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Nematode Population and Activity under Varying Cropping Ratio of Wheat and Mustard in Central Himalayan Agro Ecosystem

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***Abstract* – A study was conducted to find out whether cropping ratio has an effect on the soil nematode population and their activity at a farmers field in central Himalaya. Wheat (*Triticum aestivum*) and Mustard (*Brassica compestris*) were sown as sole crops and in three cropping ratio of 4:2, 3:3 and 2:4 in substitutive design. Nematode population decreased with increasing ratio of mustard indicating allelopathic effect of mustard root exudation. Overall bacterial feeders dominated total nematode abundance, followed closely by omnivores, predators, herbivores and fungivores. The plots planted to wheat and mustard in equal ratio showed the highest channel index of 0.79 indicating a faster bacterial driven decomposition in progress in such plots. Mustard sole crops showed a channel index of 0.77 followed by wheat mustard sown in the ratio of 2:4, 4:2, and sole wheat crops (0.63). Our results point that cropping ratio has important multitrophic effects on soil food webs at the trophic level and suggest that differences in cropping ratio may be important in driving the decomposer subsystem.**

***Keywords* – Central Himalayan Agro Ecosystem, Mustard, Nematode Channel Ratio, Soil Nematode Population, Wheat.**

I. Introduction

Sustainability is an important goal for hill agriculture i.e. maintenance of productivity of food and feed crops and of animals coupled with efficient cycling of nutrients and with biological control of pathogens. An understanding of the existing traditional agriculture systems in relation to nutrient dynamics is necessary to ensure efficient use of resources and calibrate soil fertility indices to help farmers maximize their returns on labour and other inputs. "Baranaaja" is a mixed cropping pattern practiced in the central Himalayan region. It involves cultivation of less than twelve or and sometimes more than twelve food crops in synergetic combinations [1]. This system is useful in meeting food requirements and preserving agro-biodiversity in the region and it needs to be sustained as it is an example of 'conservation agriculture". There is a need to focus our research activities on the detection of ecological patterns underlying the functioning of this healthy and durable farming system.

Nematodes are abundant and diverse invertebrates present in the soil [2]. They are an important component of the soil biotic community and assessment of nematode fauna provides a unique insight into soil biological processes [3]. Nematodes play a role in decomposition and nutrient cycling [4],[5]. Free living nematodes that feed on bacteria and fungi contribute as much as 27% of the readily available nitrogen in the soil [6] and also promote rhizosphere colonization of beneficial rhizobacteria [7]. Therefore one of the major goals of sustainable agriculture should be to enhance populations of free-living nematodes and reduce those of plant parasitic nematodes [8].

The central Himalayan farming system is largely organic in nature with little application of fertilizers, pesticides and herbicides and minimum tillage and fertility is maintained through organic amendment of plant litter manure or and dung manure. Frequent tillage is known to affect nematode trophic structure [5]. Other factors such as soil compaction, irrigation and crop rotation also impact the soil nematode community [9],[10]. Assessment of different nematode trophic groups provide comprehensive insight into soil biological processes [3]. Different species of cultivated crop have different effects on nematode population. It was found that there was greater diversity and abundance of nematodes in wheat fields than in permanent grass ecosystem [11].

This paper compares the effect of varying cropping ratios of two crops Wheat (*Triticum aestivum*) and Mustard (*Brassica compestris*) widely sown in different ratios across the central Himalayan region as winter or "Rabi " crop on the nematode population dynamics and nematode channel ratios. It was hypothesized that the differences in cropping ratios would have differential effects on the nematode community structure which may impact the biological activity of the system. To compare between the plots under different cropping ratios the following indicators were chosen : nematode feeding groups (trophic abundance) and nematode channel ratio (NCR).

II. Materials and Methods

21. Site description, treatments and field plot design

The experimental site was situated at village Agthala, Pipalkoti ($30^0$25'N; 79^0 25'E; 1233m elevation). The area has a warm temperate climate with temperatures ranging from 33^0C to freezing point. The annual average rainfall is around 1200mm. According to USDA classification the soil is an *Inseptisol*, it is sandy loam with a neutral pH, well drained and moderately fertile.

