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Research Article

Variation of nematode indices under contrasting pest management practices in a tomato growing agro-ecosystem

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ABSTRACT

This study examined the variation of generic composition and nematode indices to evaluate the soil health status under differently treated tomato growing experimental plots. The study was conducted from 2016 to 2018 in summer and winter seasons on a traditionally tomato (*Lycopersicon esculentum*) growing farm in the Northern part of the Kathmandu valley, Nepal. Soil samples were taken from four types of replicating plots (each 5 × 5 m sq.) each using a different pest control method consisting of: cattle manure (MP), chemical pesticides (PP), botanical pesticides (BP), and a control plot (CP). The nematode communities were extracted, colonizer-persister (c-p) values assigned and ecological indices such as maturity index (MI), enrichment indexes (EI), structural index (SI) and channel index (CI) were calculated. The low abundance of all indices in pesticide applied plots during both seasons (winter and summer) was recorded. This result indicated pesticide application can be linked to the sensitivity of nematode genera. Nematode indices such as maturity index (MI), enrichment index (EI) and structural index (SI) were significantly affected with lower mean value especially in chemical pesticides applied plots during both the summer and winter seasons. The decomposition channel index (CI) did not exhibit significant variation in our analysis of treatment plots in either season. There was a positive response towards moisture content, soil temperature and nitrogen–carbon ratios for these indices. Nematode genera and indices showed significant variation for pest management practices in tomato grown plots.

1. Introduction

Management of soil biodiversity of agricultural land is a vital aspect of sustainable farming. The loss of species from food webs due to agricultural intensification and extensive use of pesticides during pest management demand a more rigorous investigation into sustainable soil conservation on agricultural farms globally [1, 2]. The nematode communities either plant-parasitic or free-living may be used as ecological indicator to evaluate the soil health status since their composition related with nutrient cycling and decomposition pathway [3, 4]. Free living nematodes and the indices are able to indicate soil health status and biodiversity indicator of soil ecosystem [5, 6]. There are a number of nematode community indices [7, 8, 9, 10] which have been developed and successfully applied to monitor land use changes, management effects and environmental disturbance [8, 9, 10]. These indicators are the preferred bio-indicators used to assess soil health condition in agricultural as well as natural soil [11, 12, 13, 14]. These nematode indices have been broadly used to assess and monitor soil conditions extending from local fine scales to large landscape properties [12, 15, 16]. Furthermore, nematode metabolic foot prints are also used to indicate environmental status and can be predicted on bioclimatic change [11, 17]. Nematode indices, assigned based on colonizer-persister (c-p) values of nematode taxa ordinate on a 1–5 scale based on r-k life-history characteristics, are useful in interpreting the tropic status of the soil food web in different habitats [15, 18, 19]. Nematode indices can be used as a powerful tool to assess the structure, function and resilience of a soil food web [3, 7, 15, 20]. Thus, they have been applied in studies monitoring the effects of different management regimes in agriculture, land-use change, environmental disturbance and pollution [17, 19, 21, 22]. The effects of pest management practices on food web structure of soil have rarely been carried out in experimental approaches in tomato growing agroecosystems. These indices include: maturity index (MI), enrichment

