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Spatial Patterns of Soil Nematode Communities in Tomato (*Lycopersicon esculentum* Mill.) Growing Agro Farms in Kathmandu Valley, Nepal

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ABSTRACT
Soil nematodes play a vital role in ecological processes and soil food web. Nematodes are valuable bio-indicators of soil disturbance in agricultural practices. Therefore, they can be used to understand the biological mechanisms of the soil. The aim of the study was to observe the abundance and diversity of soil nematode communities in different tomato (*Lycopersicon esculentum* Mill.) growing agrofarm sites in Kathmandu Valley. Nematodes were extracted by CobbÈs sieving and decanting method from the collected soil samples. Altogether 39 genera of soil nematodes belonging under 19 families of five trophic groups were recorded from the Kathmandu valley, with 16 genera of each bacterivorous and plant feeder, 3 genera of omnivorous and 2 genera of each predator and fungivorous feeding group. Out of 19 families, the highest genera of nematodes were recorded from families Tylenchidae and Cephalobidae. The abundance of nematode genera is well discriminated in our analysis. One-way ANOVA was used to compare the richness of nematode genera (per plot) among these agrofarm sites and the generic richness of nematodes in Kathmandu, Bhaktapur and Lalitpur were significantly different (df = 2, MS= 13.36, F= 5.78, P=0.005). We highlighted the need for further exploration of the diversity and community dynamics of soil nematodes under different pest management practices in Tomato growing agrofarms for sustainable soil ecosystem service in Nepal.

Key words: Agrofarm; diversity; soil nematodes; trophic groups; diversity.

INTRODUCTION

Soil is a critical component in the structure and function of agroecosystems and the condition of biological communities is important to both the structure and function of soils. Nematodes are involved in fundamental ecological processes like decomposition and nutrient cycling (de Goede and Bongers 1994) and their functional composition is indicative of the major channels of energy transfer channels across the decomposition pathways in soil. Therefore, they are the major component of the agroecosystem, playing a decisive role in ecosystem services (van der Heijden et al. 2008, Gupta and Bhusal 2018) such as control of soil organic matter, physical structure along with soil community (Wolters 2000, Jouquet et al. 2006) and above ground vegetation dynamics (Soler et al. 2005, de Deyn and van der Putten 2005). It is known that the composition of soil nematodes is influenced by environmental variables such as vegetation, soil type, season, soil moisture level and soil organic matter (Liang et al. 2009, Bakonyi et al. 2007). Nematofauna plays an important role in agroecosystems and occupies a key position as a primary and intermediate consumer in soil food webs; therefore, the study of the nematode community structure offers a powerful ecological tool for assessing soil disturbances and the effects of crops (Manachini 2001, Manachini et al. 2009).

In agriculture soils, greater diversity of trophic groups is correlated with an increase in the frequency of generally-less-abundant trophic groups (i.e. fungivorous, omnivorous and predators) relative to that of generally-more-abundant trophic groups (i.e. bacterivorous and plant feeder groups) (Bongers and Ferris 1999, Yeates 2003). In general, healthier soil is composed of a diverse mixture of nematode-feeding groups (Bongers and Ferris 1999, Yeates 2003). There are a number of nematode community indices (Bongers 1990, Bongers and Bongers 1998) which have been developed based on a classification of trophic groups i.e. omnivorous, predators, bacterivorous, fungivorous and plant-feeders and successfully applied to monitor land use changes, management effects and environmental disturbance (Hunt and Wall 2002, van Der Heijden et al. 2008).

Thus, nematode community structure is a sensitive indicator of the soil status and environmental disturbances (Bongers and Bongers 1998) these are preferred bio-indicators of soil health conditions (Bongers and Ferris 1999, Zhao and Neher 2013, Gupta and Bhusal 2018). Free-living nematodes act as soil ecological indicators because they indicate the carbon and nitrogen level of the soil and they are related to the decomposition process (Neher 2001, Sun et al. 2013) and disease suppression (Yeates et al. 2000). Also used for evaluating soil health conditions under various agricultural practices such as organic and conventional farming, tillage and use of pesticides, fungicides, or herbicides (Bongers and Ferris 1999, Zhao and Neher 2013).