The experimental plots were laid out in completely randomized block design (RCBD) with each treatment plot in triplicate. Treatment being five cropping ratios. During the Rabi cropping season (October – March) wheat and mustard were sown as sole crops and in three intercropped ratio of 4:2, 3:3 and 2: 4 (replacement principle), [12]. The crop was cultivated under rainfed condition.

2.2 Soil sampling

Soil samples were collected every month across the cropping season. Three soil cores (5 cm diameter and 10 cm deep) were removed from each plot using a soil auger from three positions (i) in row (ii) in between rows and (iii) midway between the two. Samples were mixed thoroughly to form a composite sample to reduce the variance associated with aggregated spatial patterns of nematodes in the soil [13]. Large plant parts or stones were removed from samples by passing them through a soil sieve (6mm mesh). All soil samples were stored in the dark overnight at 5^0C and existing field moisture was maintained to minimize changes in nematode population [14]. Soil samples were collected and analysed for two consecutive seasons.

2.3 Nematode extraction, identification and counting

Nematodes were extracted from a 100g subsample taken from each composite soil sample using Baermann funnel technique[15]. Nematodes were collected after 72 hours, heat killed and fixed with Formalin-acetic acid fixative (FAA) solution. Specimens were identified to the genus level using an inverted microscope at 40x magnification. Diagnostic keys by [16]-[18] and the University of Nebraska Lincoln nematode identification website (http://nematode.unl..edu/konzlistbutt.htm) were used. All identified nematode genera were assigned to a trophic group (bacterivores, fungivores, herbivores, omnivores and predators) [19]. Numbers of nematodes were not corrected for extraction efficiency. Nematode genera were also assigned a colonizer-persister value (c-p value) [20]. The c-p values of 1-5 reflect the perceived gradient among nematodes from colonizing r-strategists with unstable populations to persisting k-strategists with relatively stable populations. These indices provide useful indicators of food web structure, enrichment and decomposition channel.

Nematode Channel Ratio (NCR) = B/B+F, Where, B= abundance of bacterivores nematodes, F= abundance of fungivore nematodes. Fungivore (F) to bacterivore (B) ratio was calculated to characterize decomposition and mineralization pathways using (F/B) ratio [21] and the F/(F+B) ratio [22].

Decomposition processes in soil although ultimately dependent on the plant resource base, are often allocated to either the faster bacterial-based energy channel or pathway or the slower fungal-based channel [23]. The ratio between the abundance of these two functional groups gives an index of the relative contribution of the channels which have values between 1 (totally bacterial mediated) and 0 (totally fungal mediated).

III. Results and Discussion

3.1 Effect of cropping ratio on nematode community

Nematode genera identified across the cropping season are presented in Table 1.

Approximately fifty species were identified (only full grown adults could be identified). They belonged to five orders. The genera belonged to order 1. Rhabditida 2. Tylenchida 3. Aphelenchida of the class Secernentea and 4. Dorylaimida and 5.Mononchida of class Adenophora. They were also identified into their trophic groups based on their mouth parts. They were assigned the c-p group number [20]. Majority belonged to cp-1and 2 taxa which are enrichment-opportunist bacterial feeding nematodes that respond rapidly to increase in microbial biomass. The bacterial feeding guild that characterizes basal food web is present under all soil environmental conditions. Thus they are not particularly useful as indicators of the level of bacterial decomposition. However, the enrichment opportunist *Ba1* guild is excellent indicators of bacterial response to low C: N ratio organic inputs and eutrophic conditions [24]. However the presence of Predators and Omnivores belonging to guild 5 are very important indicators since these nematodes are usually not found unless the environment is undisturbed [25]. The cumulative number of genera reported every month are presented in Fig 1. The maximum genera were reported during the moist months when the soil was under paddy cropping (experiment data not discussed in this paper). However during the Rabi season there was a gradual decline in genera abundance probably due to reduced soil moisture and temperature and presence of mustard plants. The soil nematode abundance, biomass, trophic structure and species distribution may alter considerably during the year [26],[27]. They are strongly influenced by microclimate and food availability, which can explain small over all numbers in dry summers.

