C/N ratio, soil texture: % silt, clay and sand) and Microbial Biomass C (MBC) for all 11 sampling sites. Agricultural sites in black, undisturbed sites in grey.

Clarkus, *Prionchulus and Tobrilia*) and nine were generalist predators (Qudsianematidae, Dorylaimidae, Aporcelaimidae, Nordiidae, Thornematidae, *Achromadora*, *Tripyla, Pristionchus and Mononchoides*). In general, the relative abundance of predators was significantly higher in the undisturbed cores ($p < 0.001$) compared to the agricultural cores, i.e., on average 9 ± 5% and 3 ± 2.4%, respectively.

The suppressiveness potential, as expressed by the predator/prey ratios, was significantly higher ($p < 0.001$) in the undisturbed soils (0.10 ± 0.03 vs. 0.03 ± 0.01 in the undisturbed and agricultural cores, respectively) but differed among cores (see Appendix A). Also, a higher proportion of specialist predators was found in the undisturbed cores ($p = 0.005$), on average 45 ± 80/100 cm^3 compared to 6 ± 12/100 cm^3 in the agricultural cores. Only one core, U-HHH1, did not contain a single nematode predator. There were no overall differences between natural and agricultural sites in the relative abundances and metabolic footprints of the available resident prey, i.e., herbivores, fungivores and bacterivores. The individual cores were very different in species composition and abundance, both within and across sampling sites, which resulted in the intended spectrum of predator-prey combinations. In general, enrichment metabolic footprints were highly correlated with bacterivore metabolic footprints ($R^2 = 0.75$, $p < 0.001$) but not with fungivore footprints ($R^2 = 0.008$), indicating the dominance of bacterivores as an available resource for the predators. The total predator metabolic footprint across samples was positively correlated with the resident prey biomass ($R^2 = 0.49$, $p < 0.001$).

3.3. Suppression bioassay

The survival of *S. feltiae* and *M. incognita* in the sand controls did not differ between bioassays with soils from different sites ($p \geq 0.07$ and $p \geq 0.18$, respectively), indicating that the quality of the inoculum was comparable for all runs of the experiment. In the DF and in the sand control cores, i.e., in the absence of predators and thus biological suppression, survival of the introduced prey across all sites was significantly higher ($p < 0.001$) for *S. feltiae* than for *M. incognita* (i.e., on average 163 ± 25 compared to 115 ± 19 individuals/100 cm^3 and 254 ± 48 compared to 145 ± 26 in the DF and sand controls, respectively). Survival of *S. feltiae* was significantly greater in U-FOD DF cores than in U-HCT and U-PCR1 DF cores while that in D-HAR cores was greater than that in U-PCR1 cores (all $p \leq 0.02$). There was significantly greater survival of *M. incognita* in D-HAR than U-HCT DF cores ($p = 0.002$). This indicates

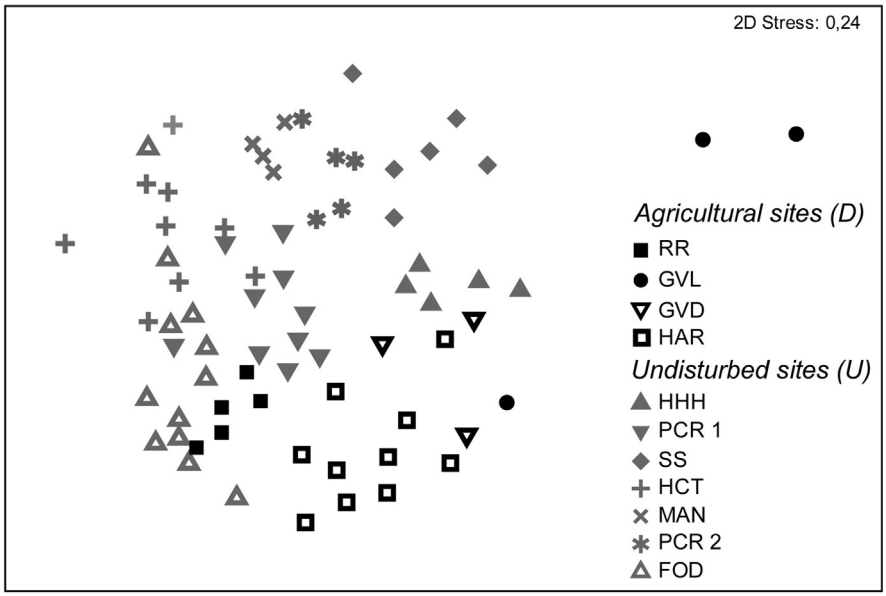

Fig. 3. MDS ordination of square-root-transformed nematode species abundances for all sampling sites. The outlier GVL samples had high numbers of *Prismatolaimus*, *Eudorylaimus* and *Helicotylenchus* which were rare in other samples.