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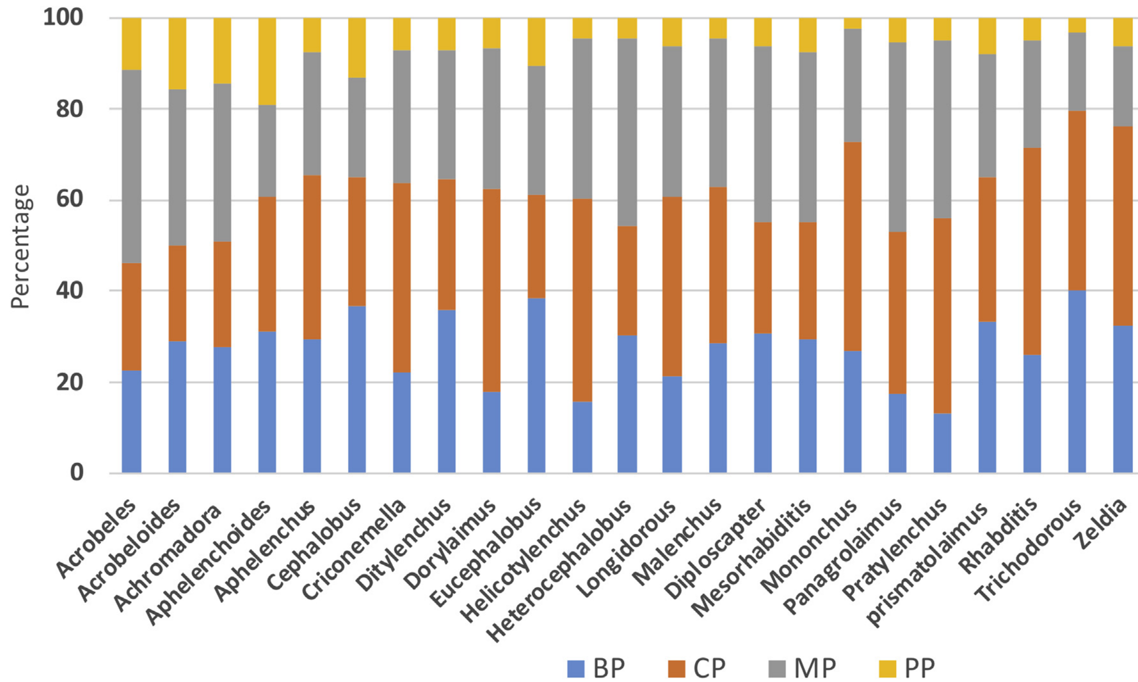

Fig. 1. Relative composition of genera in treatment plots.

Table 1
Summary table of generalized linear model (df = 3, Gaussian -identity) showing the effect of treatment plots on Maturity index (MI).

Variables	Estimate	SE	t-value	p-value
(Intercept)	0.556835	0.005012	111.103	2.00E-16
CP(Control plot)	0.018083	0.006424	2.815	0.00642
MP(Manure plot)	0.001372	0.00633	0.217	0.82907
PP(Pesticides plot)	-0.00612	0.00633	-0.966	0.33754

Table 2
Effect of treatment systems and seasons for food web indices (EI, SI and CI) in ANOVA summary.

Indices	Factors:	Sum sq	Df	F	p value
Structural index (SI)	Season	0.00366	1	0.079	0.7795(ns)
	Treatment	1.20475	3	8.6844	0.001
Enrichment index (EI)	Season	0.00639	1	0.9833	0.3252(ns)
	Treatment	0.22459	3	11.515	0.000
Channel index (CI)	Season	0.0203	1	0.1887	0.6659(ns)
	Treatment	0.0075	3	0.0233	0.9951(ns)

Table 3
Summary of generalized linear model (glm) showing effect of treatment systems for food web indices (EI, and SI).

variables	variable	Estimate	SDE	t-value	p-level
EI	CP	0.029	0.027	1.08	0.28(ns)
	MP	0.004	0.027	0.15	0.88(ns)
	PP	-0.115	0.027	-4.27	0.00
SI	CP	0.108	0.072	1.49	0.14(ns)
	MP	0.045	0.071	0.62	0.53(ns)
	PP	-0.24	0.072	-3.33	0.01

index (EI), structure index (SI), basal index (BI) and channel index (CI) [4, 8, 21, 23, 24, 25]. Maturity index calculations based on these taxa rankings have been widely used to describe the effects of different crop management practices on the soil food webs [8, 24, 25].

Globally, there is need for soil biology metrics that farmers can use as a decision support tool while managing the biological component of their farming system [2]. In developing countries have diverse challenges in agricultural systems such for the suitable use of soil ecosystem service. Extensive use of pesticides, the lack of sustainable soil biodiversity conservation policies, land use plans and eco-friendly pest management strategies are the possible threats for sustainable use of agricultural soil [26, 27, 28].

Nepal needs eco-friendly pest management practices, especially for vegetable growers in order to reduce the use of chemical pesticides; and also needs effective and sustainable eco-friendly pest management to maintain sustainability in soil ecosystems [25, 29, 30]. Many studies showed that chemical pesticides are still the primary choice for dominant Nepalese farmers for insect pest management [25, 30]. In the present study, nematofauna and their ecologically important indices were chosen to assess soil health condition under plastic film greenhouse specially to compare the impact of different pest management practices in tomato growing system. The diversity of plastic-film greenhouses offers many possibilities for use as a biological indicator of agricultural practices, soil characteristics and the degree of conservation of soils [30, 31].

