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Abstract: Livestock grazing and trampling is an important factor in the formation and development of different terrestrial ecosystems. However, despite numerous studies on soil compaction, there is still no consensus as to which kind of effect (positive or negative) animal trampling exerts on soil nematodes. The main goal of this study was to evaluate the animal trampling effect on free-living nematode abundance and diversity, and to define the attenuating effect of the tree canopies (Cupressus sempervirens, Eucalyptus camaldulensis, and Tamarix aphylla) during wet and dry periods. The nematodes were extracted from 100 g aliquots of soil samples (brown-red sandy soils) during cold-wet (CW), warm-wet (WW), and hot-dry (HD) periods during 2013-2014, in a man-made safari-zoo habitat, using the Baermann funnel procedure. Our results revealed the impact of trampling on both free-living nematode abundance and diversity, and their soil habitat. It was found that trampling, along with seasonal fluctuation and the tree-species attenuating effect on the soil medium, resulted in the creation of the spatial-temporal heterogeneity of soil properties in the study area. In turn, variation in soil properties was reflected in soil nematode abundance and diversity, revealing tight correlation with the observed soil properties. Animal trampling had an overwhelming, mostly negative impact on soil nematode abundance, genera, and trophic diversity in the open, bare area. However, the protective effect of the tree canopies, as well as seasonal fluctuations, attenuated this negative impact. The trees had a significant attenuating effect on trampling compared with the open, bare area. However, different tree species during the wet and dry periods had a variable impact on nematode abundance, genera, and trophic diversity. During the hottest period of the year, when external adverse factors dominated the trampling effect, the ability of the trees to protect nematode communities was significantly reduced. Of all the colonizer-persister (cp) continuum of nematode functional guilds, only bacteria-feeding nematodes belonging to the cp-1 guild were positively affected by trampling. In general, nematodes belonging to the r-life-strategy group (colonizers tolerant to environmental disturbance), mainly bacteria-feeding nematodes, were the most numerous (61 and 44% at the trampling and undisturbed sites, respectively). In contrast to the bacteria-feeding group, fungi-feeding nematodes were the smallest group in the study area (8 and 4% in the trampling and undisturbed sites, respectively). The undisturbed sites were a more favorable habitat for the plant-parasite nematodes (9 and 26% in the trampling and undisturbed sites, respectively). Surprisingly, the omnivore-predator nematodes belonging to the K-life strategy group and that are characterized by hypersensitivity to disturbance, were relatively numerous at the trampling (22%) and relatively undisturbed (26%) sites. The results showed that 62% of the nematode species were affected (48% negatively and 14% positively) by either direct trampling or changes in soil properties. The ecological indices confirmed that animal trampling had a negative impact on the soil biota in the study area. Our results suggested that animal trampling exerts significant a direct and indirect effects (through changes in soil properties) on soil free-living nematodes. Moreover, the wet-dry seasonal periods along with the tree canopies protective effect may significantly change the extent of animal trampling impact.

Key words: Grazing, soil disturbances, environmental factors, biological indicators, ecological index.

## **Non-standard Abbreviations**

#### Sampling Treatments

CO	control in open space;
ТО	trampling in open space;
CE	control in the Eucalyptus area;
TE	trampling in the Eucalyptus area;
CU	control of the Cupressus area;
TU	trampling in the Cupressus area;
CT	control in the Tamarix area;
TT	trampling in the Tamarix area.

## Ecological Indices

NCR	nematode channel ratio;
H'	Shannon-Weaver index;
EV	evenness;
SR	species richness;
Т	trophic diversity;
Dom	Simpson's dominance index;
MMI	maturity index modification.

#### Soil Properties

SM	water content of soil
EC; $\mu$ S g <sup>-1</sup>	electrical conductivity
WHC	water-holding capacity
OM	organic matter
soil pH	

## 1. Introduction

Intensive livestock trampling exerts a negative impact on soil physical properties by deforming existing soil structure and by leading to a flat, comparatively impermeable surface layer composed of dense, unstable clods [1-3]. Soil compaction resulting from animal trampling plays both an important direct and indirect role in the range of vegetation growth and development. Moreover, animal trampling has a simultaneous effect on both soil and range vegetation covers [4]. Trampling mainly exerts an effect on the ca. 25 cm soil depth [5-7], changes some soil properties [8, 9], vegetation covers [10, 11], and soil biotic composition, and therefore, significantly affects soil biological processes in different terrestrial ecosystems [12-14]. In addition, the seasonal effect may significantly alter the grazing and trampling impact on soil properties and soil communities [2].

Soil texture and the accompanying soil structural variations are considered among the most important factors regulating water-nutrient resources [15], which, in turn, reflect the abundance, distribution, and structure of soil biota [16]. It was reported that soil porosity, along with external environmental factors and soil chemical properties, are considered to be a significant factor in shaping soil biotic composition, density, and the interactions between them [17, 18]. The surface compaction associated with the decrease of porosity [19] exerts a restrictive effect on biotic trophic interactions in different soil habitats. Previous studies have Jones (1982), Hassink et al. (1993), and Pen-Mouratov et al. (2011) [20-22] found that in grassland and desert soils, there was a strong interdependence between bacterial and nematode biomass and pore size: the bacterial biomass positively correlated with small (0.2-1.2 µm) pores in grassland soil while the nematode biomass increased in the larger (30-90 µm) soil pores in desert soils.

However, in addition to the cylindrical pore or capillary concept, whose main ideas have been outlined above, a number of researchers adhere to the aggregate model. The main point of the model is that aggregates (isolated, water-filled spaces of soil pores) contain water that is immobile and can remain there for some time during the dry period, while the inter-aggregate space contains water that drains quickly [23-25]. Hence, the aggregates are the preferred habitat for the soil biota, especially during the unfavorable dry period [26, 27].

Numerous studies showed that soil free-living nematode communities are among the best biological tools for assessing soil disturbances, including agricultural and grazing activities in terrestrial systems

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[28-33]. In order to evaluate the animal trampling effect on a soil system in a closed, modern, outdoor zoo, soil free-living nematodes — as very sensitive bioindicators of the environment — were examined. The main goal of this study was to evaluate the animal trampling effect on a soil free-living nematode community under different environmental conditions in a man-made, safari-zoo habitat. The first objective was to measure the impact of animal trampling activity on soil free-living nematodes during the wet and dry seasons.

The second objective was to determine the protective effect of the tree canopies on the soil nematode communities that have been affected by trampling during two seasons. Along with the open area, which is constantly exposed to trampling effects, the medium under the canopies of *Cupressus sempervirens*, *Eucalyptus camaldulensis*, and *Tamarix aphylla*, as the most dominant trees, provides shelter for many ungulates inhabiting the study area. Based on previous studies [34], we hypothesized the following:

(1) Direct and indirect effects (through changes in soil properties) of trampling activity on the soil nematode community will be found.

(2) The abundance and diversity of the soil free-living nematodes, including the nematode functional guilds, will reflect the specific trampling condition decreasing with trampling intensity.

(3) The protective effect of the tree canopies, as well as seasonal fluctuations, will substantially alter the impact of animal trampling on nematode communities and their soil habitat.

## 2. Materials and Methods

## 2.1. Study Site Description

The Zoological Center Tel Aviv–Ramat Gan (commonly known as the Safari Ramat-Gan) houses the largest collection of wildlife under human care in the Middle East. The new zoo opened in 1981. The 100 ha site consists of both the African Safari Park (~

70 ha) and a modern outdoor zoo. The safari park and the zoo are home to over 1,600 animals of different species, including 83 mammalian species, 92 bird species, and 23 reptile species. The safari section is a man-made habitat for large and dynamic mixed herds of 13 species. According to the research group of the Zoological Center [35], the African Safari section is home to the following ungulates: Gozella thomsoni (200 individuals, 15-25 kg each); Capra ibex nubiana (7 individuals, 50 kg each); Oryx dammah (40 individuals, 140-210 and 91-140 kg each for the male and female, respectively); Connochaetes taurinus (24 individuals, 120-270 kg each); Kobus ellipsipryninus (9 individuals, 200-300 and 160-200 kg each for the male and female, respectively); Equus quagga (60)individuals, 272-362 and 226-317 kg each for the male and female, respectively): Taurotragus orvx (56 500-600 individuals. kg each); *Hipopptamus* (28 individuals, 1500-1800 amphibious and 1300-1500 kg each for the male and female, respectively); Ceratotherium and simum (10)individuals, 1360-3630 kg each).

