Theme: Sustainable Agricultural Innovations for One Health and Environmental Resilience

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Soil Nematode Community Structure and Food-web Condition Across Altitudinal Zones in Wheat (*Triticum aestivum* L.) Fields of the Pir Panjal Region, Jammu and Kashmir, India

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ABSTRACT

Background: Soil nematodes occupy every trophic level of the belowground decomposer food web and since individual taxa differ predictably in feeding habit, colonisation ability and life-history strategy, nematode-derived faunal and metabolic indices have become one of the most widely used, cost-effective biological tools for monitoring soil and agroecosystem condition. Elevation gradients are known to reshape nematode abundance, diversity and community structure under natural Himalayan vegetation yet their influence within actively cultivated farmer-managed cereal fields where tillage, irrigation and fertilisation are superimposed on the natural elevation gradient and where soil physicochemical properties are measured in parallel rather than assumed remains poorly documented.

Methods: Soil nematodes were sampled from wheat fields across three altitudinal zones (Lower, Middle, Upper) at two sites Kalakote and Kotranka, Pir Panjal, Jammu and Kashmir, India. Twenty-four composite samples were collected at 15-30 cm depth. Thirty-five genera were identified and analysed using the NINJA platform to derive trophic composition, faunal indices, metabolic footprints and food-web condition complemented by PCA of the five major trophic groups.

Result: Total abundance (290-320 individuals/sample), genus richness (~25/sample), Shannon diversity (H′ 3.10-3.13) and the maturity-based indices (MI, MI2-5, ΣMI, PPI) did not differ significantly among zones (all $p>0.05$). All zones fell in the enriched, structured quadrant of the EI-SI plane with no significant among-zone EI or SI differences ($p = 0.204$, 0.116). Only the fungivore footprint differed significantly ($p = 0.027$) higher in Middle than Lower on post-hoc comparison. At Kalakote, pH, moisture, temperature, nitrogen and potassium showed significant differences among elevation zones; at Kotranka, only pH, phosphorus and potassium showed significant differences among zones. PCA of the five trophic groups resolved two axes explaining 73.85% of variance (PC1 = 43.26%, PC2 = 30.59%). These results indicate Pir Panjal wheat soils sustain nutrient-enriched, structurally developed nematode food webs across the elevation range studied with elevation significantly and consistently affecting soil properties but only a limited channel-specific effect on the nematode community.

Key words: Bioindicators, Food-web indices, NINJA, Pir panjal, Principal component analysis, Soil nematodes, Soil physicochemical properties, Wheat agroecosystem.

INTRODUCTION

Soil nematodes occupy every trophic level of the belowground food web and because different taxa differ predictably in feeding habit, colonisation ability and life-history strategy (the coloniser-persister/c-p, scale) nematode-assemblage composition integrates the cumulative effects of disturbance, resource availability and successional status over time. This underlies faunal indices MI, SI, EI, CI, BI, PPI as a standard, cost-effective biomonitoring tool (Bongers, 1990; Bongers and Ferris, 1999; Neher, 2001) reinforced by evidence that nematode faunal composition reflects soil functional state more sensitively than most other faunal groups (Ritz and Trudgill, 1999; Bardgett and van der Putten, 2014). A quantitative, weather-linked relationship with nematode population dynamics has also been demonstrated directly in Indian cropping systems (Batra and Gandhi, 2021).

Mountain agroecosystems add heterogeneity because elevation co-varies with temperature, moisture and management intensity over short distances. Wheat is the dominant cereal of the mid- and lower-elevation pir panjal belts and the plant-parasitic genera recorded in regional cereal soils (*Heterodera, Globodera, Pratylenchus,*

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Helicotylenchus, Tylenchorhynchus) are recognised wheat pathogens elsewhere in the Indian Himalaya. Nationally plant-parasitic nematodes account for over one-fifth of potential crop yield loss across Indian agriculture as a whole (Singh, 2015; Jain *et al*., 2007) a general country-level figure cited only for broad economic relevance not a measurement from this research. Elevation gradients reshape nematode diversity and metabolic footprint under natural Himalayan vegetation (Choudhary *et al*., 2021) and tillage/cropping-sequence practices alter nematode densities in managed fields elsewhere (Pankaj *et al*., 2006; Lenz and Eisenbeis, 2000). What has not been examined is whether comparable patterns occur within cultivated wheat fields when soil physicochemical properties are measured in parallel rather than assumed. This study used NINJA (Sieriebriennikov *et al*., 2014) with PCA to address that gap: (i) quantifying abundance, richness and diversity across local zones; (ii) describing trophic and life-strategy composition; (iii) comparing faunal indices and metabolic footprints; (iv) reporting soil properties of each zone and (v) examining trophic-group structure by PCA.