Tomato is one of the major commercial vegetable crops in Nepal (Ghimire et al. 2001). Srijana tomato variety is mostly preferred by farmers for its wider adaptability including its suitability for off-seasonal production, superior taste as well as tolerance to bacterial wilt disease. This variety is popular among farmers and suitable for plastic house cultivation (Chapagain et al. 2011, Pokharel and Thakur 2012). The current scenario prevails diverse challenges in the agriculture system, such as extensive use of chemical pesticides and lack of sustainable soil diversity conversation policy, including land use plan and strategy (Rijal et al. 2018, Gupta et al. 2019). Nepal needs eco-friendly pest management practices, especially for vegetable growers to reduce chemical pesticides; and requires effective and sustainable eco-friendly pest management techniques to maintain sustainability in soil ecosystems (Giri et al. 2009). Application of animal and plant byproducts is the foundation of alternative eco-friendly pest management systems that aim to reduce synthetic inputs (Abawi and Widmer 2000, McSorley and Frederick 1999) although field experiment on this issue is extremely rare (Gupta and Bhusal 2018). This study aims to observe the abundance and diversity of soil nematode communities in different tomato (*Lycopersicon esculentum* Mill.) growing agrofarm sites in Kathmandu Valley.

MATERIALS AND METHODS

Study site

The study was carried out in Kathmandu Valley during 2018 and 2019. In the valley, altogether seven tomato growing agrofarm sites were selected to determine the community composition and spatial distributions of free-living soil nematodes. The selected sites were: three agrofarm sites (Tokha, Ramkot and Chobhar) in the Kathmandu district, two (Sarasawatikhel and Bode) in Bhaktapur district and two (Bhaisepati and Nakhkhu) in Lalitpur district. According to the farmers, in all study sites organic farming was practised i.e. compost manure was applied as fertilizer. The tomato varieties were the same (i.e. srijana hybrid variety) in all agrofarm sites.

Soil samples collection

Soil samples were randomly collected from the rhizosphere layer of each selected individual mature tomato plant with the help of a soil auger (diameter of 3.5 cm and depth of 20 cm). The sampling strategy of agrofarms was represented by: 9 replicates x 2 seasons x 7 sites. Hence, a total of 126 soil samples were collected.

Extraction and identification of soil nematodes

Soil nematodes were extracted from soil samples by the modified Cobb's sieving and decanting method (Jacob and van Bezooijen 1984, Ferris et al. 2001, Bongers and Ferris 1999). A subsample of 100 gm soil (wet weight) was used for the extraction of nematodes. Nematodes were collected after 52 hours in water. Thus, extracted nematodes were heat-killed and fixed in a hot solution of 4% triethanol amine formaldehyde (TAF) solution and pure glycerol (Seinhorst 1966, Shepherd 1970). Later on, the first 50 individuals per sample encountered were identified under the photographic microscope (Microscope-Nikon-Ci-L, Camera-Nikon-DS-Fi-L2) and they were photographed for later identification. Nematodes were identified at the genus level based on the identification keys of Bongers (1994), Ahmad (1996), Ferris et al. (2001), Ahmad and Jairajpuri (2010). Similarly, nematode genera were classified into five trophic groups (bacterivores, fungivores, plant feeders, omnivores and predators) using a method from that suggested by Yeates et al. (1993) and Yeates (2003) and life history strategies (c–p scales) based on Bongers and Ferris (1999) as well as functional guilds were assigned by combining the trophic groups to the c-value (Bongers and Bongers 1998, Ferris et al. 2001). In order to use nematodes as bio-indicators, they were allocated to the colonizer-persister (c-p) values of nematode taxa have been scaled (1-5 scale) based on r-k life-history characteristics that are useful in interpreting the trophic status of the soil food web in different habitats (Bonger 1990).