*Casuarina* sp., *Cupressus* sp., *Eucalyptus* sp., *Ficus* sp., and *Tamarix* sp. are the most dominant trees in the area most frequently visited by the animals in the African Safari section. The trees provide shelter for many animals, mainly the ungulates. The type of soil in the study area belongs to the brown-red sandy soils [36].

The Safari has been a full member of the European Association of Zoos and Aquaria (EAZA) since 2007, the World Association of Zoos and Aquariums (WAZA) since 1990, and a founding member of Israeli Zoo Association (IZA), established in 2002 [35].

## 2.2 Sampling

A total of 96 (n = 4) soil samples (Fig. 1) from the 0-100 mm depth were collected during the three main periods of the study, i.e., the cold-wet (CW) period (December 2013), the warmer-wet (WW) period (March 2014), and the hot-dry (HD) period (August 2014).

The Impact of Animal Trampling on Free-living Nematode Abundance, Genera, and Trophic Diversity was Attenuated by Tree Canopies



**Fig. 1** Location of the study area (Google Earth map of the sampling area, with sampling spots). Sampling spots: CO, control in open space; TO, trampling in open space; CE, control in the *Eucalyptus* area; TE, trampling in the *Eucalyptus* area; CU, control in the *Cupressus* area; TU, trampling in the *Cupressus* area; CT, control in the *Tamarix* area; TT, trampling in the *Tamarix* area.

In the CW period, i.e., the coldest month of the year 2013, sampling-day temperature ranged between 10 and 18°C. Sampling-day water content of soil (SM) and mean monthly rainfall (RF) values amounted to 18.1% and 184.3 mm, respectively. The WW was a warmer period, with the sampling-day temperatures ranging between 13°C and 25°C. The sampling-day SM and mean monthly RF values amounted to 16.1% and 67.9 mm, respectively, The HD period was the hottest month of the year, with daily temperature ranging from 25°C to 32°C. The sampling-day SM and mean monthly RF values amounted to 4% and 0 mm, respectively.

Samples were taken from the two sampling sites, i.e., the heavily trampled grazing area and the fenced control area (Fig. 1). The two study areas were separated by a fence and had the same environmental conditions. The heavily trampled area was characterized by more than six animals (ca 1470-2356 kg) per hectare during trampling for more than 30 years. The trampling soil samples were collected from the open site (TO), and from under the canopies of *Cupressus empervirens* 

(TU), *Eucalyptus camaldulensis* (TE), and *Tamarix aphylla* (TT). The control area was inaccessible to ungulates. The control soil samples were collected from the open site (CO) and from under the canopies of *Cupressus empervirens* (CU), *Eucalyptus camaldulensis* (CE), and *Tamarix aphylla* (CT).

Each soil sample, which consisted of five subsamples from the study area, was collected with a core sampler as follows: eight replicates (4 control and 4 trampling samples)  $\times$  4 sampling points (from under the canopies of trees and open spaces)  $\times$  3 seasons. Subsamples were mounted on a plastic sheet and stirred into a homogeneous mixture. An amount of 0.5 kg from each replicate was placed in an individual plastic bag and transported to the laboratory in an insulated box. The replicates were kept in cold storage in the laboratory at 4°C. Before sieving, 100 g from each replicate was used to determine the soil free-living nematode community. The remaining soil was sieved through a 2-mm mesh sieve before microbial, physical, and chemical analyses.

## 2.3 Sample Analysis

Subsamples of each replicate were subjected to the following analyses:

a. *Water content of soil* (SM, g kg<sup>-1</sup>) was measured gravimetrically as a percentage of dry mass by oven-drying to a constant weight (105°C, 48 h).

b. *Soil salinity* was determined in soil extracts and expressed as electrical conductivity (EC;  $\mu$ S g<sup>-1</sup>);

c. *Water-holding capacity* (WHC, g kg<sup>-1</sup>) was determined in 100 g soil. Soil samples were flooded with tap water in a bottom-perforated vessel for five minutes. The WHC was inferred from the amount of residual water remaining following infiltration of gravitational water. We treated the water content of soil as a fraction of the WHC (SMWHC), thus forming a new concept reflecting soil water availability that is more acceptable for biological activity assessments [37].

d. *Organic matter* (OM, g kg<sup>-1</sup>) was determined by oxidization with dichromate in the presence of  $H_2SO_4$ , without the application of external heat [38].

e. *Soil pH* was measured with a potentiometric glass electrode, using a 1:2 soil:water ratio.

f. The nematodes were extracted from 100 g aliquots of the subsamples using the Baermann funnel procedure [39]. The recovered organisms were counted and preserved in formaldehyde [40]. A maximum of 120 individuals from each sample were identified according to order, family, and genus level, using a compound microscope with optical magnifications of 200, 800, and 2000. Nematodes were classified according to known feeding habitats and morphology [41-43] into the following trophic groups: bacteria-feeding (BF), fungi-feeding (FF); plant-parasitic (PP,) and omnivore-predator (OP) [44, 45]. The total number of nematodes was counted and adjusted to 100 g dry soil.

## 2.4 Ecological Indices

The characteristics of the nematode communities were described using the following parameters and ecological indices: (a) absolute abundance of nematode individuals per 100 g<sup>-1</sup> dry soil (TN); (b) functional guild of nematodes comprising nematodes with c-p ranging from 1 to 5 and belonging to the following trophic groups [44, 45]: omnivore-predator  $(OP_x)$ ; plant-parasitic  $(PP_x)$ ; fungi-feeding  $(FF_x)$ ; and bacteria-feeding (BF<sub>x</sub>) nematodes [46]; (c)  $T = 1/\sum P_i^2$ , where  $P_i$  is the proportion of the *i*-th trophic group [47]; (d) Simpson's dominance index, Dom =  $\sum P_i^2$ [48]; (e) Shannon-Weaver index,  $H' = -\sum P_i$  (lnP<sub>i</sub>), where  $P_i$  is the proportion of individuals in the *i*-th taxon [49]; (f) maturity index modification (MMI), including plant-feeding nematodes [50]; (g) species richness,  $SR = (S-1)/\ln(N)$ , where S is the number of taxa and N is the number of individuals identified [42]; (h) the nematode channel ratio, NCR = BF/(BF+FF)[43]; (i) structure index (SI) =  $100 \times (s/(s + b))$ , where = b 0.8 х (Fu2+Ba2); s  $0.8 \times \text{Ca2+1.8} \times \Sigma(\text{X3}) + 3.2 \times \Sigma(\text{X4}) + 5.0 \times \Sigma(\text{X5}); i =$  $3.2 \times Ba1+0.8 \times Fu2$ ; and (e) enrichment index (EI) =  $100 \times (e/(e + b))$  [46, 51-53].

## 2.5 Statistical Analysis

All data were subjected to statistical analysis of variance using the SAS model (ANOVA, Duncan's multiple range test, and Pearson correlation coefficient) and were used to evaluate differences between separate means. Differences obtained at levels of P < 0.05 were considered significant.

Duncan's multiple range tests and the Pearson correlation coefficient were used to evaluate significant differences and interrelationships among separate means. A two-tailed probability index (P < 0.05) was considered to be statistically significant. Moreover, the data were tested by computing multivariate redundancy analysis (RDA) in order to provide more information by taking into account differences between planted and open spaces (CANOCO Program, Version 4.54, October 2005 — written by ter Braak (C) 1988-2005). The Monte Carlo permutation test (499 permutations were used for this study) was used to calculate the significance of a

given factor and its relevance for the measured parameter [54, 55]. The graphical output arrows, pointing roughly in the same direction, indicated a positive correlation, while arrows pointing in the opposite direction indicated a negative one. The length of the arrow indicated the relative strength of the relationship.

## 3. Results

## 3.1 Soil Properties

Some of the observed soil properties, such as WHC and EC, showed significant differences (one- and three-way ANOVA) between control and trampling area under the different tree canopies and in the open area during the three main periods of the study (Table 1). Along with the above soil properties, SM showed a difference between the control and trampling area during the CW and HD periods, while OM showed different values only during the HD and pH during the WW periods (Table 1). Moreover, WHC, SM, and OM were higher in the control area, while EC and pH were higher in the trampling area (Table 1).