MATERIALS AND METHODS

Study area, design and experimental unit

This study was carried out at the Nematode Biodiversity and Genomics Research Laboratory, Department of Zoology, Baba Ghulam Shah Badshah University (BGSBU), Rajouri, Jammu and Kashmir over two consecutive Rabi (Wheat) seasons. Sampling spanned two geographically distinct sites, Kotranka and Kalakote; zone (Lower, Middle, Upper). At Kotranka the zones spanned upper; 1130-1200 m, middle; 1000-1100 m and lower; 810-950 m and at Kalakote, upper; 1070-1150 m, middle 950-1050 m and lower; 730-920 m. The experimental unit was the composite soil sample: four independent, non-repeated composites per zone per site (12/site, 24 overall; n = 8/zone pooling sites) so ANOVA compares independent locations not repeated measures. Each composite pooled 200 to 450 g soil from village fields (48 villages overall) at 15-30 cm depth from farmer-managed, rainfed wheat fields during active growth and ripening; field-level cultivar, fertiliser and pesticide records were incomplete a limitation addressed in the discussion.

Soil physicochemical analysis

For each zone at each site soil pH was determined potentiometrically, moisture was expressed as a soil water index (SWI) and temperature was recorded in situ at sampling depth. Available nitrogen was estimated by the alkaline potassium permanganate method (Subbiah and Asija, 1956) available phosphorus by Olsen's sodium bicarbonate method (Olsen *et al*., 1954) and available potassium by flame photometry (Jackson, 1973); these are standard procedures for Himalayan agricultural soils of this kind (Kumar, 2023).

Nematode extraction, identification and functional classification

Nematodes were extracted by Cobb's sieving and decanting technique followed by a modified Baermann tray method. Each suspended sample was first poured through a coarse sieve (2 mm pore size) and the filtrate then passed through a 300-mesh sieve (53 µm pore size) then transfer to a Baermann funnel for 24-hour active-nematode migration. Recovered nematodes were heat-killed and fixed in hot 4:1 formalin-acetic acid, dehydrated through a graded glycerine-ethanol series and mounted for identification and morphometric measurement. Identification was to genus level consistent with NINJA's taxonomic input requirement; feeding habit can vary among species within a genus (*e.g*., *Ditylenchus, Aphelenchoides*) so trophic assignments reflect each genus's predominant, literature-documented feeding habit rather than species-specific confirmation a limitation discussed below.

Nematode functional and metabolic indices and PCA

Genus-level abundance data were analysed on the NINJA platform (Sieriebriennikov *et al*., 2014) which assigns feeding-type classification (Yeates *et al*., 1993) and coloniser–persister/plant-parasite (c-p/p-p) values (Bongers, 1990; Bongers *et al*., 1995) to compute MI, MI2-5, ΣMI, PPI, EI, SI, CI, BI and trophic-group metabolic footprints (Ferris *et al*., 2001; Ferris, 2010) with EI–SI plotted on the four-quadrant diagnostic graph. Sample-level abundances of the five major trophic groups were standardised to z-scores and subjected to Principal Component Analysis by eigen-decomposition of the covariance matrix; eigenvalues, loadings and scores are reported in Table 5 and 6.

Statistical analysis

Abundance, richness, diversity, evenness, all NINJA-derived indices/footprints and soil properties were compared among zones (n = 8/zone for nematode variables, pooling sites; n = 4/zone/site for soil properties) by one-way ANOVA with homogeneity of variance checked and percentage data arcsine-transformed where applicable; Tukey's HSD separated soil-property means and Bonferroni-corrected pairwise t-tests separated zone means for the fungivore footprint, the only nematode variable with a significant overall ANOVA. Significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Nematode abundance, richness and diversity

Thirty-five genera spanning herbivore, fungivore, bacterivore, predator and omnivore feeding groups were recorded, ten most abundant summarised in Table 1. Total abundance did not differ significantly among zones (293±52, 320±30, 290±59 individuals/sample; Lower/Middle/Upper, n = 8/zone; p = 0.42; Table 2, Fig 1a), nor genus richness (p = 0.90; Fig 1b) Shannon diversity (p = 0.83; Fig 1c) or evenness (p = 0.073; Table 2).

Trophic composition and faunal indices

Herbivores were numerically dominant (34.0-37.6%) followed by bacterivores (26.6-30.9%) fungivores (12.0-19.1%) omnivores (9.9-12.9%) and predators (7.0-9.8%; Fig 2) with *Pelodera, Ditylenchus, Tylenchorhynchus, Hoplolaimus, Aphelenchoides Longidorus* consistently most abundant (Table 1). MI, MI2-5, ΣMI and PPI did not differ significantly among zones (Table 2, Fig 3) nor did channel index which showed a non-significant tendency toward higher Middle-zone values (15.76, 27.25, 11.87; p = 0.067;

Table 1: Ten most abundant nematode genera (mean individuals/sample) by elevation zone, of 35 genera recorded; c-p/p-p classification follows Bongers (1990), Bongers *et al.* (1995) and Yeates *et al.* (1993) as implemented in NINJA.