In this study, we focused on the variation of nematode community indices across differently treated plots that were established with the following additives: cattle urine and manure mixture applied plot (MP), pesticides applied plot (PP) botanical pesticides applied plots (BP) and a control plot (CP) to evaluate the soil disturbance, decomposition channels, resource enrichment and food web complexity. We hypothesized that differences in pest management strategies influence soil conditions and that these changes will be reflected in nematode community structure and indices. Our objectives were: i) to assess the effect of treatment systems on generic abundance and richness ii) to compare the variation of nematode indices among the treatment plots.

2. Materials and methods

2.1. Site description

The experimental site was in Tokha Municipality (27 ^{0} 46′ 12.69″ N to

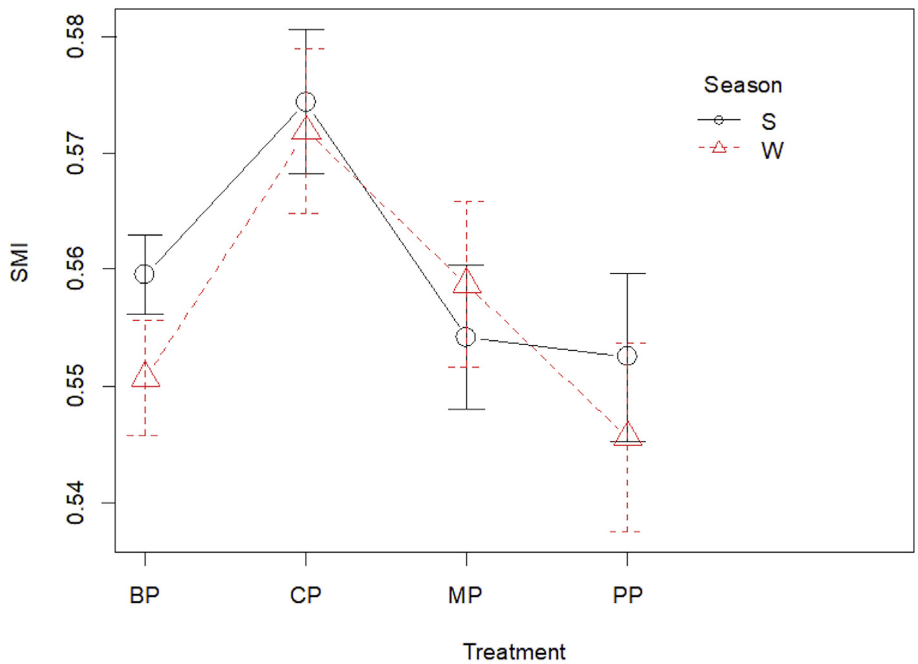

Fig. 2. Variation of mean value of Maturity index (MI) with treatment plots in summer(S) and winter (W) seasons.

85^0 19' 44.86" E) of the northern part of the Kathmandu valley, Nepal. The experiment was set up in a traditionally tomato (*Lycopersicon esculentum*) growing farmland with organically managed soil.

2.2. Experimental design

The field experiment was designed within the tomato growing plastic tunnels. For the application of pest management treatments, we established four replicating plots (5 × 5 m sq.). We applied a cattle manure treated plot (MP), a pesticide treated plot (PP), a botanical pesticides treated plot (BP) and a control plot (CP). Soils in the mature stages of tomato production were collected in2016–2018 for summer and winter seasons. Our sampling strategy was represented by: 2 seasons X 4 plots X 9 replicates X 2 times. In the cattle manure plot (MP), a mixed solution of 80% cattle urine and dung (1 lit./30 days) with 20% water was applied [32, 33]. In the botanical pesticide plot (BP), the powder of Timur (Rutaceae- *Zanthoxylum armatum*) was applied (100 gm/lit water/plot). Similarly, in the pesticide plot (PP) a mixture of chlorpyrifos 50% and cypermethrin 5% EC-2ml/lit chemical were applied [34, 35]. There was no any treatment applied in control plots. Soil samples were randomly collected from soil cores with a diameter of 3.5 cm and a depth of 15 cm in each of the designed plots.