Multivariate analysis of the soil properties (Fig. 2) showed discrimination between the sampling spots during the study. Whereas in the CW period the most observed soil properties (SM, WHC, and OM) were

found to be higher under the canopies of *Eucalyptus* camaldulensis (CE and TE in the control and trampling sites, respectively) and *Tamarix aphylla* (CT, control area), the EC values indicated an increase under the canopies of *Tamarix aphylla* (CT and TT in the control and trampling areas, respectively), *Eucalyptus camaldulensis* (TE, trampling area), and *Cupressus sempervirens* (TU, trampling area). The pH values showed an increase in the open area (CO and TO in the control and trampling areas, respectively) and under the canopies of *Cupressus empervirens* (CU and TU in the control and trampling areas, respectively) and TU in the control and trampling areas, respectively) and TU in the control and trampling areas, respectively) and *Tamarix aphylla* (TT, trampling area) (Fig. 2).

In the WW period (Fig. 2), the WHC and pH values were higher under canopies of the Cupressus empervirens (both at the control and trampling sites) and the Tamarix aphylla (TT). The high values of the SM and OM (Fig. 2), similar to the CW, still remained high under the canopies of the Eucalyptus camaldulensis (TE) and Tamarix aphylla (CT), and increased under canopy of the Tamarix aphylla (TT). Similar to the CW period, the EC values remained high under canopies of the Tamarix aphylla (TT), the Eucalvptus camaldulensis (TE), Cupressus empervirens (TU), and increased under the canopy of the Cupressus empervirens (CU) (Fig. 2).

Seasons		Ss			soil propertie	S	
			SM	EC	WHC	OM	pН
			(g kg <sup>-1</sup> )	$(\mu S g^{-1})$	(g kg <sup>-1</sup> )	(g kg <sup>-1</sup> )	
		С	22.76a*	150.57b	53.65a	0.90a	7.63a
Wet	CW	Т	16.77b	227.58a	36.99b	0.83a	7.66a
period		С	18.11a	200.12b	53.65a	1.20a	7.49b
	WW	Т	17.48a	308.54a	36.99b	1.37a	7.66a
Dry		С	6.40a	183.9b	53.653a	1.87a	8.05a
period	HD	Т	2.89b	623.69a	36.99b	1.22b	8.02a
	P values***	Ss	0.0005	0.0005	0.0005	0.011	NS
		Sp	0.0005	0.0005	0.0005	0.0005	NS
		Se	0.0005	0.0005	NS	0.0005	0.0005

Table 1 Season effect of trampling damage on soil properties (n = 96).

\*, one-way ANOVA; \*\*\*, three-way ANOVA.

Ss, sampling sites (control & trampling area); Sp, sampling points (open sites & trees area); Se, season periods.

Soil moisture (SM); electrical conductivity (EC); water-holding capacity (WHC); organic matter (OM); soil pH.

C, control plots; T, trampling plots. Cold-wet (CW), warm-wet (WW) and hot-dry (HD) periods.

Bold and different letters indicate significant differences between the trampling and control sites.





Redundancy analysis (RDA) indicated a seasonal trampling effect on the soil properties and soil biota. The length and angle of arrows indicate the strength and degree of correlation between the observed characteristics and environment. The first axis of the CW figure explains 81% of the total variability in the data, with the sum of all canonical eigenvalues amounting to 81%. The significance of these variations was confirmed by the Monte Carlo permutation test (P value = 0.01; F ratio = 4.18; number of permutations = 499). The first axis of the WW figure explains 39% of the total variability in the data, with the sum of all canonical eigenvalues amounting to 59%. The significance of these variations was confirmed by the Monte Carlo permutation by the Monte Carlo permutation test (P value = 0.03; F ratio = 3.41; number of permutations = 499). All four eigenvalues in the figures were found to be canonical and correspond to axes that are constrained by the environmental variables. The first axis of the HD figure explains 66% of the total variability in the data, with the sum of all canonical eigenvalues amounting to 68%. The significance of these variations was confirmed by the Monte Carlo permutation test (P value = 0.02; F ratio = 3.28; number of permutations = 499). All four eigenvalues amounting to 68%. The significance of these variations was confirmed by the Monte Carlo permutation test (P value = 0.02; F ratio = 3.28; number of permutations = 499). All four eigenvalues in the figures were found to be canonical and correspond to axes that are constrained by the environmental variables, the environmental variables.

Sampling spots: CO, control in open space; TO, trampling in open space; CE, control in the Eucalyptus area; TE, trampling in the *Eucalyptus* area; CU, control in the *Cupressus* area; TU, trampling in the *Cupressus* area; CT, control in the *Tamarix* area; TT, trampling in the *Tamarix* area.

Soil properties: Water content of soil (SM); electrical conductivity (EC;  $\mu$ S g<sup>-1</sup>); water-holding capacity (WHC); organic matter (OM); soil pH.

The dotted ovals indicate the relevant differences between sampling spots during the study.

	TN	BF	FF	PP	OP	BF1	BF2	BF3	BF4
С	3530.42a*	1569.18a	136.34a	911.83a	913.05a	143.6b	1298.91a	110.92a	15.75a
Т	1429.85b	872.26b	118.36a	127.40b	311.83b	394.17a	467.86b	9.62b	0.61b
P values***									
Ss	0.0005	0.008	NS	0.0005	0.0005	0.003	0.0005	0.0005	0.04
Sp	0.0005	0.0005	NS	NS	0.003	0.002	0.0005	0.005	NS
Se	0.0005	0.0005	0.04	0.002	0.001	0.001	0.0005	NS	NS
	FF2	FF4	PP2	PP3	PP4	PP5	OP3	OP4	OP5
С	89.29a*	47.05a	310.52a	508.72a	27.65a	64.94a	2.72a	427.28a	483.05a
Т	114.64a	3.72b	99.86b	26.47b	1.07b	0b	0a	157.41b	154.42b
P values***									
Ss	NS	0.0005	0.002	0.0005	0.0001	0.0005	NS	0.001	0.006
Sp	0.02	0.0005	0.01	0.03	0.0001	NS	NS	0.006	0.05
Se	0.02	NS	0.01	NS	0.0001	0.008	NS	0.002	0.04

Table 2Effect of trampling damage on total number, trophic structure and functional guilds of soil free-living nematodesduring the study period (the three-way ANOVA).

\*, one-way ANOVA; \*\*\*, three-way ANOVA.

Ss, sampling sites (control & trampling area); Sp, sampling points (open sites & trees area); Se, season periods.

C, control plots; T, trampling plots. TN, total number of free-living nematodes (n = 96, kg<sup>-1</sup> dry soil).

Trophic groups: OP, omnivore-predator; PP, plant-parasitic; FF, fungifeeding; BF, bacteria-feeding nematodes.

BF1, BF2..., c-p values of different nematode functional guilds (n = 96, kg<sup>-1</sup> dry soil).

Different letters indicate significant differences between trampling and control sites

During the HD period (Fig. 2), the most observed soil properties (SM, WHC, OM and pH) exhibited higher values under the *Eucalyptus camaldulensis* and *Cupressus empervirens* canopies in the control area. EC exhibited similar high values under the *Tamarix aphylla* and *Eucalyptus camaldulensis* canopies in the trampling area (Fig. 2).

## 3.2 Nematode Community Structure

The total number of soil free-living nematodes (TN) was significantly higher in the control area than in the trampling area during the study (Table 2). The mean abundance of the trophic groups [except for the FF and OP nematodes, which belong to c-p 2 and 3 functional guilds, respectively (Table 2)] was higher in the control area than in the trampling area during the study. In contrast to the trophic groups, the BF nematodes belonging to the c-p 1 exhibited higher density in the trampling area (Table 2). Moreover, a seasonal effect of animal trampling on the soil free-living nematode communities in the study area was found (Fig. 3). In the cold-wet period, the TN values were higher in the control area under canopies of the Cupressus empervirens and in the open area; in the WW period, the TN values were higher in the control area under canopies of the Tamarix aphylla and in the open area; while in the HD period, no statistical differences were found between the control and trampling areas (Fig. 3, Table 2). The Pearson correlation coefficient showed a significant correlation between the TN and the observed soil properties in the study area (Table 3). The TN showed correlation with SM and pH (positive and negative, respectively) in both the control and trampling areas, and a positive correlation with OM in the trampling area only (Table 3).