that physical and chemical conditions were less favorable in U-PCR1 and U-HCT DF cores especially for survival of *S. feltiae*. The HCT and PCR1 soil had a higher sand content (>55%) than most of the other soils (see Table 1). *S. feltiae* is more active (and infective) in sandy soils (Georgis and Poinar, 1983). This combined with the absence of resources may have depleted their energy in the U-HCT and U-PCR1 DF cores.

In the presence of predators, suppression of the introduced prey increased with absolute predator numbers (for *S. feltiae* see Fig. 4A). The magnitude of the increase was greater at low numbers of predators then dampened to an asymptotic level at greater predator abundance. Cores with a rather low absolute number of predators (<100) but a relatively high suppression mostly had a high proportion of specialist predators. Examples are *Prionchulus* in U-HCT 4, 6, 7, 10, 11, *Discolaimus* in U-PCR1 6, *Clarkus* in U-MAN 3 and U-HHH 5 and *Mylonchulus* in D-GVL 1 and D-HAR 2, 3, 10. At the other extreme, cores with abundant predators but lower suppressiveness were dominated by small omnivores (generalists). For example *Tripyla* in U-FOD 5, 6, 7, 9, *Microdorylaimus* in U-SS 1, 2, 3, 4, 5 and Dorylaimidae in U-PCR2 1, 3, 4.

When metabolic rates of predators were considered, suppression of introduced prey was significantly related to the total metabolic predator footprint (REG procedure in SAS, with log transformed metabolic footprint predictor, for *S. feltiae*: $t(1) = 5.09$, $p < 0.001$; for *M. incognita*: $t(1) = 3.56$, $p < 0.001$) (for *M. incognita* see Fig. 4B). The multiple regression, including a variable that indicates resource limitation for predators (i.e., the ratio of prey biomass and predator metabolic footprint) indicated that suppression of the introduced test prey was a function of predator metabolic footprint ($p \leq 0.001$) but not of resource limitation ratio ($p \geq 0.05$). The lower the latter ratio, i.e., the more resource-limited the predators, the higher the suppression. The higher the metabolic predator footprint, the higher the suppression of introduced prey (Fig. 5).

4. Discussion

With our sampling strategy and experimental design we were able to test suppression of two different introduced nematode species in a range of different predator/prey ratios representing disturbed and undisturbed soil patches with co-located predators and prey. The relatively low survival rate of the introduced prey over the 5-day incubation period in the DF and sand control cores might be explained by the lack of food, i.e. roots for *M. incognita* and insect larvae for *S. feltiae*. In general survival of *M. incognita* was lower than that of *S. feltiae*. However, it was observed that *Meloidogyne* juveniles were more active than *Steinernema* juveniles and juveniles of both genera are reported to remain active and infective in similar conditions as the current experiment up to 30 days (Ferris et al., 1982) and 8 weeks respectively (Ishibashi and Kondo, 1986).

In the presence of predators, in the NDF cores, suppression did not differ consistently for either of the two introduced nematode species. This suggests that suppression was not species-specific, but an overall regulatory force, effective against all prey present and really an integral function of the food web. Nevertheless, under experimental conditions, *M. incognita* has been reported as preferred prey of the predator *Mononchoides gaugleri* compared to several other larger-bodied adult plant-parasitic nematodes (i.e., *Hirschmanniella oryzae*, *Tylenchorhynchus mashoodi*, *Helicotylenchus indicus*, *Hoplolaimus indicus*, *Hemicriconemoides magniferae*, *Xiphinema americanum*, *Longidorus attenuatus and Paratrichodorus christiei*) (Bilgrami et al., 2005). In more natural conditions, Piskiewicz et al. (2008) concluded that various top-down control factors (i.e., micro-organisms, other nematodes or micro-arthropods) differ in their regulatory effectiveness among nematode species. In other words, suppression is not species-specific. Consequently, we infer that the contributions of various environmental factors and community interactions differ among species and that conserving soil biodiversity is crucial for the complementarity and reliability of the overall regulatory force of the food web (e.g., Ferris and Tuomisto, 2015). A disadvantage of our assay is that it focuses on suppressive forces that affect the migratory stages of a nematode life cycle and does not consider antagonistic interactions that may occur in the rhizosphere or within roots (Stirling, 2014a). Although outside the scope of this research, a possible method for confirming the observed suppressiveness is to