Soil physicochemical parameters

The average temperature, pH, and soil moisture from the selected tomato-growing agrofarm sites of Kathmandu Valley were measured in the winter and summer seasons. The soil temperature, pH and moisture were measured by using a soil pH and moisture meter. Total organic carbon (C), total nitrogen (N) and carbon-nitrogen ratio (C: N) were also estimated. Total organic carbon (C) was estimated from the soil samples by Walkley & Black method (Walkley and Black 1934) and the total nitrogen content in the soil were estimated by the Kjeldahl method. The C/N ratio was also calculated.

Statistical analysis

The diversity, variation and abundance of soil nematodes found in different agro-farms were analyzed using One-way Analysis of variance (ANOVA). The indicator value of each genus and discriminate ordination was carried out using a suitable package and function in R (R Version 3.3.1). The data were log (x +1) transferred for normalization. In all cases, statistical significance was established with $p < 0.05$.

RESULTS

Comparative community composition of soil nematodes

A total of 39 genera of soil nematodes were reported from agrofarm sites of the Kathmandu valley. Among 29 genera of nematodes recorded in Kathmandu, 14 were plant feeders, 10 omnivorous feeding groups. Out of 30 genera recorded from Bhaktapur, 12 were bacteriovorous, 12 were plant feeder, 3 were omnivorous, 2 were fungivorous and 1 genus was predatory feeding groups. Similarly, 27 genera were reported from Lalitpur, 12 were plant feeders, 9 were bacteriovorous, 3 were omnivorous, 2 were fungivorous and 1 genus was predatory feeding groups (Fig. 1).

Based on the nematode abundance, cluster analysis was performed to find the similarity and dissimilarities in the sampling sites. The Kathmandu sites were significantly different in the abundance of nematode-fauna compared to other sites (Fig. 2). Among the six different sites, the new and old sites of Kathmandu were well distinct compared to the other sites. The highest abundance of nematode genera was found in the new field of Kathmandu

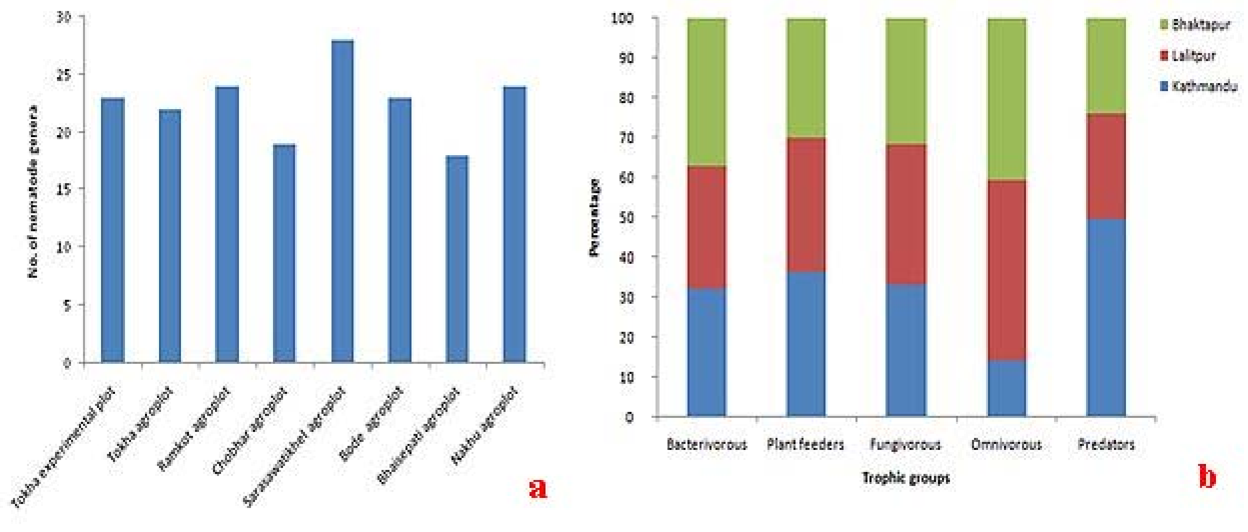

Figure 1. (a) Nematode genera in tomato agro-plots in Kathmandu Valley (b) Trophic groups of nematodes in agro-plots of Kathmandu Valleys