2.3. Nematode extraction and identification

We used modified Cobb's sieving and decanting method for the extraction of nematodes from soil samples as proposed by proposed by S. Jacob and van Bezooijen (1984) [36]. A subsample of 100 gm soil (wet weight) was used for extraction of nematodes. Nematodes were collected after 72 h and they were immediately counted using a stereomicroscope (40X) for estimation of abundance. Thus extracted nematodes were preserved in 4% formaldehyde solution. Next, at least 50 nematode individuals were randomly counted from each sample under the photographic microscope and they were photographed for later identification. The identification was done at genus level based on identification keys by Bongers [8, 10]. Similarly, genera were further allocated to a trophic group (bacterivores, fungivores, plant parasites and predatory) as suggested by Yeates [37] and to life-history strategies (c–p scales) based on Bongers and Ferris [8, 11]. The nematode indices were then assigned [10]. Total organic carbon (C) was estimated by the Walkley and Black method (1934) [13], and the total content of nitrogen was determined by the Kjeldahl method.

2.4. Calculation of nematodes indices

The maturity index (MI) for free-living taxa is the indicator for disturbance with smaller values being indicative of a more disturbed soil and larger values indicates for less disturbed [11, 37]. The proposed modification of this index based on combining free-living and plant parasitic nematodes developing summed maturity index (SMI or ΣMI2-5 -combined free-living and plant-parasitic nematodes without cp = 1) = ΣviXfi/n; where vi = colonizer-persister (c-p) value assigned to family, frequency of family (abundance), in sample (abundance), n = total number of individuals in a sample. Enrichment Index (EI), Structure Index (SI), and Channel Index (CI) were calculated according to Ferris et al. (2001) [10,17,38]. EI is based on the expected responsiveness of the opportunistic guilds (bacterivorous nematodes with c-p1) to organic resources enrichment. The SI represents an aggregation of functional guilds with c-p values ranging from 3-5 and describes whether the soil ecosystem is structured with greater trophic links (high SI) or degraded (low SI) with fewer trophic links. EI is based on the expected responsiveness of the opportunistic guilds (bacterivorous nematodes with c-p1) to organic resources enrichment. The SI represents an aggregation of functional guilds with c-p values ranging from 3-5 and describes whether the soil ecosystem is structured with greater trophic links (high SI) or degraded (low SI) with fewer trophic links [4]. Channel index (CI) is a percentage of fungivores among the total fungivores and c-p 1 bacterivores. It indicates predominant decomposition channels in the soil food web. A high CI (>50 %) indicates fungal decomposition channels, whereas low CI (<50 %) suggests bacterial decomposition channels.

2.5. Statistical analysis

We performed log (x +1) transformations for the normalization of indices data after two-way ANOVA was used to detect significant influences of treatment and season. A generalized linear model (glm) was applied to identify the effect of particular treatment type on the mean variation of nematodes indices (EI, SI, CI and SMI) in our samples. The

