




## Article

# Nematode Communities in Soils of the Same Volcanic Origin across a Gradient of Naturalization: From Intensive Agriculture to Forest

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**Abstract:** Nematodes play a key role in ecological environments. Biotic indices based on soil nematode community are effectively used for assessing soil health status. This work represents the opportunity to investigate three different management practices continuously maintained for 40 or more decades on soils with the same geological origin. This unique feature allows us to reduce variables and link biotic with abiotic factors. Therefore, the nematode communities of three neighboring volcanic soils under different managements were examined. The maximum values in soil biodiversity were found in the forest environment in which all the trophic groups were detected and well balanced. Instead, in permanent grassing and carnation crop greenhouse (CG), biodiversity indices progressively decreased with the intensification of agricultural practices. Furthermore, CG showed a stronger dominance of a specific plant-parasitic nematode identified as *Heterodera daverti*. Nematode indicators demonstrated that changes in the nematode community gradually varied from anthropic to natural environments whereas soil properties were feebly correlated to nematode community indicators. In conclusion, we demonstrated that biodiversity and ecological indices applied to the soil nematode community are effective at detecting alterations due to anthropogenic impact. Nematodes seem to be susceptible to perceiving the soil dynamics.

**Keywords:** nematode communities; biodiversity; biotic indices; carnation; permanent grassing; forest; anthropogenic impact



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## 1. Introduction

Land use for intensive agriculture produces unfavorable modifications to soil ecosystems, provoking global concern. Essential terrestrial ecosystem processes are mediated by soil nematode communities; therefore, understanding their responses to soil management becomes a priority. In fact, soil ecosystems undergo severe disturbances from agricultural practices causing unpredictable changes to community structure and influencing ecosystem function. In order to comprehend the impact of these activities on soil ecosystems, several monitoring tools have been assessed. In this scenario, soil fauna-based indices have received increasing consideration in current research [1,2]. The study of a soil community, at the individual or community levels, provides integrated information on several ecological levels and with each disturbance the nematocenosis changes [3]. Scientists have realized that the abundance and diversity of nematodes in the soils provide numerous beneficial ecosystem services, including the provision of nutrients for plants, the spread of beneficial bacteria, and the predation of herbivores. For example, frequent silvicultural thinning in artificial black pine (*Pinus nigra* Arnold) forests produces a short-term impact on the biodiversity of nematodes and microarthropods [4]. This abundance and diversity mean that the analysis of nematode communities can give important information about the biological status of soils [5]. Nematodes have a food diet that varies according to their trophic group

(algae, bacteria, fungi, plants, and other animals) and occupies a central role in the soil food web [6]. They are implicated in complex ecological networks through various associations (competition and cooperation) with other soil organisms, showing a broad range of adaptations and roles played in the soil, making them suitable for studies in ecological and evolutionary biology [7]. Plants play a central role in shaping the soil organism community. It has been demonstrated that their genotype and their exudates influence the biomass of soil microbial and nematode communities in the rhizosphere [8–11]. Free-living and plant-parasitic nematodes are effectively used to monitor the status of soil quality [12] for their ability to reveal changes in soil structure and function, as well as for their numerous morphological and functional characteristics (i.e., high abundance, diversity, etc.) [13,14]. They represent 80% of the metazoan taxa density and 1–100 kg/ha in terms of biomass [15–17], and their main functional characteristics are represented by trophic guilds and an evolutionary adaptation strategy [18,19]. Moreover, nematodes are ubiquitous in the soil, playing key roles in their sheer abundance and in nutrient mobilization [20,21]. Their identification at the family level provides an improved understanding of soil functioning, whereas their identification at the species level reveals information on biodiversity, redundancy, and other ecological aspects [22]. Studies on the biodiversity of nematode communities have also progressively been used to evaluate the status of environmental ecological conditions for several habitats (i.e., marine, soil, etc.). Life history strategies and distribution patterns are influenced by the soil matrix and its complexity; consequently, nematodes are characterized by patchy distribution patterns. Biotic indices are based on the concept that the diversity patterns of a specific community reflect the environmental conditions [23]. Investigations of soil biology are also useful to better understand phenomena such as soil erosion, desertification, and ecotonal boundaries [24,25]. More knowledge is necessary to establish the nematode functional response to environmental changes. To fill this gap, our study aims to connect environmental variables to the diversity–function response of nematodes from three neighboring soils of identical origin (volcanic soils) through (1) the determination of the spatial environmental gradients and correlated ecological information guided by the taxonomy-based assemblage distribution patterns and a (2) comparison with ecological information. This work represents the opportunity to investigate three soils from the same origin under different managements. This peculiarity allows us to reduce variables and highlight the factors linked to various environmental stresses.

## 2. Materials and Methods

### 2.1. Study Area

The Park of the Royal Palace, also known as Gussone Park (40°48′40.3″ N–14°20′33.8″ E), situated in the city of Portici (Naples, Southern Italy), occupies about 21 ha. This highly urbanized area (up to 50,000 inhabitants) along the southwestern slopes of Vesuvius was covered by lava after the eruption of 1631. Some of the native tree plant species that colonized the park after the eruption were left in place while other sites were reforested. Currently, the superior part of Gussone Park is covered by an adult high forest (age: up to 200 years) consisting primarily of *Quercus ilex* L. The climate of the study area is Mediterranean, and the altitude is 60–100 m a.s.l.; there is a cumulative mean annual rainfall of ~1000 mm, which is irregularly distributed. The mean monthly temperatures range between 25 °C (summer) and 8 °C (winter). Winter temperatures are mitigated by the nearby coastline (~1 km).

This research was carried out in three sites in the Gussone Park area. Specifically, permanent grassing (PG) and oak forest (OF) sites were located inside Gussone Park, and the cultivation of carnations (CG) (*Dianthus caryophyllus* L.) was located in greenhouses 200 m away from the Gussone Park. These sites were selected for the similar origin of their soils. PG was natural and mostly represented by grass mowed annually in spring. CG has been established for 50 years. During the past 30 years, this greenhouse had been treated with a blend of DD (1.2 dichloropropene + 1.3 dichloropropane) plus metham sodium (Vapam), followed by methyl bromide for about 20 years.