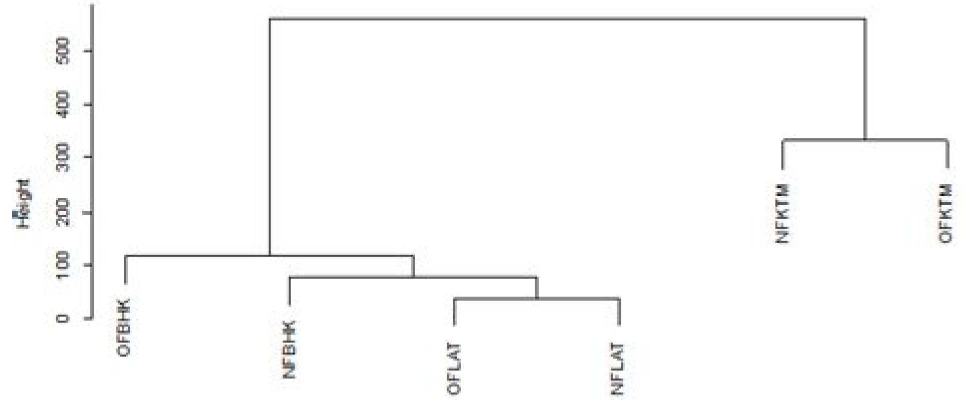

Figure 2. Cluster analysis (Method = ward, distance = Euclidian) for six observation sites based on the abundance of nematodes genera. NFKTM (New field Kathmandu), OFKTM (Old field Kathmandu), OFBHK (Old field Bhaktapur), NFBHK (New field Bhaktapur), NFLAT (New field Lalitpur), OFLAT (Old field Lalitpur)

sites whereas there were no significant differences between several nematode genera in other sites. It is probably due to disturbance in soil and the use of compost manures in the soil as well as the high content of moisture in the soil. This variation might be related to the soil physical properties of soil and the long disturbance history within the old and new fields of tomato growing farms of Kathmandu Valley. Similarly, the frequency of nematode genera within the samples was analyzed based on the Interquartile Range (IQR) (Table 1). In this analysis, the genera *Cephalobus, Hoplolaimus, Merlinius* and *Heterorhabditis* were found with high frequency, but the genera such as *Criconemella*, *Trichodorus*, *Atylenchus*, *Rhabditis* and *Achromadora* were found with low frequency in the samples.

Abundance of nematode genera

One-way ANOVA was performed to find the effect of sampling sites on the abundance of nematode genera in spatial observation of Kathmandu Valley (Table 2). The sites of Kathmandu Valley and the abundance of nematode genera were significantly varying in terms of abundance. Sampling sites represent old and new plots in Kathmandu, Bhaktapur and Lalitpur, exhibiting significant variation in the nematode's abundances.

A generalized linear model (GLM) was performed in six different sampling sites in Kathmandu Valley to find the significant effect of a particular site on the abundance of the nematode genera (Table 3). The NFKTM and OFKTM are significantly different compared to other sites of Bhaktapur and Lalitpur.

The regression of abundance and soil physical properties in the observational sites are given in Table 4. The relationship between the abundance of nematode genera and the physicochemical parameters of soil is shown in Figure 3. There was a significant negative correlation between the abundance of soil nematodes with carbon, moisture and soil temperature whereas there was a non-significant positive correlation with nitrogen and pH (Table 4). It indicates that mostly the nitrogen and pH factors in the soil are important factors affecting soil nematodes rather than other physicochemical factors.

The relationship between the abundance of nematode genera and physicochemical parameters was analyzed with the help of Principal Component Analysis (Figure 6). The soil temperature, moisture and carbon content affected the abundance of soil nematodes. A positive correlation was found between nitrogen and soil pH.