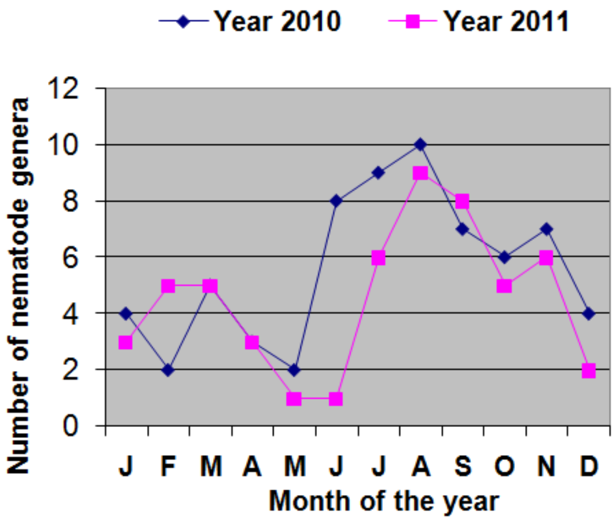

Fig.1. Cumulative number of genera reported every month

The diversity characteristics of the nematode fauna across the cropping season is presented in Table 2. Bacterivores had the highest diversity while omnivores had the lowest diversity. The Margalef richness shows that the nematode trophic structure was fairly consistent across the treatment plots and all sampling dates. Overall bacterial feeders dominated total nematode abundance, followed closely by omnivores and predators and lastly by herbivores and fungivores. Many nematode trophic group studies found that bacterial and fungal feeding nematodes

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Table 1: Genera of nematodes identified and classified into trophic groups from soil planted to wheat-mustard and their c-p ranking from 1-5 [20].

Bacterivores	Fungivores	Herbivores	Omnivores	Predators
Mesorhabditis (1)	*Tylencholaimus (4)*	*Psilenchus (2)*	*Prodorylaimus (4)*	*Clarkus (4)*
Caenorhabditis (1)	*Dorylaimoides (3)*	*Tylenchorhynchus (2)*	*Mesodorylaimus (4)*	*Prionchulus (4)*
Cephalobus (2)	*Promumtazium (4)*	*Helicotylenchus (3)*	*Thornenema (4)*	*Coomansus (4)*
Eucephalobus (2)	*Aphelenchus (2)*	*Hemicriconemoides (3)*	*Opisthodorylaimus (4)*	*Mylonchulus (4)*
Acrobeles (2)		*Xiphinema (5)*	*Morasia (4)*	*Paramylonchulus (4)*
Acrobeloides (2)		*Trichodorus (4)*	*Eudorylaimus (4)*	*Itonchus (4)*
Chiloplacus (2)		*Paratrichodorus(4)*		*Discolaimus (5)*
Zeldia (2)				*Ironus (4)*
Pseudacrobelus (2)				*Tripyla (3)*
Plectus (2)				*Coomansinema (4)*
Chiloplectus (2)				*Aporcelaimellus (5)*
Prismatolaimus (3)				*Labronema (4)*
Alaimus (4)				
Amphidelus (4)				

represent the majority of total nematode abundance however nematode community structure often varies widely within and between ecosystems and generalizations regarding nematode community composition between sites are often not clear [2]. It is reported that the predominant trophic group in cultivated land shifted between omnivorous and plant feeders implying that food chain path changed with agricultural processes [28]. They observed that Omnivorous nematodes could be markedly abundant in agroecosystems, depending on the annual crop rotation and tillage practices.