## 2.2. Sampling Methods

A sampling survey was performed in the selected sites during the winter, spring, summer, and autumn of 2017, according to the timing of the carnation crop cultivation. Specifically, samples were collected approximately one month before the last harvest (end of March); in June, 20 days after transplanting and 50 days after soil fumigation; in September, during the plant growth and development stage; and in December, during the carnation flower production stage.

Twenty soil sub-samples per site were collected randomly using a hand auger (5 cm internal Ø) at a depth of 20 cm in the top layer of bulk soil after removing surface residues (5 cm). Then, sub-samples were mixed to form a composite sample of 1 kg each. Soil samples were placed in plastic bags, labeled, and stored at 7 °C until analysis.

## 2.3. Soil Chemical and Physical Analysis

A separate soil sub-sample of approximately 100 mL, collected in February from each site, was used to determine various soil chemical and physical measures. The soil samples were air-dried at room temperature and sieved through a 2 mm mesh for pH and available P. Then, soil samples were sieved one more time through a 0.5 mm mesh for total organic carbon (TOC) and total N analysis. The soil pH was measured potentiometrically in a 1:2.5 soil–water suspension. Available P was determined according to the Bray and Kurtz methods [26]. Soil TOC was determined via hot oxidation with potassium dichromate and sulfuric acid [27]. Soil total N was analyzed according to the Kjeldahl procedure [28], using a Tecator heating block (Foss Tecator AB, Hoganas, Sweden) for sample digestion (in concentrated H<sub>2</sub>SO<sub>4</sub> + CuSO<sub>4</sub> catalyst) and a Foss 2300 Kjeltec apparatus (Foss Analytical, Hillerød, Denmark) for steam distillation/titration [29].

## 2.4. Nematode Communities

Plant-parasitic and free-living nematodes were extracted from 100 mL of soil sample using the Oostenbrink elutriator–cottonwool filter method [30]. Each nematode suspension was sieved (25 µm) and nematodes were counted under the stereomicroscope (50× magnification). Specimens were mounted on temporary slides and nematode assemblages were identified to the family or genus level [31–33]. The population density per genus, the total number of genera, and the total number of nematodes per sample were determined. Nematode genera were assigned to trophic groups (bacterivores, fungivores, plant parasites, predators, omnivores, and plant associates) based on the nematode mouth buccal morphology [34]. Life history strategies were assessed by assigning a value in a colonizer–persister scale (*c-p* scale), from 1 (colonizers) to 5 (persisters) to genera [35,36]. Generally, the colonizing strategy favors rapid rates of reproduction and growth, as well as a quite high tolerance to factors of disturbance, whereas the characteristics of persisters include a slow growth rate and a high susceptibility to disturbance.

## 2.5. Data Analysis

Nematode communities were characterized using several biodiversity and ecological indices calculated from data on nematode genera density: (i) the abundance of individuals; (ii) the richness, determined by counting the number of taxa; (iii) the Maturity (MI) and Plant-Parasitic indices (PPI) across all nematode genera after Bongers [37], calculated as the sum of the weighted relative abundances of families classified in the *cp* scale for free-living and plant-parasitic nematodes; (iv) food web indicators (EI, enrichment index; SI, structure index) after Ferris et al. [38]. EI was calculated as the weighted relative abundance of functional guilds responsive to nutrient enrichment in *cp* groups 1 and 2 and SI as the weighted relative abundance of functional guilds responsive to physical disturbance in *cp* groups 3, 4, and 5; (v) Ref. [39] the index of taxon richness [40]; (vi) the Shannon–Weaver [41] and Berger–Parker [42] diversity indices were applied to measure diversity among nematode genera; (vii) Evenness [43] was determined from the diversity index; (viii) Simpson's [44] index was used to assess nematode genera dominance in the

sample; (ix) the Brillouin formula [45]; and (x) Equitability E compared the observed diversity to the maximal theoretical diversity  $E = H/\log S$ .

A two-way ANOVA was carried out to evaluate management and seasonal effects on nematode taxa abundance. When the F-test was significant at  $p < 0.05$ , treatment means were compared using the Student–Newman–Keuls test using the CoStat Statistical Software 6.4 (2021). In addition, nematode communities were compared using multivariate methods provided by the Past analysis package, analysis of similarity (ANOSIM) and multidimensional scaling (MDS) based on the Bray–Curtis similarity index, and nearest-neighbor. Data on the nematode abundance were analyzed using square root transformation. A Bonferroni correction  $p$  value was applied. A Canonical Correspondence Analysis (CCA) was performed in order to link nematode communities and soil chemical variables. The relationship between communities and environmental variables was statistically assessed using a permutation test of the first ordination axis and by combining the first and second axes.