Genus	Lower	Middle	Upper	Overall mean
Pelodera	12.6	9.2	13.4	11.8
Ditylenchus	8.9	13.4	10.2	10.8
Tylenchorhynchus	12.0	9.6	8.5	10.0
Hoplolaimus	7.5	13.9	8.5	10.0
Aphelenchoides	9.8	14.9	5.1	9.9
Longidorus	8.9	12.4	8.4	9.9
Prionchulus	7.0	11.8	10.8	9.8
Merlinius	11.9	7.0	10.0	9.6
Heterodera	12.0	5.6	10.9	9.5
Aphelenchus	6.9	14.0	7.4	9.4

Table 2: Nematode abundance, diversity, faunal indices and metabolic footprints (mean±SD, n = 8 per zone) by elevation zone, pooling both sites.

Metric	Lower	Middle	Upper	ANOVA p
Total abundance (ind./sample)	293.4±52.3	320.1±29.5	290.4±58.6	0.416
Genus richness	25.25±2.38	24.75±1.67	24.88±2.75	0.904
Shannon diversity (H′)	3.104±0.096	3.131±0.071	3.102±0.136	0.833
Pielou's evenness (J′)	0.962±0.015	0.976±0.003	0.967±0.012	0.073
Maturity index (MI)	2.73±0.24	2.77±0.14	2.76±0.13	0.904
Maturity index (2-5)	3.20±0.21	3.17±0.17	3.35±0.15	0.114
Sigma maturity index	2.81±0.15	2.84±0.11	2.83±0.08	0.893
Plant parasitic index	2.93±0.08	2.97±0.15	2.95±0.14	0.867
Channel index	15.76±9.98	27.25±19.48	11.87±4.28	0.067
Basal index	10.48±4.18	10.76±4.91	7.10±2.66	0.154
Enrichment index	73.06±12.79	71.49±15.66	82.02±6.50	0.204
Structure index	85.00±4.80	85.28±5.06	89.47±3.77	0.116
Composite footprint	592.28±173.28	516.24±103.34	513.11±135.37	0.455
Fungivore footprint	4.81±2.27	8.44±3.03	6.79±2.00	**0.027**
Omnivore footprint	170.34±85.07	140.94±56.37	94.17±51.04	0.089

Bold p-values indicate significance (one-way ANOVA, p<0.05).

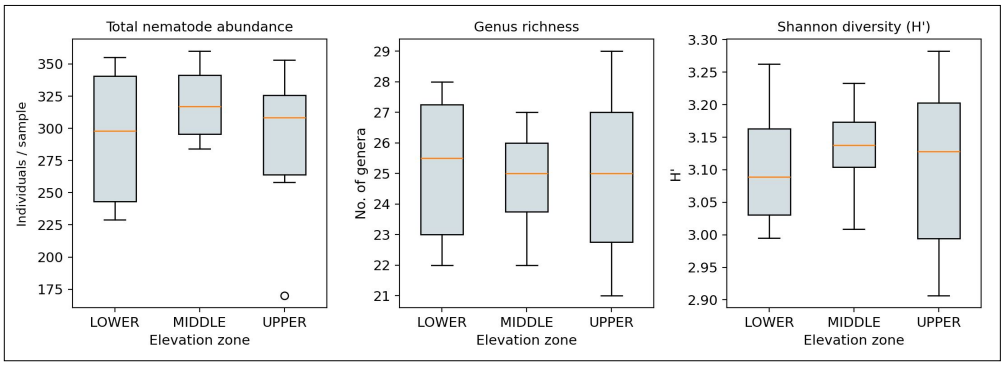

Fig 1: Total nematode abundance (a), genus richness (b) and Shannon diversity (c) by elevation zone.

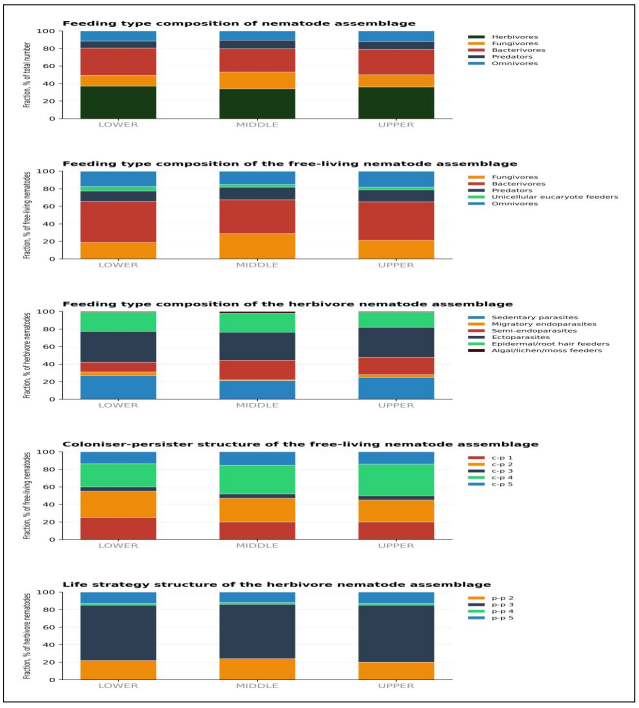

Fig 2: Trophic (feeding-type) composition of the nematode assemblage by elevation zone.