Table 2: Characteristics of nematode fauna across the Rabi season

Indices of nematode fauna	Bacterivores	Fungivores	Herbivores	Omnivores	Predators
Margalef index species richness	3.05	1.54	2.19	3.57	2.49
Shanon-Weiner's index (H')	2.78	1.19	0.46	0.36	0.93
Simpson's index	0.89	0.79	0.87	0.83	0.91
Pielou's evenness index	1.04	0.67	0.22	0.20	0.37
Maturity index MI for free living soil nematodes	1.84	5.05	3.25	5.44	2.86

Diversity indices of nematode fauna among the treatment plots is presented in Table 3. Maximum diversity of nematodes was observed in treatment plots planted to wheat and mustard in equal ratio (3:3). The diversity was minimum in wheat mustard planted in 2:4 ratio. Both wheat and mustard sole crop showed lower diversity when compared to mixed cropping. Nematode community evenness estimated from Pielou's evenness index was also affected by cropping combination. Evenness of nematode genera was higher in plots planted to wheat and mustard in different ratios while the sole cropped plots showed lowest evenness (Table 3). The maturity index (MI) ranged from 3.32 in wheat mustard plots planted in 3:3 ratio to 2.50 in wheat mustard plots planted in 4:2 ratio. The maturity index was always over 2 and is therefore considered high and the system was in a stable condition. A low value of MI is an indication of disturbance and a high one of stable conditions [20]. To generalize the nematode taxonomic richness, diversity and evenness were all greater under wheat mustard cropped in equal ratio. The bacterivores showed maximum evenness followed by fungivores and lowest evenness was showed by the herbivore and omnivore groups. Changes in trophic structure may be related to variation in food supply and

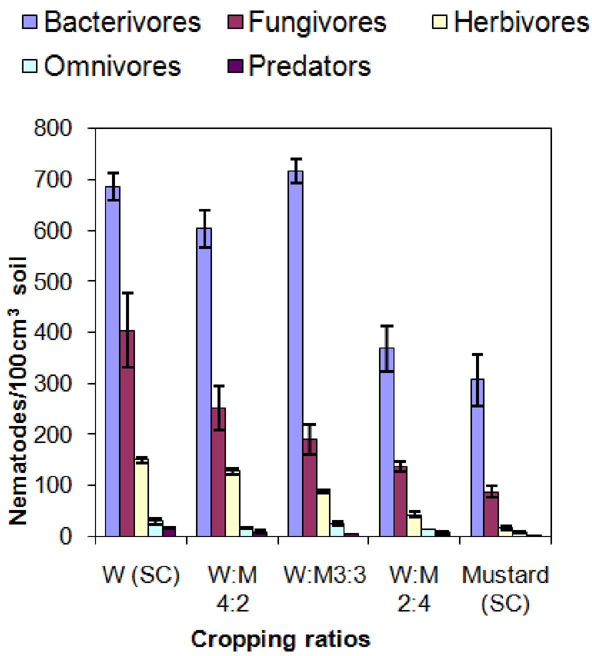

Fig.2. Distribution of nematode feeding groups under different cropping combination during Rabi season

ecosystem processes such as productivity, flow material and decomposition pathways [29], [30]. Trophic differences among nematode populations were observed to be indicative of variations in crop yields [31], [32] and also of decomposition and in particular N mineralization processes [33].

The cp classification allows calculation of the index maturity (MI) of a nematode fauna as the weighted mean frequency of the cp classes [20]. It expresses the proportional representation of nematode families as an index of environmental condition.

The variation in nematode abundance in different treatment plots is presented in Fig 2. Our data were well within the reported range from elsewhere. Nematode abundance in some European grasslands ranges between 0.5x 10^6 and 12 x 10^6 individuals /m^2 [34].

Table 3: Indices of nematode fauna under five cropping ratio plots

Indices of nematode fauna	W (sc)	W:M (4: 2)	W:M (3:3)	W:M (2:4)	M (sc)
Margalef index species richness	2.99	3.28	3.62	2.79	2.37
Shanon-Weiner's index (H')	2.37	2.41	2.52	2.29	2.39
Simpson's index	1.92	2.12	2.05	1.98	1.96
Pielou's evenness index	0.82	0.85	0.86	0.79	0.77
Maturity index MI for free living soil nematodes	3.07	2.50	3.32	2.79	2.63