Trophic groups and trophic guilds

The regression summary of the abundance of trophic guilds and physicochemical parameters of soil in observational sites is given in Table 5. Only the soil temperature was affected significantly and there was a positive correlation with soil carbon, nitrogen and moisture.

The Principal Component Analysis (PCA) between

Table 1. Summary of frequency distribution based on Interquartile Range (IQR) of nematode genera in the samples of observational sites

Genus	Mean	SD	IQR	0%	25%	50%	75%	100%	A:n
Achromadora	9.25	7.544	7.75	4	4	6.5	11.75	20	4
Acrobeles	14	14.023	16	2	4.25	10.5	20.25	33	4
Acrobeloides	16.25	17.036	15.75	5	5	9.5	20.75	41	4
Alaimus	11.5	13.102	8.5	3	4.5	6	13	31	4
Amphidelus	14.25	13.400	17.75	2	4.25	12	22	31	4
Aphelenchoides	21.75	18.318	26.75	5	7.25	19.5	34	43	4
Aphelenchus	25.75	23.683	28.75	5	8.75	20.5	37.5	57	4
Atylenchus	26.5	11.445	7	13	22.75	26	29.75	41	4
Cephalobus	110.25	151.915	83.25	29	32	37	115.25	338	4
Chiloplacus	31.5	18.841	17.5	5	25.25	36.5	42.75	48	4
Criconemella	36.25	3.774	3.75	32	34.25	36	38	41	4
Diploscapter	42	20.281	17.5	25	30.25	36	47.75	71	4
Ditylenchus	29.75	11.672	8.25	13	27.25	33	35.5	40	4
Dorylaimus	49.5	30.599	22	31	31.75	36	53.75	95	4
Eucephalobus	43.25	38.52597	27.75	2	26.75	38	54.5	95	4
Eudorylaimus	43	38.884	28	1	26.5	38	54.5	95	4
Eutylenchus	26.25	23.171	31.75	6	8.25	22	40	55	4
Filenchus	17.5	25.252	18.5	2	2.75	6.5	21.25	55	4
Helicotylenchus	35	19.849	24	10	24.25	37.5	48.25	55	4
Hemicriconemoides	35.75	19.602	21.75	10	26.5	39	48.25	55	4
Heterocephalobus	34.5	32.357	50	4	8.5	32.5	58.5	69	4
Heterodera	20.75	23.753	18.75	1	7.75	13.5	26.5	55	4
Heterorhabditis	41.75	39.651	54.25	10	10	32.5	64.25	92	4
Hoplolaimus	42.75	40.557	58.25	9	9.75	35	68	92	4
Longidorous	54	37.629	24	2	45.5	61	69.5	92	4
Malenchus	50.5	37.287	31.5	2	36.5	54	68	92	4
Merlinius	28	30.930	50	1	1.75	25.5	51.75	60	4
Mesodorylaimus	23.666	31.564	28.5	3	5.5	8	34	60	3
Mesorhabiditis	34	25.059	25	10	21	32	46	60	3
Mononchus	37.333	24.684	24	10	27	44	51	58	3
Panagrolaimus	27	19.467	18.5	5	19.5	34	38	42	3
Pratylenchus	30	21.794	20	15	17.5	20	37.5	55	3
Prismatolaimus	11.333	9.609	9.5	1	7	13	16.5	20	3
Psilenchus	32	11.135	11	20	27	34	38	42	3
Rhabditis	17	7.937	7.5	8	14	20	21.5	23	3
Trichodorous	12	7	6.5	7	8	9	14.5	20	3
Tylenchorhynchus	21	24.269	21.5	6	7	8	28.5	49	3
Tylenchus	25.666	27.153	24	9	10	11	34	57	3
Zeldia	27.666	27.061	26	6	12.5	19	38.5	58	3

SD: Standard deviation, A: Abundance

Table 2. Summary of one-way ANOVA for sites and abundance of genera in spatial observation in Kathmandu Valley

Variable	Df	Sum sq	Mean sq	F test	Pr(>F)
Sites	5	18641	3728	3.245	0.00841*

SE: Standard error, *Significance level (>0.05)