### 3. Results

#### 3.1. Soil Chemical and Physical Properties

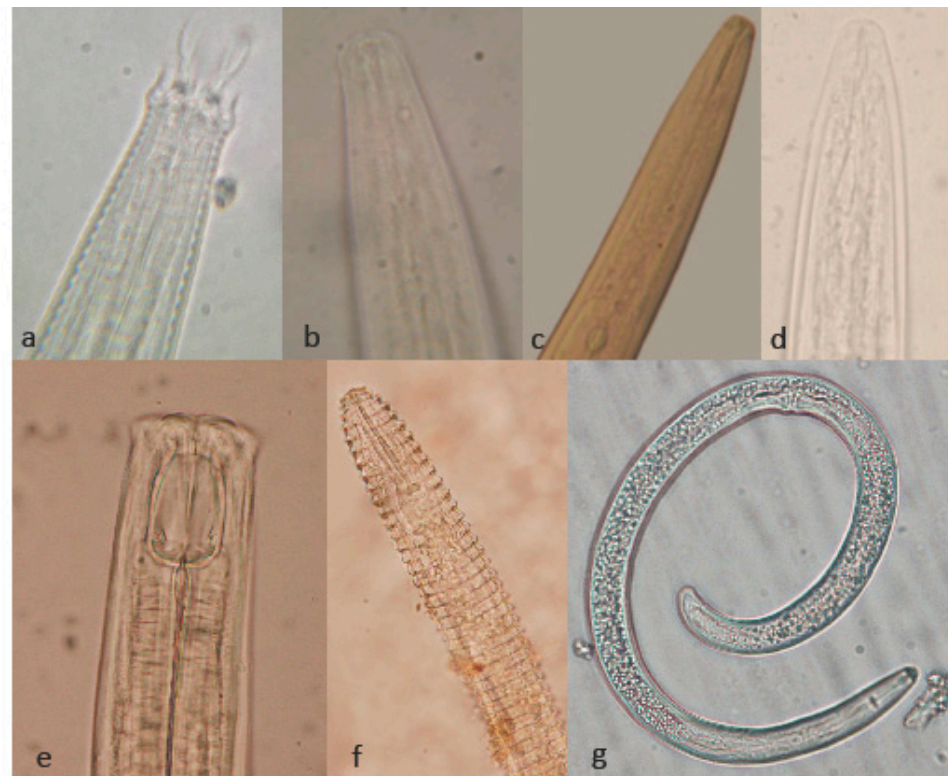
The soil parameters are reported in Table 1. The three selected sites showed several similarities, demonstrating the same volcanic origin of soils. According to the USDA soil taxonomy, the soil texture at the OF and PG sites was classified as loamy sand, while the soil at the CG site was classified as sandy loam. Soil pH values ranged from sub-acid in OF to sub-alkaline in CG. The highest contents of TOC ( $3.96 \text{ g kg}^{-1}$ ), organic matter (6.83%), total N ( $3.81 \text{ g kg}^{-1}$ ), and available P ( $45.9 \text{ g kg}^{-1}$ ) were detected at the OF site, whereas the CG site showed the lowest contents of TOC ( $0.9 \text{ g kg}^{-1}$ ), organic matter (1.55%), total N ( $0.85 \text{ g kg}^{-1}$ ), and available P ( $13.7 \text{ g kg}^{-1}$ ).

**Table 1.** Soil physicochemical properties at three sites: oak forest (OF), permanent grassing (PG), and in a neighboring greenhouse of carnation crops (CG). Average values and standard errors ( $\pm$ ) are divided per group. The impact of the single variables was estimated using ANOVA ( $p$  value  $< 0.05$ ), and letters indicate the significance of the Student–Newman–Keuls post hoc test.

	Soil Managements		
	OF	PG	CG
Sand ( $\text{g kg}^{-1}$ )	802.00 $\pm$ 0.96 a	785.00 $\pm$ 0.82 b	700.00 $\pm$ 1.41 c
Loam ( $\text{g kg}^{-1}$ )	147.00 $\pm$ 0.82 b	140.00 $\pm$ 0.82 c	201.00 $\pm$ 0.81 a
Clay ( $\text{g kg}^{-1}$ )	51.00 $\pm$ 0.82 c	75.00 $\pm$ 0.82 b	99.00 $\pm$ 0.50 a
pH (in $\text{H}_2\text{O}$ )	6.52 $\pm$ 0.006 c	7.27 $\pm$ 0.006 b	7.41 $\pm$ 0.01 a
EC ( $\text{dS m}^{-1}$ )	0.08 $\pm$ 0.02 b	0.085 $\pm$ 0.005 b	0.930 $\pm$ 0.01 a
Total calcium carbonate ( $\text{g kg}^{-1}$ )	6.00 $\pm$ 0.06 a	6.20 $\pm$ 0.20 a	5.70 $\pm$ 0.06 b
CSC (mequiv 100 $\text{g}^{-1}$ )	15.93 $\pm$ 0.02 a	15.74 $\pm$ 0.04 b	14.71 $\pm$ 0.02 c
TOC ( $\text{g kg}^{-1}$ )	3.96 $\pm$ 0.006 a	1.68 $\pm$ 0.02 b	0.90 $\pm$ 0.10 c
Organic matter (%)	6.83 $\pm$ 0.02 a	2.90 $\pm$ 0.06 b	1.55 $\pm$ 0.01 c
Total N ( $\text{g kg}^{-1}$ )	3.81 $\pm$ 0.01 a	1.61 $\pm$ 0.01 b	0.85 $\pm$ 0.02 c
C/N	1.04 $\pm$ 0.006 a	1.05 $\pm$ 0.01 a	1.06 $\pm$ 0.02 a
Available P ( $\text{mg P kg}^{-1}$ )	45.90 $\pm$ 0.06 a	32.70 $\pm$ 0.10 b	13.70 $\pm$ 0.15 c

#### 3.2. Nematode Communities

More than 30 nematode genera were identified (Figure 1), and the data are shown in Table 2. Significant differences in the abundances of several genera were recorded according to the type of soil ecosystem management. Similarly, significant differences were recorded also in the abundance of many genera in relation to the season.