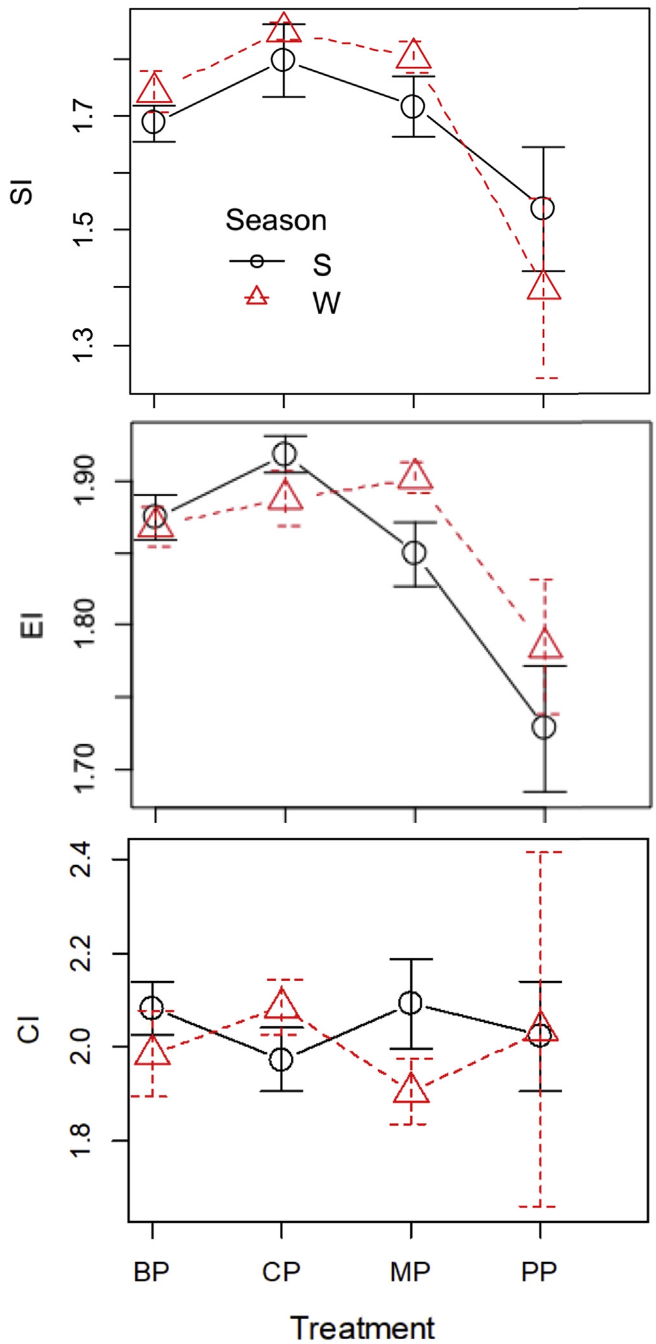

Fig. 3. Effect of treatment systems by seasons (Summer-S and Winter-W) for food web indices (enrichment index-EI, Structural index SI, and Channel index CI).

model was selected based on the Akaike information criterion (AIC) in forward/backward selection of factors. Principle component analysis (PCA) was applied to detect the correlation among nematode indices and physical parameters (soil temperature, soil moisture, and carbon-nitrogen ratio). In all cases statistical significance was observed ($p < 0.05$). Similarly, error bars indicate the standard error of the mean in all graphs.

3. Results and discussion

3.1. Relative abundance of genera

In total, 23 nematode genera were identified in our study. The relative composition of genera was determined. A high variation of nematodes genera was observed within each treatment plot. The percentage contribution of different genera varied with treatment plots viz. *Cephalobus, Eucephalobus and Trichodorus* were the major genera found in the BP; with *Dorylaimus, Rhabditis, Mononchus*the main genera found in the CP pots. Similarly, *Panagrolaimus, Heterocephalobus, Acrobeles* were dominant in the MP and *Achromadora, Acrobeloides, Aphelenchoides*in the PP. The lowest abundance of genera was observed in the pesticide applied plots (Fig. 1) followed by botanical pesticide applied plots. The highest diversity and abundance were present in manure applied plots and the control plots. This result probably can be attributed to the effect of the organically managed system in our experimental plot (MP); that is by applying the mixture of cattle urine and manure for the control of pests. This result supports the findings of a previous study of nematode genera where specific nematodes genera were identified as indicator species for describing the impact of soil management or land use change [31]. Many studies indicated that bacterial feeders were more abundant under organically managed system, while fungal feeders were more abundant in conventionally managed soil [16, 21, 37]. It is reported that the agricultural practices in organic managed farming are supposed have higher diversity, which correlates with the stability or resilience of the soil.

3.2. Effect of treatment systems on summed of maturity index (SMI)

It was determined that SMI was significantly affected only with the treatment system ($F = 5.013$, $P < 0.005$), but there was no significant variation detected with seasonality (summer and winter) for this index (Table 1). In our analysis, the mean value of SMI in the control plot (CP) exhibited significantly higher ($t = 2.81$, $P = 0.006$) compared with the mean of other treatment plots for both seasons (Fig. 1). The lowest mean value of SMI was obtained in the pesticide applied (PP) without statistical significance ($P > 0.05$). This result is revealed more structural and stable soil food web in CP than other plots indicating relatively undisturbed soil status in control plot indicating a stable soil ecosystem in term of diversity and functionality soil nematodes [37, 39]. Similarly, the mean value of SMI attributed to low structural food web in pesticides applying plots. This result indicated that the pesticide-applied plots linked to the sensitivity of nematode genera with the application of chemical compounds resulting in dominant lower cp (colonizer-persister) group [1, 3, 18, 22, 24]. For example, organic amendments applied to agricultural crops affected the nematode community, promoting the increase of nematodes like bacterial feeders [13, 21, 25].