Table 2, Fig 4). Fungivore footprint was the only significant nematode variable (4.81, 8.44, 6.79; p = 0.027; Table 2); Bonferroni-corrected pairwise comparisons showed this was attributable mainly to Middle exceeding lower (p ≈ 0.02) upper not differing from either (p>0.05). c-p group 4 taxa comprised 26.3%, 32.6% and 35.9% of the free-living assemblage respectively (Table 3, Fig 6) paralleling without independent testing, the upper zone's numerically highest Structure Index and lowest Basal Index/ omnivore footprint (Table 2).

Food-web condition

EI and SI placed all zones in the maturing, nitrogen-enriched, structured quadrant; neither differed significantly (EI: 73.06, 71.49, 82.02, p = 0.204; SI: 85.00, 85.28, 89.47, p = 0.116; Table 2, Fig 5, Fig 7).

Soil physicochemical properties

Several soil physicochemical properties differed significantly among elevation zones, with the pattern of significant differences varying between the two study sites (Table 4, Fig 12). At Kalakote pH, moisture and temperature were significantly higher in the Lower zone (pH: 7.75/6.75/5.75, p = 0.025; moisture: 0.50/0.35/0.20, p = 0.012; temperature: 28.5/17.5/7.5°C, p = 0.032) while nitrogen and potassium were higher in the Upper zone (210/275/375 kg/ha,

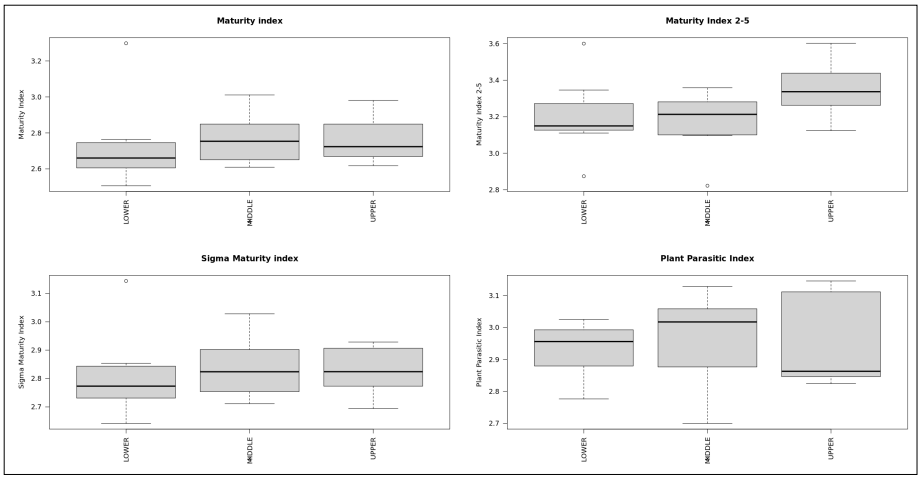

Fig 3: Maturity index, maturity index 2-5, sigma maturity index and plant parasitic index boxplots by elevation zone.

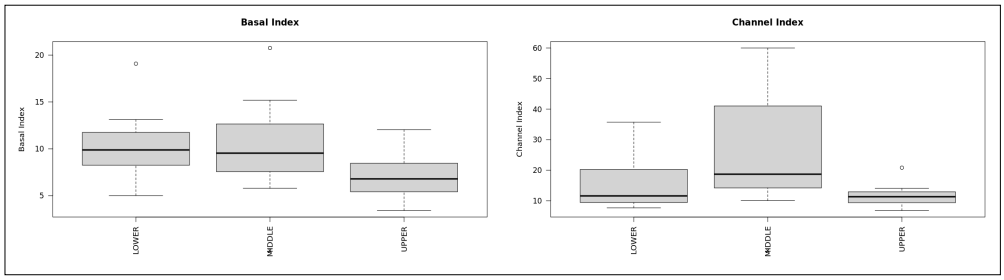

Fig 4: Basal index and channel index boxplots by elevation zone.

p = 0.027; 52.5/62.5/72.5 kg/ha, p = 0.025). At Kotranka pH was higher in the lower zone (6.95/6.65/6.05, p = 0.032), while phosphorus and potassium were higher in the Upper zone (36/62.5/91 kg/ha, p = 0.026; 92.5/170/230 kg/ha, p = 0.013); moisture, temperature and nitrogen did not differ significantly (p>0.05).