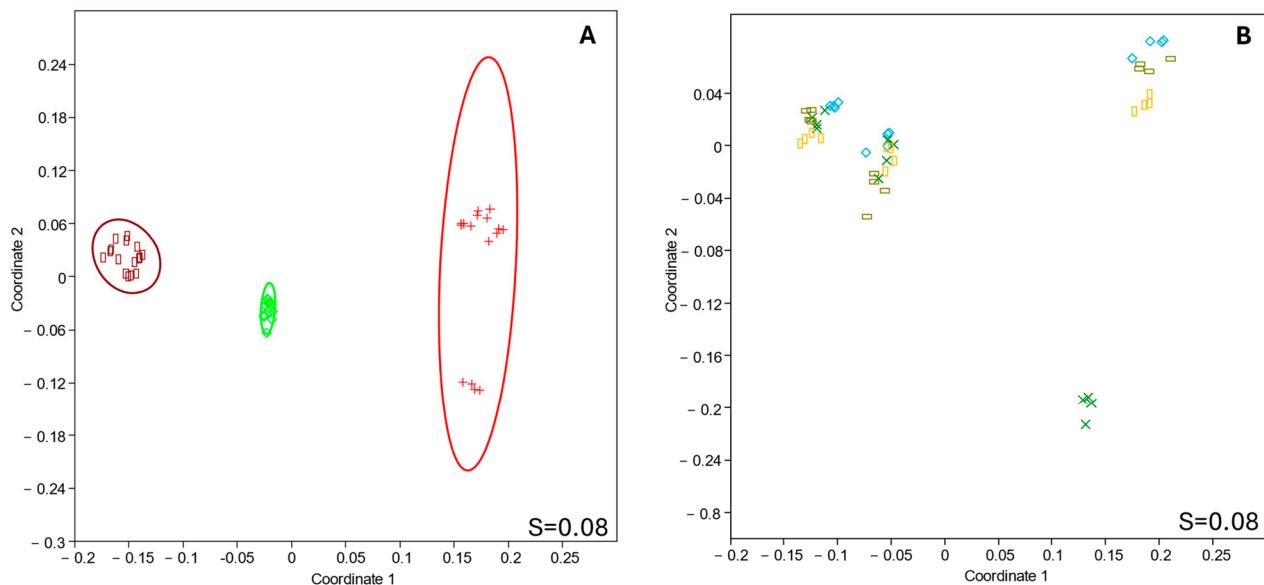
**Figure 1.** Some nematode genera collected in the research area. Bacterial feeders (B): (a) *Acrobelus* and (b) *Plectus*; hyphal feeders (H): (c) *Aphelenchus*; omnivores (O): (d) *Eudorylaimus*; predators (P): (e) *Michonchus*; plant feeders (PP): (f) *Criconemoides* and (g) *Helicotylenchus*. (Photos by Manachini B.).

**Table 2.** Trophic group composition and nematode genera linked to soil management (oak forest—OF, permanent grassing—PG, and carnation crop greenhouse—CG) and season (spring, summer, fall, and winter). Average values and standard errors ( $\pm$ ) are divided by group. Different letters for the same parameters indicate significantly different values (Student–Newman–Keuls test,  $p < 0.05$ ).