Sixty-six nematode taxa were identified in the present study: 20 taxa belonged to the bacterivore trophic group (BF), 8 were fungivores (FF), 21 were plant-parasites (PP), and 17 were omnivore-predators (OP) (Table 4). The total number of nematodes and most of the trophic groups showed statistical

differences (three-way ANOVA) between sampling sites (control and trampling area) and between sampling points (open sites and trees area) during the observed seasons (Table 4). A substantial part of nematode genera also showed statistical differences between comparable parameters (Table 4). Moreover, the total number of soil free-living nematodes (TN) was significantly higher under tree canopies than in the open bare space in the trampling area (Table 4). While nematode abundance and the diversity of nematode genera and trophic groups were much higher in the control sites in comparison with the trampling sites in the open bare area, this ratio was more complex under the tree-canopy area (Table 4).

Multivariate analysis of the nematode trophic groups showed clear nematode trophic discrimination between the control and the trampling sites during the wet and dry periods (Fig. 4). In the CW period, most of the observed nematode trophic groups showed a trend toward increasing abundance in the control area (Fig. 4). However, the OP nematodes belonging to the c-p 3 exhibited higher values in the trampling sites under the canopies of Eucalyptus camaldulensis, Cupressus empervirens, and in the open area, as well as in the control and trampling sites under the canopy of Tamarix aphylla in the CW period (Fig. 4). The PP nematodes (c-p 4) were found in both the control and trampling sites (Fig. 4). The BF and FF nematodes belonging to the c-p 1 and 2, respectively, were more numerous in both the control and trampling sites under the canopies of Eucalyptus camaldulensis and Tamarix aphylla in the same season (Fig. 4). In the WW period, the total number of the BF and FF nematodes belonging to c-p 1 and 2 functional guilds, respectively, showed higher values only in the trampling sites under the canopies of Eucalyptus camaldulensis and Cupressus empervirens, while the total number of the BF (c-p 2), PP (c-p 3 and 5), and OP (c-p 4 and 5) nematodes showed high values in the control area under the canopies of the same trees (Fig. 4). The total number of the following trophic groups



Fig. 3 Effect of different habitat conditions on total number of soil free-living nematodes.

C, control spots; T, trampling spots; O.s., open space; E.s., *Eucalyptus camaldulensis*; C.s., *Cupressus empervirens*; T.a., *Tamarix aphylla*.

Different letters indicate significant differences between trampling and control sites.

Table 3	Pearson correlation	coefficients betwee	n soil properties	and total n	number of soil	free-living net	matodes in	the stu	ıdy
area (n =	96).								

	SM	EC	WHC	OM	pН
Control	0.28*	NS	NS	NS	-0.38**
Trampling	0.62***	NS	NS	0.46***	-0.46***

Soil properties: soil moisture (SM); electrical conductivity (EC);

water-holding capacity (WHC); organic matter (OM); soil pH.

Locations		CO <sup>d</sup>	ТО	CE	TE	CU	TU	CT	TT	Ss	Sp	Se
Trophic groups/ger	us										(three-way ANOVA)	
TN <sup>a</sup>		2518.9±1254.2ª	141.8±195.6 <sup>b</sup>	5680.5±4472.3ª	1724.8±2131.5 <sup>b</sup>	3651.5±2303.2ª	2131.1±2280.8 <sup>b</sup>	2270.8±1744.9ª	1721.7±1083.5ª	0.0001	0.0001	0.0001
Bacterivores <sup>b</sup>	c-p <sup>c</sup> values	$544.7 \pm 378.8^{a}$	116.1±193.1 <sup>b</sup>	2643.1±2728.4ª	1259.2±1611.7ª	2068.8±2303.3ª	1238.8±1692.2ª	1020.2±1744.1ª	875.0±647.7 <sup>a</sup>	0.008	0.0001	0.0001
Achromadora	3	108.74±95.62	0	0	0	0	0	13.48±46.67	0	0.0001	0.0001	NS
Acrobeles	2	0	0.43±1	383.6±555.4	0	738.3±1184.7	40.01±90.42	197.5±268.44	0	0.001	0.035	NS
Acrobeloides	2	40.74±71.26	16.47±25.33	529±1194	$13.43 \pm 24.32$	138.0±122.8	152.5±170.6	85.18±78.55	16.91±39.98	NS	NS	NS
Alaimus	4	$37.91{\pm}71.26$	0	0	0	0	$2.43 \pm 5.42$	25.11±63.61	0	0.036	NS	NS
Cephalobus	2	33.00±77.11	16.0±21.99	$163.5 \pm 208.8$	$6.33{\pm}16.98$	82.87±125.2	116.5±166.2	$34.98{\pm}75.61$	$39.29{\pm}71.87$	NS	NS	NS
Cervidellus	2	0	0	0	4.80±1662	0	$107.7 \pm 105.2$	$74.45{\pm}144.8$	33.79±35.31	NS	0.001	0.002
Chiloplacus	2	$30.51{\pm}76.10$	0	$69.04{\pm}108.1$	$58.43{\pm}118.9$	0	$138.4{\pm}194.2$	127.1±214.6	234.4±348.7	0.04	0.0001	0.0001
Chronogaster	3	3.86±13.36	0	0	0	41.36±98.9	$4.27 \pm 14.80$	47.84±87.23	$14.09 \pm 48.81$	0.049	0.051	0.017
Eucephalobus	2	71.17±72.49	$15.43 \pm 33.14$	96.04±111.2	235.2±542.4	246.9±253.2	165.7±216.0	17.35±47.38	156.7±189.4	NS	NS	0.028
Eumonhystera	2	$70.10{\pm}105.44$	0	1046±1397	0	223.4±258.0	22.77±48.50	149.7±304.4	17.35±42.84	0.0001	0.002	0.047
Heterocephalobus	2	0	0	0	195±349	207.6±327.6	30.10±86.56	0	28.84±70.13	NS	0.007	0.001
Mesorhabtidae	1	0	1.09±3.1	75.29±204.2	43.96±111.04	69.04±178.9	8.03±27.82	137.8±199.6	54.70±158.2	NS	0.042	0.022
Monhystera	2	24.13±47.44	0	0	0	153.8±163.3	8.89±17.10	6.76±24.41	0	0.0001	0.0001	0.005
Panagrellus	1	0	0.72±1.35	0	10.72±20.11	0	0	0	0	0.005	0.0001	0.002
Panagrolaimus	1	38.23±68.59	65.91±153.1	204.68±483.01	684.5±732.9	0	414.1±831.8	42.16±58.86	264.5±223.0	0.0001	0.002	0.004
Paradiplogaster	1	0	0	7.18±24.89	0	0	0	0	0	NS	NS	NS
Plectus	2	45.54±86.61	0	0	0	0	0	21.24±60.45	0	0.009	0.035	0.004
Prismatolaimus	3	40.73±80.51	0	0	0	148.1±241.5	12.82±44.40	39.57±89.98	0	0.007	0.029	NS
Rhabditis	1	0	0	0	6.33±16.98	0	7.32±17.55	0	14.42±35.01	0.012	NS	0.002
Wilsonema	2	0	0	69.43±150.6	0	19.40±67.21	0	0	0	NS	NS	NS
Fungivores		223.6±161.1ª	2.54±5.02 <sup>b</sup>	124.3±202.9ª	109.9±224.7ª	19.10±57.16 <sup>b</sup>	182.8±243.6ª	178.4±213.1ª	178.2±177.4ª	NS	NS	0.044
Aphelenchoides	2	8.74±27.74	0	0	77.65±127.0	19.10±57.16	78.56±101.2	21.61±59.76	141.4±188.1	0.001	0.027	0.044
Aprutides	2	0	0	0	32.29±111.9	0	0	0	0	NS	NS	NS
Aphelenchus	2	18.88±52.26	1.24±3.06	82.11±198.2	0	0	14.90±25.57	117.4±190.5	9.12±31.58	0.024	NS	NS
Ditylenchus	2	8.65±29.41	0	42.19±85.63	0	0	49.31±92.97	17.37±60.17	11.37±48.81	NS	NS	NS
Nothotylenchus	2	0	0	0	0	0	33.94±109.9	0	0	NS	0.049	NS
Paraphelenchus	2	0	0	0	0	0	5.71±20.9	22.02±38.34	0	NS	0.0001	0.0001
Tylencholaimus	4	168.99±128.88	1.30±3.27	0	0	0	0	0	16.30±36.25	0.001	0.0001	NS
Tylencholaimellus	4	18.34±37.89	0	0	0	0	0	0	0	NS	NS	NS

 Table 4
 Mean abundance (ind. per 100 g dry soil, n = 96) and standard deviation of nematode genera at the control and trampling study sites.