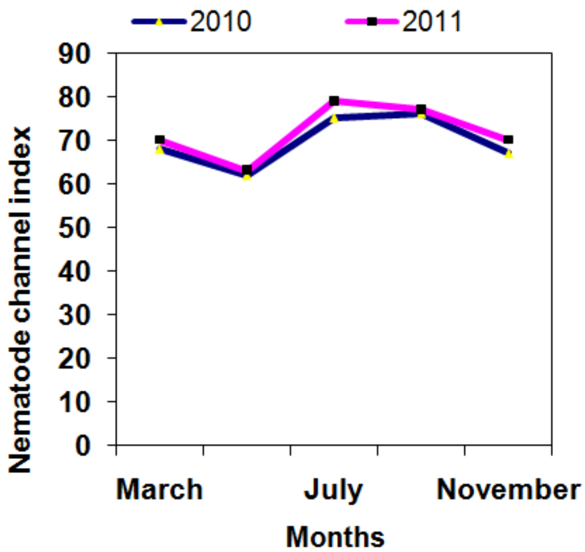

Fig.3. Temporal dynamics of nematode channel index (CI)

3.2 Effect of cropping ratio on bacterial – vs fungal based energy channels

The ratio of bacterial feeding to fungal feeding nematode abundances indicating the relative importance of the bacterial and fungal energy channels were responsive to treatment effects (Table 4). The plots planted to wheat and mustard in equal ratio showed the highest channel index of 0.79 indicating a faster bacterial driven decomposition in progress in such plots. Mustard sole crops showed a channel index of 0.77 and followed by wheat mustard sown in the ratio of 2:4 and then 4:2, the lowest channel index was recorded in plots planted to sole wheat crops (0.63).

Table 4: Nematode Channel Ratio (NCR)= B/ (B+F) for Rabi cropping season

S. No.	Treatment	NCR
1.	W (sc)	0.63
2.	W:M (4:2)	0.70
3.	W:M (3:3)	0.79
4.	W:M (2:4)	0.73
5.	M (sc)	0.77

Depending on which primary decomposers predominate the decomposition pathways or channels are described as bacterial (fast cycle) or fungal (slow cycle) [35],[36]. The bacterial feeding nematodes have higher carbon: nitrogen (C: N) ratio than their substrate [37] so that in consuming bacteria they take in more N than necessary for their body structure. The excess nitrogen is excreted as ammonia [38],[39]. The C: N ratio of fungal feeding nematodes is closer to that of their food sources. However for nematodes of both feeding habits a considerable portion of the C consumed is used in respiration. The N associated with respired C that is in excess of structural needs is also excreted. The excreted N is available in the soil solution for uptake by plants and by microbes. Because microbivorous nematodes exhibit a wide range of metabolic rates and behaviourial attributes the contribution of individual species to nitrogen cycling and soil fertility may vary considerably. Channel index also acts as an indicator of relative flow of substrate along bacterial and fungal decomposition pathways [25], [40]. In the present study mustard treated plots show considerable decrease in nematode abundance and at the same time they show highest channel index this indicates that presence of mustard prefers bacterivores than fungivores probably due to allopathic effect. The temporal dynamics of the channel index is presented in Fig. 3. It was maximum during the moist season and lower in dry summer and winter. This was probably that during the rainy season there was increase in enrichment opportunist bacterial-feeding nematodes.

IV. Conclusion

Results obtained indicate trophic responses to the planting ratio. Nematode abundance was higher in plots planted to wheat in comparison to plots planted to mustard in combination or sole mustard crop probably due to allelopathic effect of mustard root exudation. It has been reported that a significant decrease in nematode population under mustard crops occur due to presence of allelochemical, glucosinolate [41].

It is possible that different plant species sown in different proportion select for different combinations of nematode species which may in turn vary in their palatability to enchytraeids and microbe feeding nematodes. Herbivorous nematode populations also differed among treatments presumably reflecting differences among species in resource quality.

The present study of nematode diversity in a traditional agroecosystem under different cropping pattern and their inherent role in decomposition cycle will undoubtedly be of immense benefit towards the proper management of traditional farming in the Himalayas, primarily through asserting the right mix of crops and nematode population as through mixing of soils rich in nematodes along nutrient gradients in soil or identifying cropping combination that promote or inhibits beneficial nematodes i.e. bacterivores and fungivores which play a major role in decomposition cycle. Several such cropping combinations need to be tested for conclusive policy decisions.

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