In our study, a more consistent pattern of MI value was obtained in all treatment systems for both seasons. However, Ilieva-Makulecet al. (2016) [31] suggested that the value of MI is probably vary with farming systems. In our system, a relatively higher value of MI was obtained from the organically managed plots (MP, BP) than from chemical pesticide-applied plots, suggesting that an organically managed agro-ecosystem is a well-structured food web and comparatively stable conventionally application system. Many researchers indicated that the soil disturbance caused by external inputs for pest management results changing on soil microbial communities thereby affecting the food-web dynamics of soil [6, 15, 33, 34].

3.3. Variation of food web indices by treatment and season

We evaluated the variation of food web indices such as enrichment index (EI), structural index (SI) and channel index (CI) across both summer and winter seasons. The effect of treatment plots and seasons on mean values of EI, SI and CI were examined performing two-way ANOVA (Table 2). We determined that only treatment systems cause significant affect, rather than seasonality, especially for EI and SI. However, there was no statistical significant change within the mean of channel index (CI) with either treatment systems or with seasonal change. After that, we applied a generalized linear model (glm) to determine the effects of a

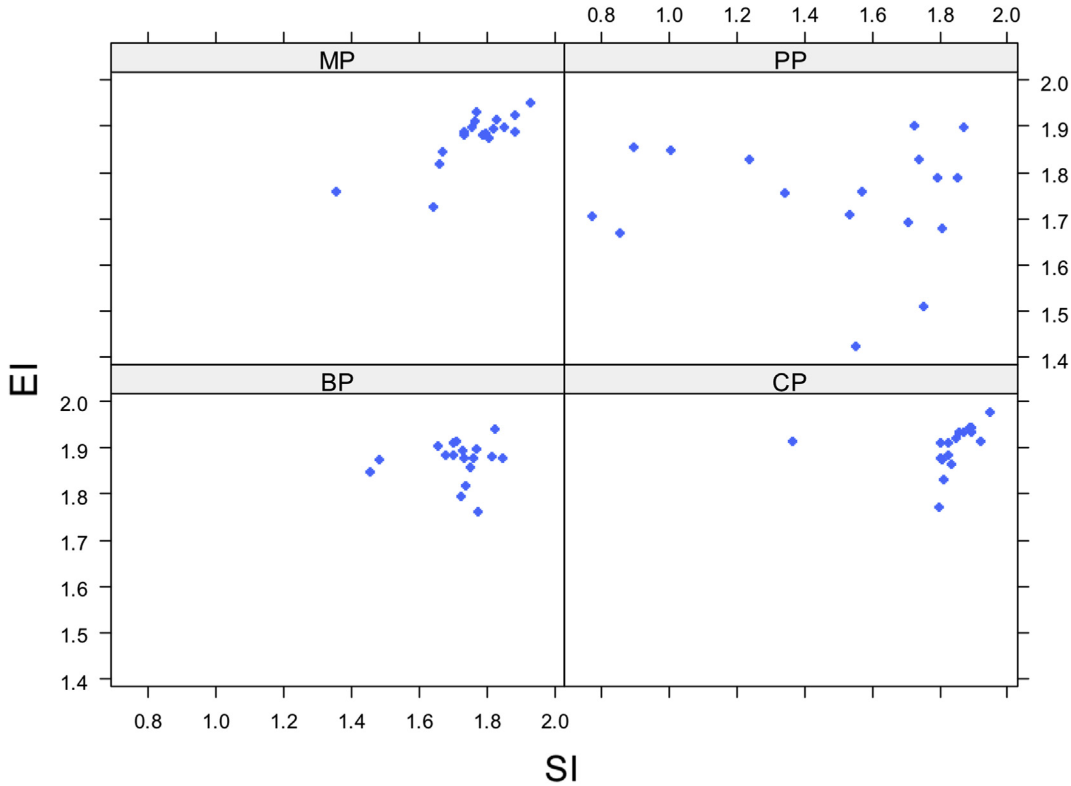

Fig. 4. A food web structure on enrichment index (EI) and structure index (SI) trajectories.