Table 4 to be con	tinued											
Plant-parasites		1110±1109ª	4.73±7.05 <sup>b</sup>	1181±1019ª	36.92±110.9 <sup>b</sup>	569.4±497.1ª	267.9±234.9ª	786.9±729.8ª	200.1±182.5 <sup>b</sup>	0.0001	NS	0.002
Aglenchus	2	$28.43 \pm 51.86$	0	0	0	0	0	$7.74{\pm}26.82$	0	0.029	0.049	NS
Basiria	2	9.97±34.55	0	0	0	0	$2.89 \pm 9.99$	0	$7.36 \pm 25.51$	NS	NS	NS
Boleodorus	2	$10.45 \pm 36.20$	0	0	0	19.10±57.16	0	6.76±23.41	0	NS	NS	NS
Cephalenchus	2	0	$0.42 \pm 0.97$	0	0	0	$19.63{\pm}68.12$	0	0	NS	NS	NS
Coslenchus	2	$41.17 \pm 60.88$	0	$7.18 \pm 24.89$	0	0	0	0	0	0.005	0.001	0.021
Filenchus	2	389.17±449.03	$1.54 \pm 3.31$	$28.25 \pm 68.01$	0	$182.9 \pm 228.9$	$98.30 \pm 94.94$	42.42±71.63	85.20±93.89	0.003	0.005	NS
Helicotylenchus	3	0	$0.56 \pm 1.94$	$91.05 \pm 209.2$	0	$19.63 \pm 39.80$	0	0	0	NS	NS	NS
Longidorella	4	82.35±132.07	0	0	0	0	$4.27 \pm 14.80$	$18.28 \pm 34.58$	0	0.0001	0.0001	0.0001
Longidorus	5	$23.13 \pm 52.95$	0	112.5±172.7	0	$22.04 \pm 52.28$	0	$13.52 \pm 46.82$	0	0.001	0.02	0.026
Malenchus	2	$78.20{\pm}104.27$	0	0	0	$34.05 \pm 88.97$	$44.29 \pm 89.56$	$7.74{\pm}26.82$	$79.02{\pm}107.6$	NS	NS	0.01
Meloidogyna	3	69.49±111.36	0	0	0	0	0	0	0	0.038	0.006	NS
Paratylenchus	2	$149.05 \pm 290.71$	0	$88.02 \pm 304.9$	0	67.53±131.7	0	0	0	0.014	NS	NS
Pratylenchoides	3	71.26±120.37	0	110.0±338.7	0	31.27±74.42	0	220.1±398.3	0	0.001	NS	0.0001
Pratylenchus	3	8.50±29.41	0	$361.3 \pm 758.5$	0	0	$25.72 \pm 58.76$	$141.6 \pm 248.1$	0	0.026	NS	NS
Psilenchus	2	0	0	0	0	0	$38.20{\pm}74.23$	$43.94{\pm}105.7$	0	NS	NS	NS
Rotylenchus	3	0	0	0	0	$9.23 \pm 31.98$	0	0	0	NS	NS	NS
Trichodorus	4	9.97±34.55	0	0	0	0	0	0	0	NS	NS	NS
Trophurus	3	$55.86 \pm 88.06$	$0.64 \pm 2.22$	0	32.29±111.9	0	0	0	0	NS	NS	NS
Tylenchorhynchus	3	67.96±117.04	$1.56\pm4.77$	$382.8 \pm 537.3$	0	$134.8 \pm 152.7$	21.96±33.69	$260.1 \pm 360.8$	23.16±62.90	0.0001	NS	0.045
Tylenchus	2	0	0	0	0	0	$12.61 \pm 24.25$	0	$5.31 \pm 18.38$	0.044	NS	NS
Xiphinema	5	$15.03 \pm 52.06$	0	0	0	48.75±113.9	0	$24.80 \pm 85.92$	0	NS	NS	NS
Omnivores-predate	ors	640.8±588.1ª	$18.51 \pm 31.73^{b}$	1732±1601ª	$318.8{\pm}463.8^{b}$	994.2±897.1ª	441.6±776.3ª	$285.3{\pm}251.7^{b}$	$468.5{\pm}755.7^{a}$	0.0001	0.003	0.001
Anatonchus	4	0	0	$7.18 \pm 24.86$	0	0	0	0	0	NS	NS	NS
Aporcelaimellus	5	$98.02{\pm}61.18$	$1.67 \pm 5.8$	$323.5 \pm 592.1$	0	278.7±226.7	9.06±18.33	3.87±13.41	0	0.0001	0.042	NS
Aporcelaimium	5	$18.90{\pm}44.75$	0	0	0	0	0	0	0	NS	NS	NS
Aporcelaimus	5	$97.45 \pm 77.92$	$0.42{\pm}1.45$	0	0	$224.58 \pm 230.23$	$98.71{\pm}182.7$	0	0	0.014	NS	NS
Axonchium	5	$188.26{\pm}400.59$	$0.30 \pm 0.78$	0	0	0	0	0	$0.82 \pm 2.79$	NS	NS	NS
Clarcus	4	8.49±29.41	0	0	0	0	0	0	0	NS	NS	NS
Discolaimium	5	10.89±37.72	$0.27 \pm 0.7$	0	0	0	0	$47.98{\pm}117.1$	0	NS	NS	NS
Discolaimoides	5	0	0	0	0	0	0	0	0	NS	NS	NS
Dorilaimoides	4	17.88±31.02	3.61±6.24	126.7±174.1	55.26±128.3	383.3±409.2	226.8±506.1	9.58±22.96	148.4±163.6	NS	0.0001	0.049
Epidorylaimus	4	0	$0.42{\pm}1.45$	0	4.80±1.45	0	0	0	97.02±103.3	0.0001	0.0001	0.0001
Eudorylaimus	4	69.36±54.63	0	130.2±268.4	5.30±17.31	0	0	$61.31{\pm}114.6$	43.07±149.2	0.023	NS	NS

Table 4 to be continued

Mesodorylaimus	5	17.88±31.02	11.62±24.54	17.36±31.02	212.5±351.6	0	62.02±104.1	16.73±30.16	179.2±438.1	0.012	NS	NS
Microdorylaimus	4	$31.66{\pm}65.48$	0	201.6±260.3	0	$98.38{\pm}223.0$	44.94±73.79	$134.9{\pm}146.8$	0	0.0001	NS	0.026
Mononchus	4	0	0	48.4±106.0	0	0	0	0	0	NS	0.043	NS
Nygolaimus	5	$36.04 \pm 86.91$	$0.20\pm0.7$	$114.9 \pm 219.7$	$40.93{\pm}113.1$	9.23±31.98	0	0	0	NS	0.011	NS
Thonus	4	$45.92{\pm}72.01$	0	257.1±594.3	0	0	0	0	0	NS	NS	NS
Tobrilus	3	0	0	0	0	0	0	$10.88 \pm 37.75$	0	NS	NS	NS

CO, control of the open space; TO, trampling of the open space;

CE, control of the Eucalyptus area ; TE, trampling in the Eucalyptus area

CU, control of the Cupresus; TU, trampling in the Cupresus area

CT, control of the Tamarix; TT, trampling in the Tamarix area

Ss, sampling sites (control & trampling area); Sp, sampling points (open sites & trees area); Se, season periods.

TN<sup>a</sup>, total number of soil free-living nematodes.

<sup>b</sup> Trophic groups according to Yeates *et al.* (1993).

<sup>c</sup>c-p values, characterized by life history characteristics, are adapted from Bongers (1990).

<sup>d</sup> comparative changes of abundance of trophic groups in the control and trampling sites

Different letters indicate significant differences between trampling and control plots.