Principal component analysis

The first two components explained 73.85% of variance (PC1 = 43.26%, PC2 = 30.59%; Table 5, Fig 8). PC1 loadings were positive for bacterivores (0.540) omnivores (0.520) and plant parasites (0.510) weakly positive for fungivores (0.136) negative for predators (-0.399); PC2 loadings were strongly positive for fungivores (0.694) and predators (0.475) negative for omnivores (-0.372) (Table 5). The scatter plot (Table 6, Fig 9) separates Kotranka and Kalakote broadly along PC1; the biplot (Fig 10) and contribution plot (Fig 11) confirm bacterivores (29.3%), omnivores (27.2%) and plant parasites (26.0%) as PC1's dominant contributors, fungivores dominating PC2, consistent with the elevated Channel Index and fungivore footprint in the Middle zone.

Elevation and community stability

The principal finding is that abundance, richness, diversity and the maturity-based indices did not differ significantly among zones (Table 2) despite soil properties changing significantly and consistently with zone at both sites (Table 4, Fig 12). This is not without precedent: managed-agroecosystem nematode communities often respond more to management history than background environmental gradients (Freckman and Ettema, 1993; Yeates and Bongers, 1999) and bacterivore/fungivore-mediated nutrient cycling feeds back on the very soil properties changing here (Bouwman *et al*., 1994; Ferris *et al*., 1995) potentially dampening a simple soil to nematode relationship. Farm management-irrigation, fertiliser, pesticide, tillage may plausibly buffer the community but as unrecorded, this remains untested. The pattern contrasts with unmanaged Himalayan gradients where nematode abundance, diversity and metabolic footprint decline significantly with elevation (Afzal *et al*., 2021; Choudhary *et al*., 2021; Kergunteuil *et al*., 2016), at least consistent with cultivation having a stabilising influence.

Channel-specific effects

The only significant nematode variable was the fungivore footprint, higher in Middle than Lower (p ≈ 0.02), Upper intermediate. The non-significant channel index tendency (p = 0.067) is consistent with without establishing a greater fungal decomposition role at that zone (Yeates, 2003; Ferris and Bongers, 2006) perhaps reflecting favourable moisture for genera like *Ditylenchus* and *Aphelenchoides*-untested here. Fertility-linked shifts concentrated in specific trophic channels not the whole community occur elsewhere in Indian cereal systems

Table 3: Coloniser-persister (c-p) structure of free-living nematodes and life-strategy (p-p) structure of herbivore nematodes (%), by elevation zone.

c-p class	Lower	Middle	Upper
c-p 1	21.0	18.6	25.1
c-p 2	33.0	32.9	23.9
c-p 3	8.7	9.0	8.2
c-p 4	26.3	32.6	35.9
c-p 5	11.0	6.9	6.9
p-p class	Lower	Middle	Upper
p-p 2	22.5	24.3	22.4
p-p 3	69.2	64.6	69.0
p-p 4	0.0	0.0	0.0
p-p 5	8.3	11.1	8.6

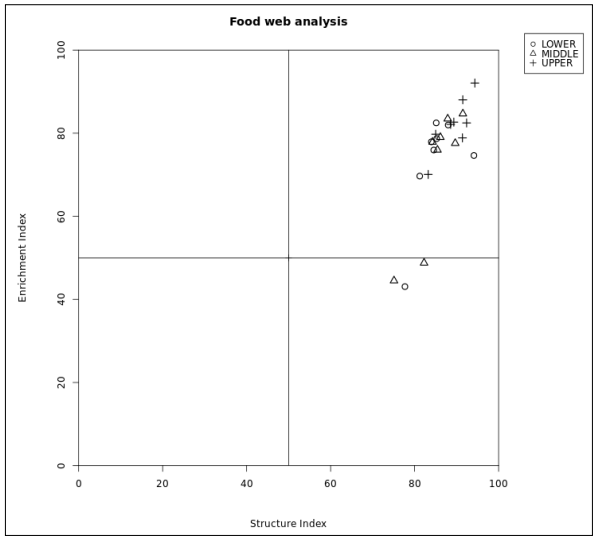

Fig 5: Food-web diagnostic (Enrichment index-structure index) scatter plot of individual samples by elevation zone.

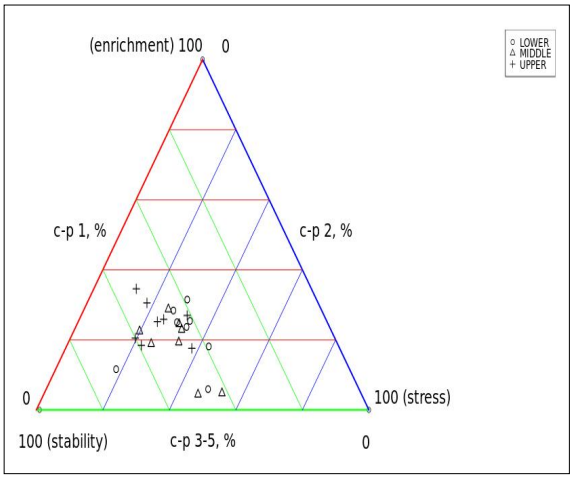

Fig 6: Coloniser-persister (c-p) triangle showing the relative proportions of c-p groups by elevation zone.