Table 4
Summary of covariates in PCA axes.

Variables	Axis.1	Axis.2
Tem	0.825	0.68
SM	0.783	0.613
CN	0.744	0.554
EI	-0.568	0.323
SI	-0.469	0.22
CI	0.324	0.105

particular treatment on the mean of these indices. The model was selected based on the lowest value of Akaike information criterion (AIC) in forward/backward selection of variables (Table 3).

In our analysis, the means of all indices were significantly lower in pesticide-applied plots (PP) compared to other treated plots in both seasons. The highest mean value was obtained in the control plot (CP) followed by botanical pesticides (BP), PP, and MP. This result indicates that the treatment systems effect the nematodes soil food webs irrespective of seasonality. The CP has higher enrichment status as well as the higher mean value of SI followed by other treatments (Fig. 2).

The soil ecosystem in these plots is structured with greater trophic links (high SI) or degraded (low SI) with fewer trophic links. In pesticide-applied plots (PP), the soil is resource-depleted, probably due to inputs of pesticides. The EI is a measure of the abundance of enrichment opportunists relative to the abundance of basal taxa. In our result, significantly higher ($P < 0.05$) mean value of EI presented in the non-pesticide-applied plots. It attributes to the higher contribution of bacterivores nematodes that increase when organic matter in the form of compost is applied [8, 12,19,14,17] or in intensive irrigation process [8, 27]. In our study, the EI ranged from moderate to high in MP and CP but that was always low in PP followed by BP. This case was more similar with several studies on tomato or vegetable production systems [40, 41, 42]. This indicates the level of resource enrichment and serves as an indicator of soil productivity [43, 44].

Similarly, the channel index (CI), an indication of whether organic matter decomposition pathways are dominated by bacteria or fungi, did not differ significantly between treatments. A higher value of CI was identified in the BP and MP during the summer, but a contrasting situation was obtained in the CP plot. There was a similar CI value obtained in PP plots for both seasons. This result indicates that the BP and MP have fungal-dominated decomposition [24, 33, 39, 42] pathways, especially in summer, but there is no mean variation of CI in pesticide-applied plots for either season.

3.4. *Structure versus enrichment indices for each plot*

The Enrichment Trajectory and the Structure Trajectory, both based on the indicator importance of functional guilds of nematodes, are descriptors of food web condition. Plotting of EI versus SI provides a model framework of nematode faunal analysis is an indicator of the likely conditions of the soil food web [21, 45, 46, 47]. Use of these indices has provided critical information about below-ground processes in distinct agro-ecosystems [12, 17, 24, 48, 49]. Plotting EI versus SI revealed a majority of data points in the CP, MP and BP plots which exhibited enriched and structured food web webs status, but not in the pesticide-applied plots (Fig. 3). Our analysis determined the most degraded food web as well as the most resource-depleted situation took place in the pesticide-applied plots. The SI in this study was influenced by a decline in omnivores and predators. The use of synthetic fertilizers and pesticides probably contributed to the declining SI and increasing EI.

In this context, greater c-p value nematodes are generally associated with low stress and undisturbed environments [13, 27, 35, 50]. A higher buildup of greater c-p (3–5) value nematodes in the treatment plots resulted in higher SI values suggesting more structured food webs. The variation of indices in our system was attributed to changing characteristics of soil as well as the effect of resource variation in experimental plots, especially found to be decreased in pesticide-applied (PP) plot [41, 50, 51]. As seen in the food webs under all treatment systems on pesticides applying plots the quadrate indicating strong disturbance, which