Table 3. Summary of Generalized Linear Model (GLM -AIC= 1431) for abundance and sampling sites. Model was fitted: glm (formula = abundance ~ sites, family = Gaussian (identity), data = X)

Sampling site (Intercept)	Estimate	SE	t-value	Pr(>|t|)
NFBHK	16.722	7.989	2.093	0.0382
NFKTM	23.593	9.225	2.557	0.0116*
NFLAT	11.5	11.299	1.018	0.3105
OFBHK	3.722	11.299	0.329	0.7423
OFKTM	31.222	11.299	2.763	0.0065*
OFLAT	1.333	11.299	0.118	0.9062

SE: Standard error, *Significance level (>0.05)

Table 4. Regression of nematodes genera abundance and soil physical properties in observational sites

Coefficients	Estimate	SE	t-value	P (>|t|)
(Intercept)	197.756	123.464	1.602	0.111501
Carbon	-34.657	14.356	-2.414	0.017083*
Moisture	-139.954	43.79	-3.196	0.001727**
Nitrogen	-6.993	14.799	-0.473	0.637281
pH	-16.253	17.925	-0.907	0.366124
Temp.	4.414	1.119	3.943	0.000128***

SE: Standard error; *, **, *** Significance level (>0.05, >0.01 and 0.001, respectively)
Residual standard error: 33.72 on 138 degrees of freedom, Multiple R-squared: 0.1147, Adjusted R-squared: 0.08262, F-statistic: 3.576 on 5 and 138 DF, p-value: 0.004516.

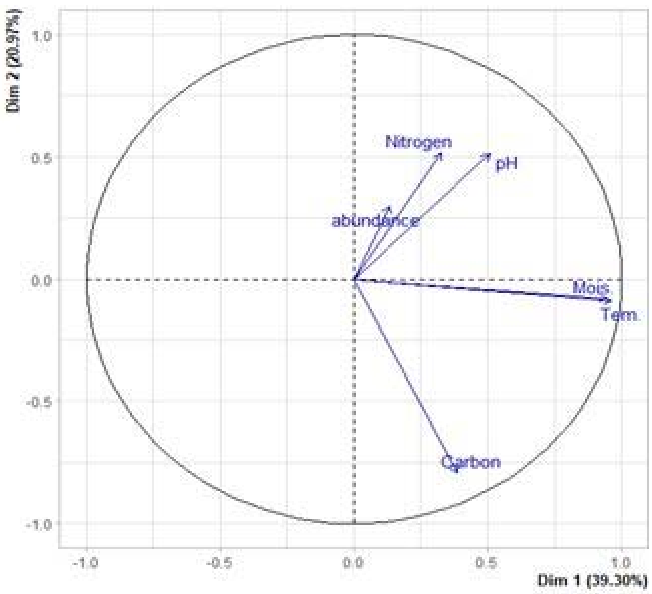

Figure 3. Principal component analysis (PCA) between the abundance of nematodes genera and physicochemical parameters of soil

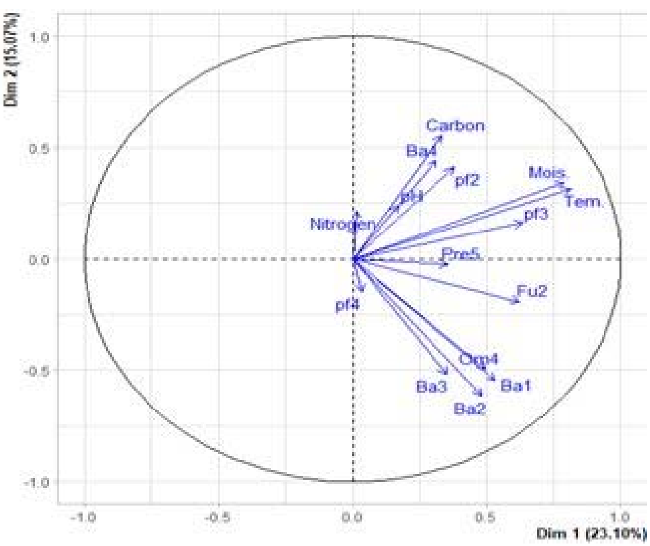

Figure 4. Principal Component Analysis (PCA) between the abundance of trophic guilds and physicochemical parameters in observational sites