[BF (c-p 3 and 4), FF (c-p 4), and PP (c-p 2 and 4)] showed higher values in both the control and trampling areas (Fig. 4). In the HD period, most of the observed nematode trophic groups showed a clear discrimination between the control and trampling spots, with nematodes preferring the control to the trampling area (Fig. 4). However, the trophic composition of nematodes under the canopy of *Tamarix aphylla* in the trampling area was similar to that in the control vegetated area, and vice versa.

The Pearson correlation coefficient showed a significant correlation between soil properties and trophic groups belonging to different functional guilds in the control and trampling sites (Table 5). Correlation coefficient between SM and trophic groups such as BF (c-p 1, 2, 3 and 4), FF (c-p 2 and 4), and OP (c-p 3

and 5) was quite changeable and showed different degree of dependence in the control and trampling area (Table 5). Correlation coefficient between EC and the following trophic groups, as BF (c-p 1), FF (c-p 4), and PP (c-p 2 and 4), also revealed changeable dependent on location and showed different correlation values in the control and trampling sites (Table 5). Correlation coefficient between WHC and BF (c-p 4) and PP (2, 3, and 4) trophic groups showed different correlation in the control and trampling sites (Table 5). Correlation coefficient between OM and trophic groups such as BF (c-p 1, 2, 3), PP (c-p 2), and OP (c-p 5) showed different degree of dependence in the control and trampling sites (Table 5). Similar to the mentioned soil properties, correlation coefficient between pH and the following trophic groups such as



Fig. 4 Effect of different habitat conditions on trophic structure of a soil nematode community.

Redundancy analysis (RDA) indicated a seasonal trampling effect on the trophic structure of a soil free-living nematode community. The length and angle of arrows indicate the strength and degree of correlation between the characteristics and environment. The first axis of the CW figure explains 81% of the total variability in the data, with the sum of all canonical eigenvalues amounting to 81%. The significance of these variations was confirmed by the Monte Carlo permutation test (P value = 0.01; F ratio = 4.18; number of permutations = 499) The first axis of the WW figure explains 39% of the total variability in the data, with the sum of all canonical eigenvalues amounting to 59%. The significance of these variations was confirmed by the Monte Carlo permutation test (P value = 0.03; F ratio = 3.41; number of permutations = 499). All four eigenvalues in the figures were found to be canonical and correspond to axes that are constrained by the environmental variables. The first axis of the HD figure explains 66% of the total variability in the data, with the sum of all canonical eigenvalues amounting to 68%. The significance of these variations was confirmed by the Monte Carlo permutation test (P value = 0.02; F-ratio = 3.28; number of permutations = 499). All four eigenvalues in the figure explains 66% of the total variability in the data, with the sum of all canonical eigenvalues amounting to 68%. The significance of these variations was confirmed by the Monte Carlo permutation test (P value = 0.02; F-ratio = 3.28; number of permutations = 499). All four eigenvalues in the figure explains 66% of the total variability in the data, with the sum of all canonical eigenvalues amounting to 68%. The significance of these variations was confirmed by the Monte Carlo permutation test (P value = 0.02; F-ratio = 3.28; number of permutations = 499). All four eigenvalues in the figures were found to be canonical and correspond to axes that are constrained by the environmental variab

Sampling spots: CO, control in open space; TO, trampling in the open space; CE, control in the *Eucalyptus* area; TE, trampling in the *Eucalyptus* area; CU, control in the *Cupressus* area; TU, trampling in the *Cupressus* area; CT, control in the *Tamarix* area; TT, trampling in the *Tamarix* area.

Soil properties: Water content of soil (SM); electrical conductivity (EC; µS g<sup>-1</sup>); water-holding capacity (WHC); organic matter

(OM); soil pH.

The dotted ovals indicate the relevant differences between sampling spots during the study.

Table 5 Comparable analysis of correlation between soil properties and trophic groups belonging to different functional guilds (n = 96).

Properties	Location	BF1	BF2	BF3	BF4	FF2	FF4	PP2	PP3	PP4	PP5	OP3	OP4	OP5
SM	С	ns	ns	0.40**	0.27*	ns	ns	0.36**	ns	ns	ns	0.26*	ns	ns
	Т	0.66***	0.58***	ns	ns	0.49***	0.24*	0.25*	ns	ns	ns	ns	ns	0.24*
EC	С	0.29*	ns	ns	ns	ns	-0.45**	-0.27*	ns	-0.32*	ns	ns	ns	ns
	Т	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
WHC	С	ns	ns	ns	ns	ns	ns	ns	-0.29*	ns	0.26*	ns	ns	ns
	Т	ns	ns	ns	0.29*	ns	ns	0.45**	ns	ns	ns	ns	ns	ns
ОМ	С	ns	-0.31*	-0.35**	ns	0.44**	ns	-0.28*	ns	ns	ns	ns	ns	ns
	Т	0.49***	0.41**	ns	ns	0.36*	ns	ns	ns	ns	ns	ns	ns	0.39**
рН	С	ns	ns	ns	-0.30*	ns	ns	-0.35**	ns	-0.44**	-0.26*	ns	-0.25*	ns
	Т	-0.32*	-0.44**	ns	ns	-0.33*	ns	-0.31*	ns	ns	ns	ns	-0.31*	-0.26*

SM, soil moisture; EC, electrical conductivity; WHC, water-holding capacity; OM, organic matter; soil pH.

C, control plots; T, trampling plots.

Trophic structure: OP, omnivore-predator; PP, plant-parasitic; FF, fungifeeding; BF, bacteria-feeding nematodes.

BF1, BF2..., c-p values of different nematode functional guilds

\*, \*\*, \*\*\*Correlation coefficient significant at p<0.05, 0.01 and more than 0.001, respectively.

Grey color indicates difference between control and trampling areas.

BF (c-p 1, 2, 4), FF 9c-p 2), PP (c-p 4 and 5), and OP (c-p 5) showed different correlation values in the control and trampling sites (Table 5).

Among the 66 nematode genera observed in the study area, 22 were found only in the control area and 7 only in the trampling area. Ten genera showed a decrease and 2 genera showed an increase in the trampling area. The other 25 nematode genera were found to be insensitive to the trampling effect and were present both in the control and trampling areas (Table 4).

The Acrobeles (1360 ind. 100 g<sup>-1</sup> dry soil), Eumonhystera (1529 ind. 100 g<sup>-1</sup> dry soil), and Panagrolaimus (1714 ind. 100 g<sup>-1</sup> dry soil) were the most dominant bacterivore nematodes (Table 4). However, while the Acrobeles and Eumonhystera showed their highest values under the canopies of the trees in the control area, the Panagrolaimus exhibited higher values in the trampling area (Table 4). The Aphelenchoides (542 ind. 100 g<sup>-1</sup> dry soil) and Aphelenchus (417 ind. 100 g<sup>-1</sup> dry soil), being the most common among the fungivores, and the Filenchus (894 ind. 100 g<sup>-1</sup> dry soil) and Tylenchorhynchus (985 ind.100 g<sup>-1</sup> dry soil), being the most common among the plant-parasite nematodes, reached maximal values in both the control and trampling sites (Table 4). The *Aporcelaimus* (725 ind. 100 g<sup>-1</sup> dry soil), *Aporelaimellus* (788 ind. 100 g<sup>-1</sup> dry soil), and *Dorilaimoides* (1058 ind. 100 g<sup>-1</sup> dry soil) were the most dominant among the omnivore-predator nematodes (Table 4). In contrast to *Aporcelaimus* and *Aporcelaimellus*, which reached maximal values in the control area, the *Dorilaimoides* showed maximal values both in the control and the trampling areas (Table 4).