Trophic G.	Genera	Management			Season				p Values		
		OF	PG	CG	Spring	Summer	Fall	Winter	M	S	M + S
B	<i>Acrobelus</i>	32.0 ± 3.8 a	17.0 ± 2.0 b	0 c	20.7 ± 5.5 a	17.3 ± 4.0 a	19.3 ± 6.0 a	8.0 ± 2.0 b	0.00001	0.00001	0.00001
B	<i>Alaimus</i>	8.0 ± 2.3 a	2.3 ± 2.8 b	0 b	7.3 ± 3.2 a	2.3 ± 0.7 b	4.0 ± 1.1 b	0 c	0.00001	0.00001	0.00001
B	<i>Cephalobus</i>	28.0 ± 2.3 a	20.3 ± 3.2 b	1.8 ± 0.45 c	21.7 ± 4.8 a	14.0 ± 4.9 b	14.7 ± 2.8 b	16.3 ± 3.7 b	0.00001	0.007	0.00001
B	<i>Diplogaster</i>	2.5 ± 0.3 a	0.5 ± 0.3 b	0 b	2.7 ± 1.2 a	0 c	0 c	1.3 ± 0.5 b	0.00001	0.00001	0.00001
B	<i>Mermis</i>	1.3 ± 0.6 a	0 b	0 b	1.7 ± 0.8 a	0 b	0 b	0 b	0.0001	0.00001	0.00001
B	<i>Plectus</i>	34.3 ± 3.1 a	0 b	0 b	15.0 ± 6.6 a	13.0 ± 5.7 a	7.3 ± 3.3 b	10.3 ± 4.7 ab	0.00001	0.008	0.002
B	<i>Rhabditis</i>	20.5 ± 3.1 c	162.5 ± 14.6 b	319.5 ± 40.4 a	129.0 ± 24.1 b	211.0 ± 53.3 a	204.3 ± 64.5 a	125.7 ± 25.0 b	0.00001	0.00001	0.0001
B	<i>Steinernema</i>	13.5 ± 2.4 a	0 b	0 b	8.3 ± 3.8 a	2.3 ± 1.10 b	2.3 ± 1.14 b	5.0 ± 2.4	0.00001	0.0005	0.00001
B	Others Bact.	24.3 ± 1.8 a	6.0 ± 1.6 c	10.5 ± 2.2 b	17.7 ± 1.8 a	9.3 ± 3.8 b	10.7 ± 3.1 b	16.7 ± 2.9	0.00001	0.0002	0.00001
H	<i>Aphelenchoides</i>	39.0 ± 2.2 a	12.0 ± 1.3 b	0 c	17.3 ± 4.6	17.7 ± 5.4	18.0 ± 6.4	15.0 ± 4.1	0.00001	0.88	0.04
H	<i>Aphelenchus</i>	31.3 ± 2.6 b	41.3 ± 4.4 a	1.3 ± 0.4 c	26.7 ± 5.7 ab	31.0 ± 8.1 a	21.7 ± 5.0 bc	19.0 ± 4.8 c	0.00001	0.0008	0.00001
H	<i>Ditylenchus</i>	28.8 ± 2.0 a	4.5 ± 1.1 b	0 c	11.0 ± 3.2 b	14.7 ± 4.9 a	9.7 ± 4.3 b	9.0 ± 3.6 b	0.00001	0.008	0.004
P	<i>Discolaimus</i>	22.8 ± 1.8 a	1.3 ± 0.3 b	0 c	9.0 ± 3.5	6.7 ± 3.1	9.7 ± 3.9	6.7 ± 2.8	0.00001	0.17	0.42
P	<i>Iotonchus</i>	1.5 ± 0.5 a	0 b	0 b	1.0 ± 0.6 a	1.0 ± 0.6 a	0 b	0 b	0.0007	0.04	0.02
P	<i>Labronema</i>	4.8 ± 1.1 b	17.5 ± 2.0 a	0 c	10.7 ± 3.5 a	8.7 ± 2.6 ab	3.7 ± 1.1 c	6.7 ± 2.5 bc	0.00001	0.0005	0.0008
P	<i>Mononchus</i>	10.0 ± 1.3 a	1.5 ± 0.3 b	0 b	2.3 ± 0.7	4.3 ± 1.8	4.0 ± 1.7	4.7 ± 1.7	0.00001	0.18	0.06
P	<i>Seinura</i>	17.0 ± 1.7 a	7.8 ± 1.3 b	0.3 ± 0.2 c	7.3 ± 1.2	8.3 ± 2.2	7.7 ± 3.1	10.0 ± 2.5	0.00001	0.44	0.01
P	Others Pred.	10.0 ± 1.7 a	1.8 ± 0.4 b	0 b	2.7 ± 1.0	4.7 ± 2.1	5.3 ± 2.3	3.0 ± 1.1	0.00001	0.18	0.02
O	<i>Dorylaimus</i>	34.8 ± 2.7 a	7.3 ± 0.9 b	2.3 ± 0.6 c	18.0 ± 5.5 a	16.0 ± 5.5 a	14.7 ± 4.2 a	10.3 ± 3.1 b	0.00001	0.002	0.00001
O	<i>Eudorylaimus</i>	30.3 ± 2.1 a	6.8 ± 0.8 b	0 c	10.3 ± 3.0	17.7 ± 4.9	10.7 ± 4.0	13.7 ± 4.4	0.00001	0.04	0.006
O	<i>Diphtherophora</i>	15.3 ± 1.7 a	0 b	0 b	5.3 ± 2.4 ab	3.0 ± 1.4 b	7.0 ± 3.1 a	5.0 ± 2.4 ab	0.00001	0.06	0.03
O	Others Omniv.	10.0 ± 1.6 a	6.3 ± 0.8 b	0 c	7.0 ± 1.9	6.7 ± 2.2	4.7 ± 1.3	3.3 ± 1.0	0.00001	0.06	0.03
PP	<i>Criconemoides</i>	10.0 ± 2.2 a	0 b	0 b	5.0 ± 2.3 a	1.7 ± 0.9 b	5.7 ± 2.9 a	1.0 ± 0.6 b	0.00001	0.009	0.002
PP	<i>Criconema</i>	5.3 ± 1.4 a	0 b	0 b	0 b	1.7 ± 0.9 ab	3.7 ± 1.9 a	1.7 ± 0.9 ab	0.00001	0.02	0.04
PP	<i>Helicotylenchus</i>	6.5 ± 1.6 a	9.0 ± 1.3 a	0 b	9.0 ± 2.32 a	4.3 ± 1.6 b	5.0 ± 1.6 b	2.3 ± 0.8 b	0.00001	0.003	0.17
PP	<i>Heterodera</i>	0 b	0 b	1702.2 ± 307.2 a	1035.7 ± 444.5 a	0 d	799.7 ± 341.7 b	434.3 ± 188.9 c	0.00001	0.00001	0.003
PP	<i>Longidorus</i>	3.0 ± 0.7 a	3.3 ± 0.4 a	0 b	1.7 ± 0.6	2.3 ± 0.8	1.7 ± 0.6	2.3 ± 0.8	0.00001	0.51	0.02
PP	<i>Paratylenchus</i>	8.5 ± 2.3 b	16.5 ± 1.3 a	0 c	11.3 ± 2.9 a	8.0 ± 2.2 ab	8.0 ± 3.2 ab	6.0 ± 2.0 b	0.00001	0.005	0.00001
PP	<i>Pratylenchus</i>	0 b	8.5 ± 0.7 a	0 b	2.3 ± 1.1	2.3 ± 1.0	3.0 ± 1.4	3.7 ± 1.6	0.00001	0.010	0.07
PP	<i>Tylenchus</i>	12.8 ± 2.4 a	6.5 ± 1.6 b	0 c	12.3 ± 3.7 a	7.3 ± 2.2 b	3.3 ± 1.0 b	2.7 ± 1.4 b	0.00001	0.0001	0.03
PP	<i>Tylenchorhynchus</i>	26.3 ± 2.3 b	76.8 ± 6.5 a	3.5 ± 0.6 c	33.7 ± 8.9 a	42.3 ± 12.3 a	23.7 ± 5.9 b	42.3 ± 11.8	0.00001	0.0004	0.0002
PP	<i>Tylenchorhynchus</i>	14.8 ± 2.9 b	209.8 ± 14.1 b	0 b	98.0 ± 35.7 a	76.3 ± 32.1 ab	57.0 ± 22.5 b	68.0 ± 28.3 b	0.00001	0.006	0.03
PP	<i>Tricodorus</i>	1.5 ± 0.7 a	0 b	0 b	0 b	0 b	1.7 ± 1 a	0.3 ± 0.3 b	0.02	0.009	0.002
PP	<i>Xiphinema</i>	29.3 ± 2.7 a	9.3 ± 1.4 b	0 c	17.3 ± 4.5	10.3 ± 3.5	11.7 ± 3.6	12.0 ± 4.8	0.00001	0.06	0.27
PP	Others Plant-Par.	1.5 ± 0.6 b	4.8 ± 0.4 a	0 c	3.0 ± 0.8	1.3 ± 0.6	2.0 ± 0.7	2.0 ± 0.7	0.00001	0.10	0.50

Most genera of the free-living and plant-parasitic nematodes were mainly found in OF (34 genera) and to a lesser extent in PG (28 genera). Only eight genera were found in CG. The genera *Mermis*, *Plectus*, *Steinernema*, *Iotonchus*, *Diphterophora*, *Criconemoides*, and *Criconema* were peculiar in oak forest; instead, the genus *Heterodera* was recorded only in CG. While the plant feeders *Tylenchorhynchus*, *Tricodorus*, and *Xiphinema* were not recorded in CG, they were present in PG and OF.

Overall, the two-way ANOSIM analysis on nematode abundance showed significant differences for management ( $R = 1$ ,  $p < 0.0001$ ) and season ( $R = 0.81$ ,  $p < 0.0001$ ). The MDS analysis confirmed a spatial separation among the three management systems and a partial separation between summer and the other seasons (Figure 2).