the abundance of trophic guilds and physicochemical parameters in observational sites of Kathmandu Valley was performed (Fig. 4). The results show that there was a positive correlation with soil carbon, nitrogen and moisture especially with Pf2, Pf3, Ba4 and Pf5 trophic guilds.

DISCUSSION

Soil nematode community richness, diversity and trophic structure were directly affected by several environmental, biological and anthropogenic factors related to resources Renco (2011). A total of 39 genera of soil nematodes were reported from agrofarm sites of the Kathmandu valley (i.e. Kathmandu, Bhaktapur and Lalitpur). Our result is in a similar line to Keshari et al. (2018), a total of 23

Table 5. Regression summary of the abundance of trophic guilds and physicochemical parameters of soil in observational sites of Kathmandu Valley

Coefficients	Estimate	SE	t-value	Pr(>|t|)
(Intercept)	60.203	72.738	0.828	0.409
C	-9.660	8.457	-1.142	0.255
N	3.205	8.719	0.368	0.714
pH	-4.319	10.560	-0.409	0.683
Temp.	1.552	0.659	2.354	0.02*
Moisture	-32.062	25.798	-1.243	0.216

SE: Standard error; *Significance level (>0.05) Residual standard error: 19.86 on 138 degrees of freedom, Multiple R-squared: 0.091, Adjusted R-squared: 0.058, F-statistic: 2.775 on 5 and 138 DF, p-value: 0.0202

genera of soil nematodes belonging to 14 families were encountered. Among them bacteriovores with 8 genera and plant feeders with 7 genera were dominant over fungivorous with 4 genera, predators with 3 genera and omnivorous nematode with only one genus. These results also agreed with Gupta et al. (2019 a), Gupta and Bhusal (2018), Mir and Tanveer (2016), Vaid et al. (2014), Zheng et al. (2011), Pen-Mouratov et al. (2008), Ferris et al. (1998). They have found herbivores and bacterivores with high populations and omnivores and fungivores with low populations in different agroecosystems. The nematode *Helicotylenchus* is the most dominant genus over others in all seven tomato cropping agrofarm sites in Kathmandu Valley. According to Keshari et al. (2018), *Helicotylenchus* was the most abundant genus among herbivores, *Rhabditis* among bacteriovores, *Aphelenchoides* among fungivores and *Mononchus* among predators. Our results were also similar to that of Gupta et al. (2019 b), Gupta and Bhusal (2018), Osei et al. (2012), Hu and Qi (2010), Maria et al. (2007). The diversity of cephalobidae was high in previous studies (Zheng et al. 2011, Yeates et al. 2000). In this study, the diversity of cephalobidae and Tylenchidae were high and the plant feeder *Helicotylenchus* became dominant in those tomato cropping plastic tunnel agro-farm sites which was also reported by Gupta et al. (2019 a), Keshari et al. (2018), Dong et al. (2007). We expected that organic farming practices will be beneficial for nematode abundance and trophic diversity. Our results show that the abundance, generic richness, diversity and ecological indices of nematode communities greatly vary in each tomato cropping agrofarm. The number of soil nematodes and the abundance of trophic groups were different in soils with different tomato cropping agro-farm sites in this study. The abundance of bacteriovores was significantly higher due to the common application of manure amendment to nourish the bacteria, providing a food base for the nematodes in mostly tomato cropping agrofarm sites in our study. These results were similar to those of Gupta and Bhusal (2018), Mir and Tanveer (2016), Dong et al. (2007), Bulluck et al. (2002), Bongers and Ferris (1999), McSorley et al. (1999), Neher (1999), Yeates et al. (1999), Ferris et al. (1996). Our results also suggested that soil nematode community structure can reflect the degree of human disturbance derived mainly from continuous tomato cropping in plastic tunnel agro-forms. Many studies indicated that bacterial feeders were more abundant in organically managed systems, while fungal feeders were more abundant in conventionally managed soil (Gupta and Bhusal 2018, Ilieva-Makulec et al. 2016, van der Heijden et al. 2008, Briar et al. 2007, Yeates 2003, Neher and Olson 1999, Ferris et al. 1996, Freckman and Ettema 1993).