## 3.3 Ecological Indices

The most applied ecological indices showed statistical differences (three-way ANOVA) between sampling sites (control and trampling areas) and between sampling points (open sites and trees area) during the observed seasons (Table 6). Ecological indices, such as trophic diversity (T), Simpson's dominance index (Dom), Shannon–Weaver index (H'), maturity index modification (MMI), species richness (SR), structure index (SI), and enrichment index (EI), were sensitive to trampling impact and showed different values between the control and trampling sites during the study (Table 6). However, the

Simpson's dominance (Dom) and enrichment (EI) indices showed higher values in the trampling area (Table 6). Redundancy analysis (RDA) indicated correlation between the habitat seasonal variables and soil biotic community structure. Seasonal changes in the ecological-index values (Fig. 5) were found. Thus, for example, Dom and EI indicated higher values in the trampling area, while the T, H', SR, SI, and MMI

	8		8			,		
	Т	Dom	Н'	MMI	SR	NCR	SI	EI
С	2.56a*	0.14b	2.24a	2.87a	1.31a	0.85a	73.01a	26.11b
Т	1.93b	0.26a	1.69b	2.23b	1.01b	0.9a	54.14b	62.3a
P values***								
Ss	0.0005	0.0005	0.0001	0.0001	0.0001	NS	0.0001	0.0001
Sp	NS	0.011	0.0001	0.023	0.0001	0.001	0.0145	0.0001
Se	0.026	NS	0.03	0.025	NS	0.015	0.0054	0.0001

Table 6 Comparative change in values of the ecological indices in the study area (n = 96).

\*, one-way ANOVA; \*\*\*, three-way ANOVA.

C, control plots; T, trampling plots.

T, trophic diversity index; Dom, Simpson's dominance index; H', Shannon-Weaver index: MMI, maturity index modification; SR, species richness; NCR, nematode channel ratio; SI structure index; EI, enrichment index.

Ss, sampling sites (control & trampling area); Sp, sampling points (open sites & trees area); Se, season periods.

Different letters indicate significant differences between trampling and control sites

Grey color indicates a higher value in the trampling area.





In contrary to the HD period, during the wet period, differences between the trampling and control sites reached a maximum value. The length and angle of arrows indicate the strength and degree of correlation between the ecological indices and the environment. The first axis of the CW figure explains 65% of the total variability in the data, with the sum of all canonical eigenvalues amounting to 74%. The significance of these variations was confirmed by the Monte Carlo permutation test (P value = 0.002; F-ratio = 8.39; number of permutations = 499). The first axis of the WW figure explains 64% of the total variability in the data, with the sum of all canonical eigenvalues amounting to 70%. The significance of these variations was confirmed by the Monte Carlo permutation test (P value = 0.004; F-ratio = 7.03; number of permutations = 499). All four eigenvalues in the figures were found to be canonical and correspond to axes that are constrained by the environmental variables.

The first axis of the HD figure explains 34% of the total variability in the data, with the sum of all canonical eigenvalues amounting to 53%. The significance of these variations was confirmed by the Monte Carlo permutation test (P value = 0.002; F-ratio = 6.81; number of permutations = 499). All four eigenvalues in the figures were found to be canonical and correspond to axes that are constrained by the environmental variables.

Sampling spots: CO, control in open space; TO, trampling in the open space; CE, control in the *Eucalyptus* area; TE, trampling in the *Eucalyptus* area; CU, control of the *Cupressus* area; TU, trampling in the *Cupressus* area; CT, control in the *Tamarix* area; TT, trampling in the *Tamarix* area.

Ecological indices: NCR, nematode channel ratio; H', Shannon-Weaver index; EV, evenness; SR, species richness; T, trophic diversity; Dom, Simpson's dominance index; MMI, maturity index modification.

The dotted ovals indicate the relevant differences between sampling spots during the study.

			Control					Trampling		
	SM	EC	WHC	ОМ	pН	SM	EC	WHC	OM	pН
Т	-0.32*	ns	ns	0.36**	0.26*	NS	NS	0.43**	NS	NS
D	ns	0.25*	ns	ns	ns	NS	0.53***	-0.37*	NS	0.27*
Н'	ns	-0.32*	ns	ns	ns	NS	-0.43**	0.47**	NS	-0.30*
MMI	ns	-0.44**	ns	ns	ns	-0.31*	NS	NS	NS	NS
SR	ns	-0.32*	ns	ns	ns	NS	-0.42**	NS	-0.41**	NS
NCR	0.30*	ns	ns	-0.45**	ns	NS	NS	-0.50**	NS	NS
SI	ns	-0.39**	ns	ns	ns	NS	NS	NS	0.32*	NS
EI	-0.46**	ns	ns	0.55***	0.39**	0.28*	NS	NS	0.36**	NS

Table 7 Pearson correlation coefficients between ecological indices and soil properties in the study area (n = 96).

Soil properties: soil moisture (SM); electrical conductivity (EC); water-holding capacity (WHC); organic matter (OM); soil pH. Ecological indices: T, trophic diversity index; D, Simpson's dominance index; H', Shannon-Weaver index: MMI, maturity index modification;

SR, species richness; NCR, nematode channel ratio; SI structure index; EI, enrichment index.

\*.\*\*\*, \*\*\* Correlation coefficient significant at p < 0.05, 0.01 and more than 0.001, respectively.

Bold indicates the significant different values

indices showed higher values in the control area during the CW and WW periods (Fig. 5). Moreover, the nematode channel ratio (NCR) was found to have shifted from the trampling area in the CW period toward the control area in the WW period (Fig. 5).

In contrast to the previous periods, during the HD period, the ecological indices showed differences between control and trampling sites only under the Eucalyptus camaldulensis canopies (Fig. 5). In this hottest period, the Dom index indicated special environmental conditions under the Tamarix aphylla canopies in both the control and trampling areas (Fig. 5). The nematode ecological indices showed different correlations with soil properties in the trampling and control sites (Table 7). The T index showed a negative correlation with SM and a positive correlation with pH and OM in the control area, and a positive correlation with WHC in the trampling area (Table 7). The Dom showed a negative correlation with WHC in the trampling area (Table 7), while it was positively correlated with EC in the control and trampling areas and with pH in the trampling area (Table 7). The H' showed a negative correlation with EC in the control area, while in the trampling area, then it showed a positive correlation with WHC and a negative

correlation with EC and pH (Table 7). The MMI was negatively correlated with the EC in the control area, while in the trampling area, it showed a negative correlation with SM (Table 7). The SR was negatively correlated with EC in both compared areas and showed a negative correlation with OM in the trampling area (Table 7). The NCR showed a positive correlation with SM and a negative correlation with both OM in the control area and WHC in the trampling area (Table 7). The SI was negatively correlated with EC in the control area, and positively correlated with OM in the trampling area (Table 7). The EI was negatively correlated with SM and positively correlated with OM and pH in the control area, while in the trampling area, it showed a positive correlation with SM and OM (Table 7).

## 4. Discussion

## 4.1 Effect of Ungulate Trampling Intensity on Soil Properties

In general, grazing may exert a direct and/or indirect impact on soil properties caused mainly by trampling and excretion and because of changes in vegetation structure and function. Previous studies that initiated research on the effect of grazing on a soil medium reached the conclusion that animal compaction leads to change in soil porosity, increased bulk density, reduced water infiltration, and aboveand below-ground organic-matter input [56-59]. Moreover, even a low intensity of grazing will reduce infiltration and, hence, increase susceptibility to erosion [9]. However, Evans et al. (2012) [60] confirmed that a moderate intensity of long-term grazing [0.6 animal (beef cattle) unit months ha<sup>-1</sup>] did not have critical detrimental effects on soil properties.

Our results obtained in this study revealed that varying degrees of trampling affect the following soil properties: soil water content of soil, soil salinity, water-holding capacity, organic matter, and soil acidity. Moreover, we showed that trampling effect on soil properties is closely related to seasonal fluctuations. Therefore, for example, differences in were found between the trampling SM and undisturbed sites only in the cold-wet (CW) and hot-dry (HD) periods, with no differences found between these sites in the warmer-wet period (WW). OM values increased in the undisturbed sites only in the HD period, and pH values were higher in the trampling sites in the WW period. At the same time, reduction of the water-holding capacity in the soil under the effect of trampling activity [61] during the entire study, points to the compaction of soil.

## 4.2 Effect of Ungulate Trampling Intensity on a soil Free-living Nematode Community

According to previous investigations, soil compaction induced by ungulate activity mainly affected the habitat of soil nematodes by reducing the size of soil pores and changing the physical soil environment. In addition, it can be assumed that the reduction in species density and the diversity of soil nematodes in different habitats should cause the growth of soil microorganisms. Therefore, for example, microorganisms in fine-textured soils are more protected from predation than those in coarse-textured soils due to the restriction of the activity of predators, such as the soil free-living nematodes, by pore size [22]. However, despite numerous studies dedicated to soil compaction, there is still no consensus on the impact of the trampling on soil nematodes. Some of the studies affirmed that trampling, along with grazing, has a negative effect on soil nematode communities, while others do not come to such a conclusion or even found a positive effect on soil nematode communities [62-66]. It can be assumed that at least two main factors affect the findings in such studies: different environments (e.g., seasonal and vegetation effects) and differences between approaches used to study the functional differentiation of the free-living nematodes [46, 67].