**Figure 2.** MDS analysis based on the Bray–Curtis similarity index of nematode community abundance from soil for (A) management and for (B) season. Symbols represent different managements (oak forest, brown rectangle; permanent grassing, green diamond; carnation crop greenhouse, red cross) and seasons (spring, light-blue diamond; summer, dark-green cross; fall, light-brown rectangle; winter, yellow rectangle). Stress value (S) is indicated.

The highest total abundance of nematodes was found in the carnation greenhouse and the lowest value was in OF. The analysis of the trophic groups showed that plant-parasitic nematodes (mainly belonging to the genus *Heterodera*) and to a lesser extent bacterial feeders (mostly belonging to the family Rhabditidae) were the main cause of the nematode population increment in CG. OF was shown to be a stable environment in which hyphal feeders, omnivores, and predators evidenced the highest abundance and biodiversity compared to the other managements. The PG showed an intermediate situation; plant-parasitic and bacterial feeder nematodes were the trophic groups most represented as in the carnation greenhouse, but a higher richness of genera was present, and the  $r$ -strategy species were more abundant than the  $k$ -strategy species. *Rhabditis* and *Tylenchorhynchus* were dominant genera, respectively, in the bacterial and plant-parasitic nematode assemblages (Table 3).

A seasonal fluctuation in the trophic group composition was detected. The same trend was exhibited by bacterial and hyphal feeders; in fact, their abundance was higher in summer and fall in comparison to the other seasons. Plant-parasitic nematodes were very abundant only in spring, while omnivores and predators remained constant during the whole year.

**Table 3.** Effect of the three different managements on soil nematode community assembled for the trophic groups. Samples were collected from oak forest (OF), permanent grassing (PG), and greenhouse carnation crop (CG) during spring, summer, fall, and winter. Standard errors are reported. Average values and standard errors ( $\pm$ ) are divided by group. Different letters for the same parameters indicate significantly different values (Student–Newman–Keuls test,  $p < 0.05$ ).

Trophic Group	Management			Season				$p$ Values		
	OF	PG	CG	Spring	Summer	Fall	Winter	M	S	M + S
Bacterial feed.	164.3 $\pm$ 11.0 c	208.5 $\pm$ 16.8 b	331.8 $\pm$ 39.2 a	224.0 $\pm$ 17.1 b	269.3 $\pm$ 39.0 a	262.7 $\pm$ 54.1 a	183.3 $\pm$ 13.3 c	0.00001	0.00001	0.00001
Hyphal feeders	99.0 $\pm$ 4.6 a	57.8 $\pm$ 6.1 b	1.3 $\pm$ 0.4	55.0 $\pm$ 12.1 ab	63.3 $\pm$ 14.3 a	49.3 $\pm$ 14.9 bc	43.0 $\pm$ 9.4 c	0.00001	0.0003	0.00001
Predators	66.0 $\pm$ 3.9 a	29.8 $\pm$ 3.4 b	0.3 $\pm$ 0.2 c	33.0 $\pm$ 7.5	33.7 $\pm$ 9.4	30.3 $\pm$ 10.5	31.0 $\pm$ 7.4	0.00001	0.83	0.002
Omnivores	90.3 $\pm$ 3.5 a	20.3 $\pm$ 1.5 b	2.3 $\pm$ 0.6 c	40.7 $\pm$ 11.7 a	40.3 $\pm$ 13.6 a	37.0 $\pm$ 11.6 ab	32.3 $\pm$ 9.5 b	0.00001	0.02	0.0007
Plant-paras. f.	119.3 $\pm$ 8.4 c	344.3 $\pm$ 20.1 b	1705.7 $\pm$ 307.3 a	1229.3 $\pm$ 406.2 a	158.0 $\pm$ 48.3	926.0 $\pm$ 316.2 b	578.9 $\pm$ 162.3	0.00001	0.00001	0.00001
Tot. abundance	538.8 $\pm$ 20.8 c	660.5 $\pm$ 44.5 b	2041.2 $\pm$ 298.6 a	1582.0 $\pm$ 370.0 a	564.7 $\pm$ 43.8 d	1305.3 $\pm$ 346.5 b	868.6 $\pm$ 143.9 d	0.00001	0.00001	0.00001

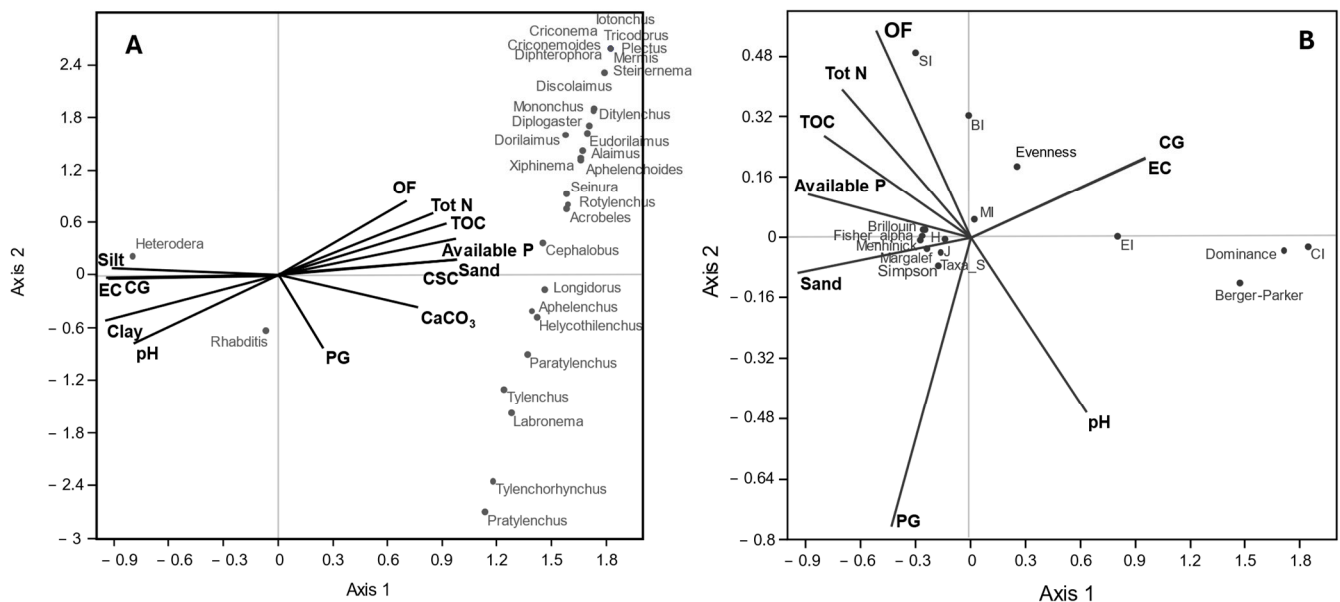
The averages of soil nematode indicators for each management and relative statistical analysis are summarized in Table 4. In general, these values showed a gradient from natural to anthropic environments, in which OF, and to a lesser extent PG, showed the highest values for Shannon, Simpson 1-D, Evenness, Brillouin, Menhinick, Margalef, Equitability, and Fisher-alpha, indicating a rich environment in biodiversity. At the same time, the low values of Dominance and Berger–Parker demonstrated the absence of dominance. On the contrary, the CG showed an opposite trend with high values for Dominance and Berger–Parker due to the dominance of the *Heterodera* genus; instead, the other biodiversity indicators exhibited the lowest values. In addition, the Maturity index confirmed that OF showed high values, indicating the high presence of *k*-strategy individuals. PG exhibited characteristic values for the agricultural environment, demonstrating disturbed soil. Instead, CG evidenced a much-degraded environment due to the exponential growth of invasive plant-parasitic nematodes. The food web indicators showed high values for SI and BI in OF, while EI was high only in CG for the presence of generalist and opportunistic species. The CI index indicated that the dominant decomposition pathways were bacterial for the CG and fungal for the OF and PG.