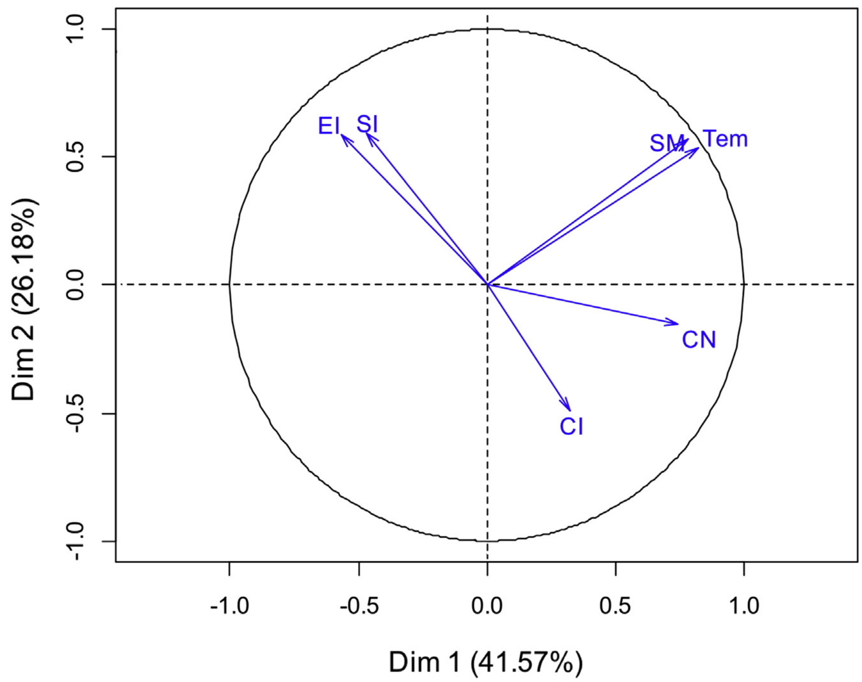

Fig. 5. Principle component analysis (PCA) for EI, SI and CI and their correlation with soil moisture (SM), temperature (TEM) and carbon nitrogen ratio (C: N).

means that they are highly N-enriched, and bacterial dominated, with a low C: N ratio.

3.5. *Correlation of indices with physical parameters*

In the previous study, several soil health/quality metrics were applied to the tomato production context [2, 50]. Nutrient enrichment and other physical parameters are the keys factors that affect the soil community by altering the physical and chemical properties of the soil by influencing pH, soil porosity, and organic fractions. Principle component analysis (PCA) was applied to find the correlation among indices (EI, SI, CI) and physical parameters (soil temperature, soil moisture, and carbon nitrogen ratio). In two PCA axes (dimension) higher variation (41.57%)was seen in the first axis (Fig. 4). All the variables are significant towards the first axis except CI. Whereas, the negative relation of EI and SI with physical parameters was exhibited in the first axis (Table 4). The physical parameters, such as soil moisture (SM), soil temperature (TEM) and carbon nitrogen ratio (C/N) were positive for channel index (CI), since the decomposition process of the soil is affected by physical status and management effect (Fig. 5). Increasing the value of the channel index is indicative of fungal-dominated decomposition pathways that are responsive to carbon nitrogen ratio, moisture regime and soil temperature. Fungal-based food webs are more resistant to drought than bacterial-based ones, since fungivorous nematodes are less affected by drought than bacterial ones [19].

4. Conclusion

Our results highlight the effect of treatment systems on the biological characteristics of the soil in our experimental plots. We observed prominent variation within experimental plots for nematodes ecological indices such as Maturity Index, Enrichment index, Structure Index, and Channel Index. Over all nematode indices are responsive to pest management practices found in tomato farming. Pesticide applied plots showed low maturity and strongly disturbed soil indicating degraded food web comparing to other experimental plots. However, further work is needed in order to testify the synergetic effect of pesticide intensity, treatment types and soil characteristic, strain of tomato crops on nematodes diversity and food web structures with space and time. Pest management practices affect the rate of decomposition and food web structures especially degrading status obtained in pesticides applying plots. Management practices have a significant impact on soil ecosystems as revealed through this variation in nematode indices and food web functioning. In sum up, pest management practices which is more organic based and ecofriendly such as botanical pesticides and cattle origin manure should be implemented for sustainable soil conservation and management of insect pests in Nepalese tomato growing farms.

Declarations

Author contribution statement

Dipak Gupta: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Shivish Bhandari: Analyzed and interpreted the data.

Daya Ram Bhusal: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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