In the present study, we found that trampling exerts a significantly different seasonal effect on the abundance and diversity of nematodes belonging to different functional guilds. Nematode abundance was lowest in the trampling area during the study. However, the tree covering during the wet and dry seasonal periods had a variable impact on nematode abundance in the study area. For example, during the study, the trees had a significant attenuating effect on trampling in comparison with the open bare area. Moreover, no difference in nematode abundance was found between the trampling and undisturbed sites under the Tamarix aphylla canopies in the CW period and under the Cupressus empervirens and Eucalyptus camaldulensis canopies in the WW period. However, during the hottest period of the year, when the external adverse factors dominated the trampling effect, the ability of the trees to protect nematode communities was significantly reduced. During this period, nematode abundance was not statistically different between the comparable areas. Soil organic matter was found to be more important for the total number of nematodes in the trampling area than in the undisturbed control area.

Out of the colonizer-persister (cp) continuum of nematode functional guilds [67], only bacteria-feeding nematodes belonging to the cp1 guild were positively affected by trampling during the study. Increased abundance of the BF1 guild might occur when resources become available due to favorable shifts or disturbances in the environment [46, 68], including of the favorable habitat of soil destruction microorganisms (the soil pore caves), thus increasing the predation success of soil free-living nematodes [22]. In general, nematodes belonging to the r-life-strategy group (colonizers. tolerant to environmental disturbance), mainly BF1 and BF2, were the most numerous in the study area, and amounted to about 61 and 44% in the trampling and undisturbed sites, respectively.

In contrast to the above-mentioned group of nematodes, the fungi-feeding nematodes were the smallest group in the study area numerically, and amounted to about 8 and 4% in the trampling and undisturbed sites, respectively. The undisturbed sites were a more favorable habitat for the plant-parasite nematodes, with their density amounting to 9 and 26% in the trampling and undisturbed sites, respectively. Surprisingly, the omnivore-predator nematodes that belong to the K-life strategy group and that are characterized by hypersensitivity to disturbance [32, 69, 70], were relatively numerous in the trampling (22%) and undisturbed (26%) sites. In addition, the results indicate that predators (soil nematodes) in the undisturbed ecosystem (characterized by a more complex and branched degree of development of trophic relationships and species diversity), are more closely related to their prey (microorganisms) than in the ungulate-disturbed trampling ecosystem (with more simple trophic relationships and poorer species diversity) [71, 72].

Our data indicate that about 62% of the nematode species were affected (48% negatively and 14% positively) by either direct trampling effect or changes in soil properties to varying degrees. Among them, *Paradiplogaster*, *Plectus*, *Wilsonema*, *Tylencholaimellus*, *Aglenchus*, *Boleodorus*, *Coslenchus*, *Longidorus*, *Meloidogyna*, *Paratylenchus*, *Pratylenchoide*, Pratylenchus, Rotylenchus, Trichodorus, Xiphinema, Anatonchus, Aporcelsaimium, Clarcus, Discolaimoides, Mononchus, Thonus, and Tobrilus were found only in the undisturbed control spots, while Panagrellus, Rhabditis, Aprutides, Nothotylenchus, Cephalenchus, Tylenchus and Epidorylaimus were found only in the trampling area. In contrast to Panagrolaimus and Mesodorylaimus (excluding the open area), Acrobeloides, Eumonhystera, Monhystera, Prismatolaimus, Tylenchorhynchus, Aporcelaimellus, Aporcelaimus, Eudorylaimus, Microdorylaimus, and Nygolaimus showed a clear tendency to decrease in the trampling area.

## 4.3 Ecological Indices

The widely used ecological indices applied in this study were sensitive to soil ecosystem changes in the trampling areas. The trophic diversity index, along with species richness, confirmed that the trampling sites were more unfavorable habitats for soil biota compared with the undisturbed area. Moreover, species richness indicated that the soil medium under the Cupressus empervirens canopy is more favorable for soil free-living nematodes in the HD period. The diversity indices [with the Shannon index sensitive to rare taxa and the Simpson's index used to measure common taxa [70], indicated an increase of the contribution of rare species to the undisturbed area as a well-developed ecosystem with a complex food web, while in the trampling area, the common nematodes were the main contribution to the soil ecosystem. Maturity indices have been successfully used to distinguish between well-functioning and disturbed ecosystems [31, 33], showing that the trampling area was a more unfavorable habitat for soil biota than the undisturbed control area. The nematode channel ratio (NCR) [with variation between 1 (bacterial-feeding nematode dominance) and 0 (fungi-feeding nematode dominance) [43, 73] indicated that the bacterial-based decomposition process was dependent on seasonal changes taking place in the observed ecosystem, as

well as shifts from the dominance in the trampling area in the CW period toward the undisturbed control area in the WW and HD periods. The structure index (SI), which is dependent on the presence of omnivore-predator nematodes [74] and suggests the presence of a food web with more trophic linkages [51], revealed a negative impact of animal trampling on the soil biotic food web, leading to the simplification and shortening of trophic linkages in the soil community. However, SI indicated that the negative animal trampling impact on the food web can be weakened due to seasonal fluctuations and the protective effect of the tree canopies. Therefore, e.g., the SI values were elevated in the trampling area under the Cupressus empervirens and Eucalyptus camaldulensis canopies in the WW period and under the Eucalyptus camaldulensis canopy in the HD period. Moreover, the SI showed that the different soil properties exerted different effects on the diversity of the trophic linkages in the study area. Our data showed the negative effect of electrical conductivity on trophic linkage diversity in the undisturbed sites and the positive effect of organic matter on trophic linkage diversity in the trampling spots.

The enrichment index (EI), which assesses food web responses to available resources as well as the response of primary decomposers to those resources [51], indicated that the interaction between primary decomposers and soil resources was more effective in the animal trampling area during the wet period. Moreover, EI values indicated that this interaction was more effective under the canopies of the Eucalyptus camaldulensis (trampling area) and Tamarix aphylla (undisturbed area), along with the open space in the HD period. In addition, the EI showed that the interaction between primary decomposers and soil resources in the trampling sites was positively dependent on SM, unlike the interaction at the undisturbed sites, with their negative correlation with SM and positive correlation with pH.

found to be significantly dependent on the trampling activity of ungulates in the study area. However, the negative impact of animal trampling on soil nematode communities has been smoothed and attenuated due to both the protective effect of tree canopies and seasonal fluctuations. This study showed that trampling, along with seasonal fluctuations and the tree-species attenuating effect on a soil medium, created the spatial-temporal heterogeneity of soil properties in the study area, which, in turn, reflected soil nematode abundance and diversity. Our data indicate that about 62% of the observed nematode species were affected (48% negatively and 14% positively) to varying degrees by either direct trampling effect or changes in soil properties. Among the soil free-living nematodes, only bacteria-feeding nematodes belonging to the cp-1 guild were positively affected by trampling during the study. The ecological indices confirmed the sensitivity of the free-living nematodes to environmental disturbances caused by ungulate activity. Our findings demonstrate that impact of the animal trampling on free-living nematode communities should be carried out in different seasons of year including the wet and dry periods and considering the attenuating effect by tree canopies on the trampling impact. Moreover, abundance and diversity of the the nematode functional guilds give the additional useful information for estimation of environmental condition of the study area.

Our results showed that animal trampling had

significant a direct and indirect effects (through

changes in soil properties) on soil biota. In agreement

with previous studies, soil properties and soil

free-living nematode abundance and diversity were

## **Conflict of Interest**

The authors declare that they have no conflicts of interest.

Acknowledgements this project was supported by the Israel Repatriate Scientists Program (KAMEA), which played no role in the research described in this

## 5. Conclusions

article. We would like to express our sincere thanks and appreciation to the staff members of the Zoological Center Tel Aviv-Ramat Gan (the Ramat Gan Safari) for both the opportunity to conduct research and for their cooperation during the research. The authors also thank Ms. Sharon Victor for her helpful comments. We appreciate the helpful comments of the anonymous reviewers.

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