The proportions of the different feeding groups in the soil nematode community vary between treatment systems and seasons and they are influenced by a variety of factors physical and biological characteristics of the soil that influence the abundance of nematodes (Gupta et al. 2019a, Treonis and Wall 2005, Ekschmitt et al. 2003, Ferris et al. 2001). Our result enrichment-opportunist bacterivore guilds (bac l) are more associated in enrich condition of soil than other trophic groups, this result agreed with Renco and Balezentiene (2014).

The abundance samples of function guilds of nematodes are well separated in the cluster analysis indicating the effect of nematodes abundance in different treatment systems within tomato cropping agrofarms. The response of functional guilds with treatment system and nutrient inputs attributes the response of functional guilds varies differently with treatment system (Briar et al. 2007, Nehar et al. 2006) also explained the manure added plots possess greater bacterial feeding nematodes than those managed with conventional practices (Briar et al. 2007) the result of the present study is in similar line. The variation of abundance of nematode feeding groups with agrofarm sites can only partly be attributed to microclimatic and microhabitat change due to management practices. Eisenhauer et al. (2012) indicated that elevated nitrogen availability significantly increased the density of opportunistic bacterial communities. Plots resource dynamics and microphysical parameters with seasons attribute the change in taxonomic and functional composition of nematodes that play the leading role in shaping nematode communities' composition (Bardgett et al. 2008, Bongers and Ferris 1999). From a number of laboratory and field studies it is known that nematodes and nematode communities sharply respond to temperature as the latter affects nematode activity, feeding rates, and community structure (Bhusal et al. 2015, Malherbe and Marais 2015, Bakonyi et al. 2007, Ekschmitt et al. 2003) and some evidence such as an abundance of fungal to bacterial feeding nematodes may be a sensitive indicator of management changes (Yeates and Bongers 1999) in agro-farms.

CONCLUSION

The application of organic byproducts as pest management can increase soil nematode diversity and abundance probably through the stimulation of microorganisms and other favourable biotic and abiotic factors in food webs. Effect of pest management system in Tomato growing had an influence on changing abundance, diversity and communities of nematodes. It is associated with characteristics in soil due to changes in management inputs. Cattle dung and urine are effective ingredients to increase the diversity of nematodes. Similarly, the application of compost manure could increase the number and proportion of bacterivorous nematodes and decrease the number and proportion of plant parasitic nematodes compared to chemical pesticides alone. Therefore, long-term application or compost manure could be suggested as a nematode management strategy as it provides an increase in pest suppression by reducing the impact on soil-plant equilibrium compared to chemicals and inorganic fertilizers.

Further understanding is needed regarding the soil biota and nematode diversity and community composition for the stability of the soil ecosystem while performing the insect pest management practices at least for the application of cattle urine mixture and botanicals to increase the diversity of bacterivorous and plant feeder nematodes to maintain the better soil health condition in the agricultural field than other chemical substances in Nepalese tomato cropping farms. This study insight the need for sustainable pest management techniques should be applied therefore to prevent the loss of species and loss of soil functionality for sustainable Tomato cropping agro-farms in Kathmandu Valley. The choice of appropriate agricultural practices in order to preserve soil biological activity seems to be still a challenge for organic farming in Nepal. It is strongly recommended that the monitoring of soil status is urgently needed before treating any pest management practices in study sites.

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Authors' contributions: The first authors conceived designed and performed the experiments and in collaboration with the second author analyzed and interpreted the data, wrote the paper.

Conflict of interest: Authors declare no conflict of interest

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