(Liu *et al*., 2015; Pan *et al*., 2010) broadly consistent with this pattern.

Food-web condition

All zones remained in the maturing, nitrogen-enriched, structured quadrant of Ferris *et al*. (2001) despite significant soil differences (Table 4, Fig 12) suggesting the food web's functional character may be comparatively robust to these gradients, consistent with nematode faunal profiles as integrators of management and organic-matter status over time (Ritz and Trudgill, 1999; Bardgett and van der Putten, 2014) rather than single soil variables and with recent syntheses treating nematode-based indices as integrated, multi-variable signals rather than single-nutrient proxies (Du Preez *et al*., 2022); confirming this would need soil and nematode data tested in one combined model, beyond the present scope.

Multivariate structure

The scatter plot (Fig 9) and biplot (Fig 10) show Kotranka and Kalakote separating broadly along PC1, the contribution plot (Fig 11) confirming bacterivores,

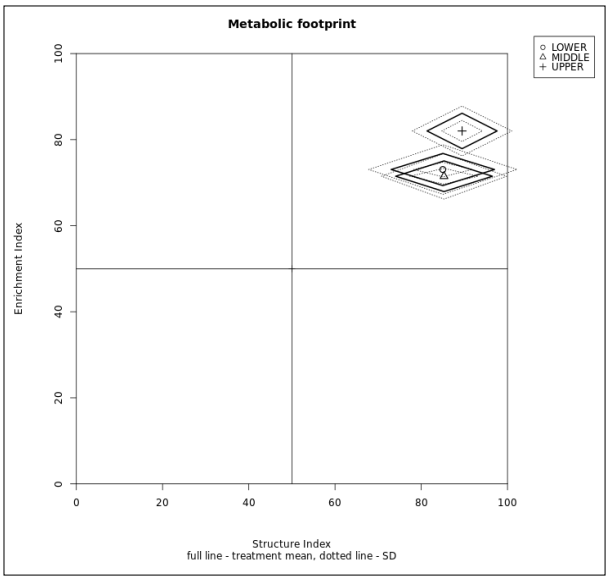

Fig 7: Metabolic footprint diamond plot on the enrichment index-structure index plane.

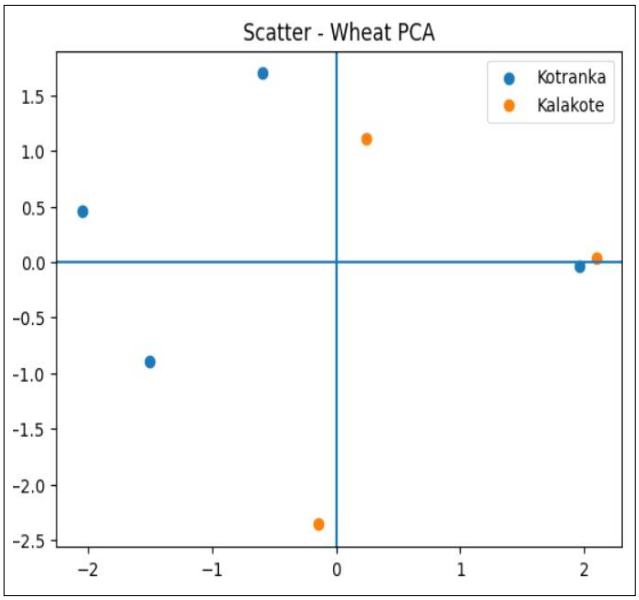

Fig 9: PCA scatter plot (PC1 vs PC2) of samples by site (Kotranka, Kalakote).

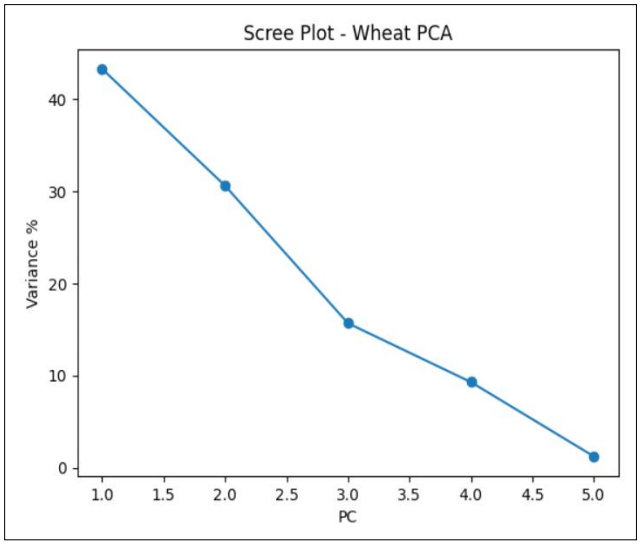

Fig 8: Scree plot showing the percentage variance explained by each principal component of nematode trophic-group composition.