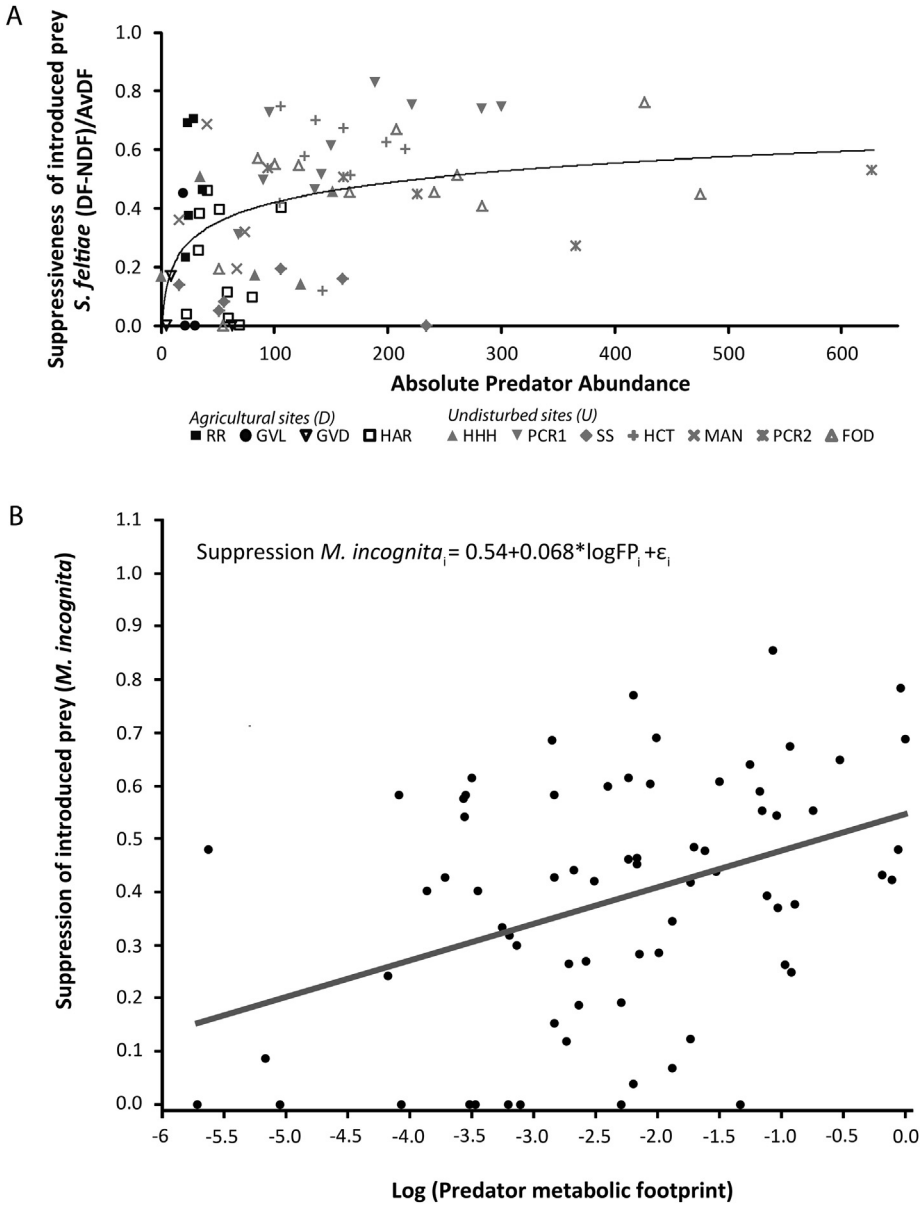

Fig. 4. A. Relationship between suppression of *Steinernema feltiae* and the absolute predator abundance in all cores. The most abundant predator is displayed for some of the cores and specialist predatory nematodes are indicated with an asterisk (*). B. Relationship between suppression of *Meloidogyne incognita* and the log of the predator metabolic footprint (FP). Linear regression is significant ($p \leq 0.001$) and $R^2 = 0.27$.

include plant assays, as in Min and Toyota (2013). An important asset of our assay was that the actual local suppression of the introduced prey, given the resident predators and prey in a specific soil patch was measured.