**Table 4.** Biodiversity and ecological indices of the nematode community in soils under different soil managements: oak forest (OF), permanent grassing (PG), and carnation crop greenhouse (CG).

	Soil Managements		
	OF	PG	CG
Biodiversity indices			
Taxa_S	34	28	8
Individuals	2156	2643	8173
Dominance_D	0.04	0.18	0.72
Simpson_1-D	0.96	0.82	0.28
Shannon_H	3.26	2.23	0.50
Evenness_e`H/S	0.76	0.33	0.21
Brillouin	3.21	2.20	0.50
Menhinick	0.73	0.54	0.09
Margalef	4.30	3.43	0.78
Equitability_J	0.92	0.67	0.24
Fisher_alpha	5.73	4.37	0.88
Berger–Parker	0.07	0.32	0.83
Ecological indices			
Maturity Index MI	3.29	2.25	1.05
Enrichment Index EI	3.96	3.67	6.09
Structure Index SI	0.58	0.13	0.04
Channel Index CI	0.04	0.29	1.55

### 3.3. Relationship between Soil Variables and Nematode Community Structure

The CCA conducted between nematode taxa abundance and soil variables evidenced that axis 1 was dominated by sand (0.98), silt (−0.91), clay (−0.95), TOC (−0.95), available P (0.97), EC (−0.95), and CG (0.95), while axis 2 was driven by pH (−0.78), OF (0.85), and PG (−0.83) (Figure 3A). The *Rhabditis* genus was poorly affected by the soil parameters. The *Heterodera* genus was positively influenced by EC, pH, silt, and clay; all the other genera were positively affected by TOC, Tot N, available P, CSC, and sand. Moreover, the OF site mainly favored the bacterial feeders belonging to the genera *Plectus*, *Diplogaster*, *Alaimus*, *Acrobeles*, and *Cephalobus*, as well as the presence of nematode parasites of arthropods such as some juveniles of *Mermis* and *Steinernema*; the fungal feeders belonging to the genera *Aphelenchoides* and *Ditylenchus*; the predators belonging to the genera *Iotonchus*, *Mononchus*, *Seinura*, and *Discolaimus*; the omnivores belonging to *Dorilaimus*, *Eudorilaimus*, and *Diphterophora*; and the plant-parasitic nematodes belonging to the genera *Criconema*, *Criconemoides*, *Tricodorus*, *Xiphinema*, and *Rotylenchus*. On the contrary, PG favored the presence of fungal feeders belonging to the genus *Aphelenchus*; the predators belonging to *Labronema*; and the plant-parasitic nematodes belonging to the genera *Longidorus*, *Helicotylenchus*, *Tylenchus*, *Paratylenchus*, *Tylenchorhynchus*, and *Pratylenchus*.



**Figure 3.** Scatter plot of CCA ordination showing (A) relationships between soil properties and nematode taxa abundance; the percentage of variance was 65.82% for axis 1 ( $p < 0.001$ ) and 34.18% for axis 2 ( $p < 0.001$ ). (B) Soil nematode indicators; the percentage of variance was 96.55% for axis 1 ( $p < 0.001$ ) and 3.44% for axis 2 ( $p < 0.001$ ).

The biplot of CCA between soil nematode indicators and soil variables displayed that axis 1 was driven by sand (−0.94), EC (0.50), TOC (−0.80), available P (−0.89), and the management CG (0.95), while axis 2 was dominated by pH (−0.46) and the ecosystem OF (0.55) (Figure 3B). The biodiversity indices such as Shannon, Simpson, Brillouin, Menhinick, Margalef, Equitability, and Fisher-alpha and the ecological indicators such as the Maturity index were few influenced by the environmental gradient established. SI and BI were positively related to OF, Tot N, and TOC, while Evenness, Berger–Parker, Dominance, EI, and CI were positively affected by EC.

## 4. Discussion

Soil nematode community structure and diversity are strongly correlated with soil functional parameters. In this work, several variables such as soil physicochemical properties, climate, and temperature, are common to the three investigated soil ecosystems



whereas differences are due to plant covering, pesticide treatments, soil cultivation, and, consequently, organic matter content. As reported by several authors, sustainable land use applied for a long time increases organic matter [46,47]. Specifically, more sustainable land use increased the contents of organic carbon, total nitrogen, and available P, especially in OF.