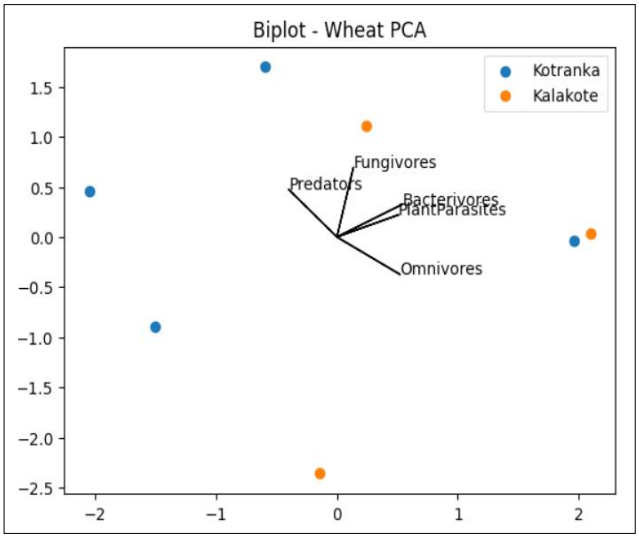

Fig 10: Biplot of samples and nematode trophic-group loadings on the first two principal components.

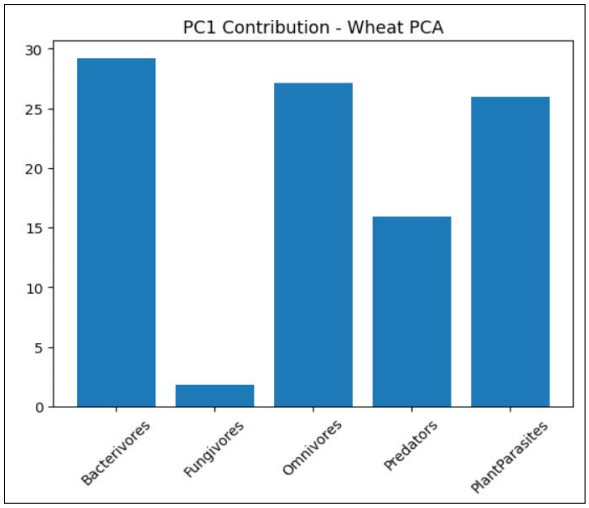

Fig 11: Contribution of the five nematode trophic groups to PC1.

omnivores and plant parasites as its dominant contributors and fungivores dominating PC2 (Table 5), PC1 a general abundance axis, PC2 an ecological-interaction axis, comparable to managed cereal soils elsewhere where bacterivore/omnivore abundance co-varies most strongly with nematode biomass (Yeates and Bongers, 1999).

Plant-parasitic pressure

Herbivores including *Heterodera, Meloidogyne, Pratylenchus* and *Hoplolaimus*, exceeded a third of abundance in every zone, PPI not varying significantly with elevation-consistent rather than elevation-specific. This study did not quantify plant-parasitic densities against yield or damage/pathogenicity; the oft-cited capacity of predatory/omnivorous nematodes to suppress plant-parasitic populations in intact food webs (Bongers and Ferris, 1999; Ahmad, 1990; Bilgrami and Brey, 2005)

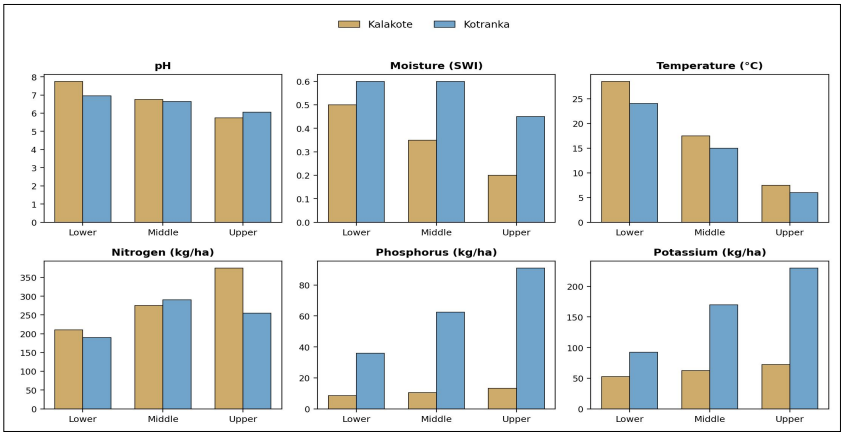

Fig 12: Soil pH, moisture, temperature, nitrogen, phosphorus and potassium by elevation zone at Kalakote and Kotranka.