The lack of certainty regarding co-location of predators and prey, coupled with the high level of soil disturbance during sampling, is probably the reason that Min and Toyota (2013) concluded that the suppression of plant-parasitic nematodes did not always coincide with the presence of higher trophic groups. Despite a rather large composite sample size, that might have compromised co-location of organisms, Sánchez-Moreno and Ferris (2007) found suppression of plant-parasitic nematodes was greater in soil food webs with more trophic links and abundant predatory nematodes than is simpler food webs. Our results suggest that global (field scale) suppressiveness largely depends on local (patch) suppressiveness and the functional connectance (Ferris et al., 2012b) within the patches, i.e., the proportion of patches in which predator and prey are co-located.

Besides predator abundance or biomass and the exigency of co-location of predator and prey, several other factors contribute to the suppressive potential of a soil community. Suppressiveness may also be determined by temporal and spatial aspects of the bioassay system and/or satiation of the predators. For example, non-nematode predators such as tardigrades change their foraging behavior according to their level of satiation (Hohberg and Traunspurger, 2009) and starved nymphal stinkbugs are more effective bio-control agents of tomato leafminer (Torres et al., 2002). In our study, the greatest suppressiveness was observed in the U-PCR1 1 and 2, the U-FOD and the U-HCT NDF cores. Although not measured in the individual microcosms, proxies of resource

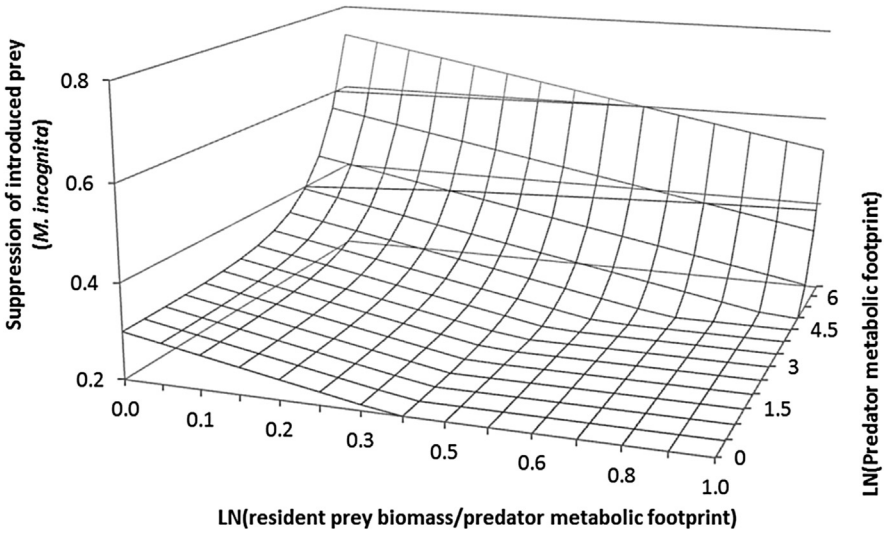

Fig. 5. Relationship between the suppression of the introduced prey, *Meloidogyne incognita*, the log of the predator metabolic footprint and the log of the resource availability factor (i.e. resident prey biomass/predator metabolic footprint).

availability were greatest in the additional cores from the same soils (MBC in U-PCR1, U-PCR2, U-FOD and C% in U-HCT, Table 1). The predator metabolic footprint was also greatest in these cores and was positively correlated with resident prey biomass. This is in accordance with the positive relation between predator and amplifiable prey (= resident prey in this study) abundances described in Ferris et al. (2012a) since they constitute endemic resources for predator organisms. Similarly, Stirling et al. (2011) found a positive relationship between predator abundances and suppression of *Radopholus similis* and also suggested that the system was sustained by input C (=food resource). Consequently, resident prey biomass is an indicator of the resource availability to the predators as well as a proxy for the pressure (i.e. suppression potential) on introduced prey species which, together with the predator metabolic footprint, determines the magnitude of the suppression. In the present study, the non-significance of the resource availability factor in the multiple regression may be due to activity of non-nematode predators of nematode prey and availability of alternate resources for the generalist predator nematodes in the soil patches (Ferris et al., 2012a).