#### 4.1. Effect of Different Soils on Nematode Community Structure

In intensive agroecosystems, in which profound and continuous changes occur as a result of ordinary agricultural practices, a deep alteration in physicochemical properties occurs with effects on the composition and role played by PPN populations [48]. In addition, it should not be underestimated that the monoculture associated with continuous chemical treatments, in particular fumigation, greatly reduces the soil biodiversity, where often, only one plant species survives disease (the most resistant), which manages to take over the other species and makes these soils increasingly dependent on chemistry [49–51]. These premises are consistent with our data, from which emerged a higher abundance of poorly diversified nematodes, represented by a higher presence in autumn of *Heterodera daverti* Wouts and Sturhan when only phytopathogenic species were recorded.

The composition and diversity of nematode communities in grasslands depended more on their age and use than on geographical, climatic, and soil site conditions [52]. The PPNs were the dominant trophic group in PG. According to our data, bacteriophages were a sub-dominant trophic group only in new meadows in arable soils, where the soil was characterized by greater resources of organic matter. On the other hand, omnivores, characterized by a long life cycle and a low abundance, particularly in crop lands, were a sub-dominant trophic group in PG, confirming what was reported by Čerevková (2011) [53] in permanent meadows and pastures.

In forest environments, the nematode population, variable in relation to different factors, has a numerical consistency represented by all the trophic categories. These deductions are consistent with our data where, regardless of the season, all the trophic groups were present and in balance with each other. These communities, which are generally less known than those of agricultural ecosystems, are characterized by an interesting variety of biological and parasitic behaviors. The abundance and richness of nematode species can be affected by changes in the environment and may be useful in determining changes in soil and its properties. Moreover, the low presence of PPNs and the high abundance of EPNs could be explained by the concentration of plant root exudates. In fact, as reported by Hiltbold et al. [54], in natural soils, the root exudates might have a dual effect, inducing quiescence for PPNs and, simultaneously, invigorating EPNs.

The nematode indicators demonstrated that changes in the nematode community varied as a result of a gradient from the anthropic to natural environments. Instead, the absence of a temporal trend for omnivores and predators characterized by high *c-p* values made the evaluation of the trend in these indicators during the different seasons irrelevant. The oak forest, characterized by the longest and the most conservative natural land use, presented the uppermost biodiversity indices values and the lack of dominance. Instead, in the two other agroecosystems investigated (the PG and CG sites), biodiversity indices decreased with the intensification of agricultural practices. Our results on biodiversity and ecological indices in CG confirm what was reported by other several authors in intensive cultivation, where biodiversity indices showed very low values and a strong dominance [55–57]. Moreover, the ecological indicators added more information, especially MI and SI, which were the most useful indices to characterize different managements. In fact, only in OF did the MI value exceed threshold three, which indicates good soil quality, and the SI value signaled a stable structure of the soil nematode community. As reported by Landi et al. [52], the anthropic activity created a disturbance both in grassland and carnation cultivation, the MI was under the threshold fixed at three, and the SI indicated a disturbed structure in the soil nematode community. Moreover, these two indicators gradually decreased when moving from PG to CG. Similar results were also obtained using

biomolecular techniques to compare different nematode communities from chestnut forests, grasslands, and maize monocultures [58].

#### 4.2. Soil Factors Influencing Soil Nematode Structure

Most nematode families were positively influenced by organic carbon, total nitrogen, and available P, which have different levels in the three soil ecosystems, showing a decrement from a more complex agroecosystem and less disturbed to the most disturbed and least rich in biodiversity (OF to CG). According to Landi et al. [52], predators and fungal feeders were mainly favored by organic matter. In fact, most genera of predators were related to OF rather than PG. Plant-parasitic nematodes were also affected by soil parameters. CCA suggested that the edaphic variables of soil texture, pH, and organic carbon were the primary determinants that influence the structure and diversity of the plant-parasitic nematode community. The *Heterodera* genus is reported to be negatively affected by organic carbon and favored by high silt content, EC, and soil pH [59]. Our data confirm the preference of *Heterodera* and highlight that the other plant-parasitic nematode genera were related to the high content of sand and soil organic matter. The land use also differentiated their distribution in the ordination diagram. The genera *Pratylenchus* and *Tylenchorhynchus* were related to the PG soil ecosystem; instead, the *Xiphinema* genus, characterized by *k*-strategy individuals, was linked to OF, the most stable environment investigated in this study.

Feeble correlations were found between nematode community indicators and soil properties. A positive correlation between EC and carnation management with the indicators Dominance, Berger–Parker, and CI was found. These correlations suggest that the intensification of agricultural practices reduced biodiversity and favored the dominance of some species such as the individuals belonging to *Heterodera*. Moreover, our results highlight that intensive agriculture does not only lead to a decline in species richness but also to numerous functional responses according to taxonomic groups, e.g., the reduction in predators that can serve as biocontrol agents against phytophagous nematodes through predation. In this study, taxonomic group species richness does not vary prominently along the gradient. Depending on the landscape structure and farming systems, this gradient could likely be truncated and does not permit the demonstration of the main variations in species richness considering other taxa [60,61].

## 5. Conclusions

Through the study of three different sites located in the city of Portici with the same pedological origin and characterization, it was possible to assess how different land use impacts the soil nematode community. Among the selected indices, MI and SI seemed to have the biggest potential as indicators for unhealthy or healthy systems. Moreover, the biodiversity indices provided additional information on regulating ecosystem processes referring to the role of biodiversity in maintaining the balance and functioning of the ecosystem. These indicators gradually increased based on a gradient from intensive agroecosystem to urban forest due to organic carbon, total nitrogen, and available phosphorus increments and reduced agricultural practices. Moreover, here, we demonstrated that nematode communities and indices related to its study are susceptible enough to perceive changes in biodiversity and ecological indices in relation to anthropogenic impact, with nematodes probably being more likely to detect the dynamics of the investigated disturbances than other taxa. More consideration should be given to these aspects when developing policies for sustainable agriculture or nature conservation.

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