Table 4: Soil physicochemical properties (mean±SE, n = 4 per zone) by elevation zone at the two study sites.

Parameter	Lower	Middle	Upper	F-value	P-value
Kalakote					
pH	7.75±0.25^{a}	6.75±0.25^{ab}	5.75±0.25^{b}	15.87	**0.025**
Moisture (SWI)	0.50±0.00^{a}	0.35±0.05^{ab}	0.20±0.00^{b}	27.00	**0.012**
Temperature (°C)	28.5±3.5^{a}	17.5±2.5^{ab}	7.5±2.5^{b}	13.37	**0.032**
Nitrogen (kg/ha)	210±10^{a}	275±25^{ab}	375±25^{b}	13.35	**0.027**
Phosphorus (kg/ha)	8.5±0.5	10.5±0.5	13.25±1.25	8.273	0.060
Potassium (kg/ha)	52.5±2.5^{a}	62.5±2.5^{ab}	72.5±2.5^{b}	15.85	**0.025**
Kotranka					
pH	6.95±0.05^{a}	6.65±0.15^{ab}	6.05±0.15^{b}	13.26	**0.032**
Moisture (SWI)	0.60±0.10	0.60±0.20	0.45±0.15	0.31	0.754
Temperature (°C)	24±4	15±3	6±2	8.379	0.059
Nitrogen (kg/ha)	190±10	290±40	255±25	3.323	0.173
Phosphorus (kg/ha)	36±6^{a}	62.5±7.5^{ab}	91±7^{b}	15.49	**0.026**
Potassium (kg/ha)	92.5±7.5^{a}	170±10^{ab}	230±20^{b}	25.63	**0.013**

Means sharing a superscript letter within a row do not differ significantly (Tukey's HSD, $p<0.05$); bold P-values indicate significance (one-way ANOVA).

Table 5: Eigenvalues, explained variance and factor loadings of the five nematode trophic groups on the principal components of the integrated PCA.

Component	Eigenvalue	Variance %	Cumulative %		
PC1	2.524	43.26	43.26		
PC2	1.784	30.59	73.85		
PC3	0.914	15.66	89.51		
PC4	0.541	9.28	98.79		
PC5	0.071	1.21	100.00		
Trophic group	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
Bacterivores	0.540	0.325	0.478	-0.117	0.600
Fungivores	0.136	0.694	-0.190	0.644	-0.221
Omnivores	0.520	-0.372	0.450	0.224	-0.581
Predators	-0.399	0.475	0.535	-0.408	-0.404
Plant parasites	0.510	0.221	-0.497	-0.596	-0.299

Table 6: Principal component scores for an illustrative subset of samples.

Site	Zone	Sample	PC1	PC2
Kotranka	Upper	S1	-1.508	-0.897
Kotranka	Upper	S2	-2.048	0.459
Kotranka	Middle	S5	-0.599	1.697
Kotranka	Lower	S9	1.957	-0.036
Kalakote	Upper	S1	-0.144	-2.362
Kalakote	Middle	S5	0.243	1.109
Kalakote	Lower	S9	2.098	0.030

is noted only as background not a demonstrated outcome here.

LIMITATIONS

Genus-level identification may obscure within-genus feeding-habit variation (*e.g.*, *Ditylenchus, Aphelenchoides*). Field-level cultivar, fertiliser, pesticide and irrigation records were incomplete so management's buffering role remains untested.

CONCLUSION

Across Lower, Middle and Upper zones of wheat fields at two Pir Panjal sites, total nematode abundance, genus richness, diversity and the principal maturity and plant-parasite-based faunal indices did not differ significantly and every zone was classified within the same nitrogen-enriched, structured food-web quadrant. By contrast, soil physicochemical properties showed parameter-and site-specific differences among elevation zones: pH differed significantly at both sites; moisture, temperature, nitrogen and potassium differed significantly at Kalakote, whereas phosphorus and potassium differed significantly at Kotranka. The only significant nematode variable was the fungivore footprint, higher in the middle than lower zone. A complementary PCA resolved a dominant axis linking bacterivore, omnivore and plant-parasitic abundance (43.3% of variance) and a secondary axis distinguishing fungivore and predator-rich samples (30.6%). These findings suggest the physicochemical environment of Pir Panjal wheat fields changes markedly with local elevation while the resident nematode community's functional architecture remains comparatively stable, supporting nematode faunal analysis as a useful though not alone sufficient, complement to direct soil testing in Himalayan wheat agroecosystems. Whether agronomic management explains this stability and whether it holds across seasons remains an important direction for future research.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions.

Informed consent

This study involved only soil sampling and laboratory extraction of soil nematodes from farmer managed wheat fields, it did not involve human participants or vertebrate animals and no ethical approval was therefore required.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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