As the rate of increase of suppression was greater at low numbers of specialist predators than at greater generalist predator abundance, our results illustrate the differences in the magnitude of the ecosystem service of pest regulation between these two functional guilds. The generalist predators were generally smaller than the specialist predators. That was recognized in the use of metabolic footprints as the independent variable to provide a functional metric for the magnitude of the ecosystem service (Ferris, 2010b). The metabolic footprint was of great importance for calculation of the resource availability for predators and understanding its relationship with suppressiveness. Nevertheless, when calculating the metabolic footprint, there is the issue of dealing with populations of different age structure which is inherently present in all samples. In order to reach a reasonable consensus, in some cases, as was done for the Dorylaimidae in the U-PCR2 cores, it may be useful to measure a representative range of individuals of each species and to calculate biomass and metabolic footprints based on the age structure and actual size of nematodes in the samples. That approach, however, might be prohibitive in terms of available time.

In conclusion, nematodes can serve as indicators of soil suppressiveness to pest species; co-location of predator and prey, the size of the predator population (metabolic footprint) and the predator/prey ratio (resource limitation of predators) on a small microcosm scale (100 cm^3) are decisive indicators of the magnitude of the top-down regulation on introduced species. The characteristics of the microcosms that were more suppressive provide a template to be achieved in the management of larger scale agricultural systems where enhancement of this ecosystem service is desired. Field scale application would thus require altering management practices and providing resources for stewardship of populations of predator nematodes. Key practices associated with suppressive agroecosystems are: i) maintaining a permanent plant residue cover by crop residues, cover crops or green manures, ii) continuous and diverse input of organic matter using organic amendments, iii) a diverse rotation crop rotation system (including legumes for natural nitrogen fixation), iv) reduced tillage to minimize disturbance and v) restrict farm machinery to traffic zones to minimize compaction problems (Stirling, 2014b). Other predators, for which nematodes are bioindicators, will also respond to the stewardship and overall suppressiveness to pest species will increase. Ultimately the practical implications of this study are that soil suppressiveness to pest species may be enhanced by increasing resources to non-target prey species, removing chemical and physical constraints to the survival and increase of predators, and altering management practices so that predators and target prey are co-located in time and space. Restoring depleted higher trophic levels and the regulatory balance in the soil food web due to mismanagement will take time (Stirling, 2014b) as the predator and omnivorous nematodes have long generation times (months rather than weeks or days), low reproduction rates and recolonization occurs slowly (Bongers and Bongers, 1998). Nevertheless, and supported by this study because the genus was found in two of the studied agricultural sites (i.e. D-HAR and D-GVL), the mononchid *Mylonchulus* was reported to be relatively tolerant of cultivation (Fiscus and Neher, 2002). Essentially such management alterations as mentioned above attempt to create an environment similar to that in undisturbed natural sites. Using nature as a model, applying biological and ecological principles in a multi-and interdisciplinary framework is the main focus of agroecology, which includes

implementing advances levels of Integrated Pest Management (IPM) (Brewer and Goodell, 2012). Conversion to agroecological practices is promoted by redesigning existing management, including *inter alia* increasing crop diversity, applying cover crops and organic amendments to increase organic inputs, and reducing tillage (Wezel et al., 2014). All of these potentially enhance the ecosystem functioning of beneficial nematodes and other organisms of which they are bioindicators and thus the related ecosystem services.

Contribution of the authors

The project was conceived and designed by both authors as an extension of a long-term research objective in the Ferris lab. Both authors participated in development of the hypotheses to be tested. Soil and nematode sampling at each study site was conducted by both authors. Nematode extractions and identifications were performed by Steel. Ecosystem service experiments were conducted by Steel with input on experimental design by Ferris. Faunal analyses were conducted by Steel using software for which Ferris participated in the development. Steel conducted statistical analyses and wrote the first draft of the manuscript. Ferris assisted in editing each draft. Steel produced the final manuscript for submission.

All authors have approved the final article.

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Appendix A. Supplementary material

Supplementary material related to this article can be found at http://dx.doi.org/10.1016/j.actao.2016.03.004.

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