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Temporal Dynamics of Soil Macrofauna and Their Association with Macronutrient Availability in Wheat-Based Agroecosystems of Punjab, Pakistan

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Soil macrofauna are dynamic ecosystem engineers that affect the structure of soil, the cycling of nutrients, and the productivity of crops. This study used 64 samples of soil from wheat fields in two tehsils (Jaranwala and Samundri) of Faisalabad District, Punjab, Pakistan, during the 2023–24 wheat season. Macrofauna were collected fortnightly using monolith sampling (25 × 25 × 30 cm), hand-sorting, and Berlese-Tullgren funnel extraction methods. Soil samples were examined for macronutrients (N, P, K, and S) using standard chemical methods (Kjeldahl, Bray I, ammonium acetate). A two-way ANOVA exposed a significant effect of month ($p = 0.030$) and tehsil × month interaction ($p = 0.053$) on nitrogen levels, with Isoptera diversity differing significantly between tehsils ($p = 0.011$). Correlation analysis showed a positive association between Haplotaxida and nitrogen, moderate associations between Coleoptera and both potassium and sulfur. No significant trends were found for phosphorus. The results indicate that macrofaunal activity varies seasonally, especially in earthworms, which are crucial for the mineralization of nitrogen. The findings emphasize the importance of soil macrofauna in nutrient dynamics, as well as the need to incorporate conservation techniques into wheat agroecosystems to promote soil fertility and sustainability.

Keyword: Soil macronutrients, soil macrofauna, Nutrient cycling, Wheat agroecosystem, Conservation agriculture

Introduction

Soil is a fundamental natural resource essential for maintaining global food security and agricultural sustainability (Toor et al., 2021). Soil productivity and fertility largely depend on the availability of key nutrients that govern plant development from seedling emergence to maturity (Kirkby et al., 2023). Macronutrients such as nitrogen (N), phosphorus (P), sulfur (S), and potassium (K) are needed in comparatively large quantities and play an essential role in the physiological and biochemical processes of plants (Javed et al., 2022). They significantly affect various functions, including chlorophyll synthesis, enzyme activation, root growth, and reproductive development, which together determine crop yield and quality(Ahmed et al., 2023; Wang et al., 2020).

Wheat *(Triticum aestivum)*, globally one of the most cultivated cereal crops, plays an essential role in human nutrition by contributing roughly 20% of the daily dietary and protein intake (Rehman et al., 2018). Wheat accounts for 98% of Pakistan's total grain production, which makes it Pakistan's most important food crop. Wheat was grown on around 9.04 million hectares during the 2022–2023 period, which amounts to 1.9% of the national GDP and 8.2% of the agricultural GDP (Islam et al., 2023). As global requirement for food continues to rise, driven by climate variability and population growth, through sustainable and biologically informed practices can increase wheat production becomes imperative (Gliessman et al., 2022).

While chemical fertilizers are extensively used to meet the nutrient requirements of wheat crops, dependence on chemical inputs has led to nutrient imbalances, soil degradation, and to overcome long-term fertility issues (Panhwar et al., 2019). An economically viable alternative in this situation is the biologically mediated nutrient cycling, especially by soil macrofauna (Franco et al., 2020). Soil macrofauna, such as earthworms, termites, ants, and ground beetles, play a crucial role in the mobilization of nutrients, the decomposition of organic material, and the structural improvement in soil, thereby contributing to the overall soil health and also in the productivity (Bagyaraj et al., 2016).

Earthworms (Haplotaxida) perform a critical role in soil ecosystems by enhancing the structure, aeration, and also the nutrient availability (Le Bayon et al., 2017). Their burrowing and feeding activities produce casting that rich in nutrients which improves the fertility of the soil. These castings also stimulate the microbial activity (Blouin et al., 2013). Their burrowing activities and the feeding promote the nutrient cycling by speeding up the nitrogen mineralization process, while at the same time enhancing the structure of soil, aeration, and microbial processes (Eriksen-Hamel & Whalen, 2007).

Termites (Isoptera) are another significant soil macrofauna, particularly abundant in the tropical and the subtropical areas (Ayuke et al., 2011). They have strong influence on the soil water infiltration and retention while fostering the growth of the stable microaggregates by using the construction of biogenic structures like galleries and the nests (Jouquet et al., 2011). Moreover, termite mound activity has been established to improve the water retention and alter the soil hydraulic conductivity in seasonally dry habitats, confirming their significance as a ecosystem engineers (Ashton et al., 2019).

Ants (Hymenoptera), with an estimated 25,000 species worldwide, are extremely diverse and play various functions in the soil ecosystems (Farji-Brener & Werenkraut, 2017). They are both the predators and the detritivores, because of the way they nest, soil particles migrate both horizontally and vertically, which promotes the heterogeneity (Shukla et al., 2016). In arid and the semi-arid areas, ants may execute their roles similar to those of the earthworms, forming macropores and improving the soil hydrology (Shukla et al., 2016).

Ground beetles (Coleoptera) are the generalist predators and significant components of the agroecosystems (Eyre et al., 2013). In addition to controlling the pests, numerous species have an indirect influence on the nitrogen cycling by feeding on soil microbes and redistributing organic matter (Gailis & Turka, 2014). Their seasonal movement among margins of the crops and in fields has implications for both pest regulation and the soil organic activity (Eyre et al., 2013).

Ground spiders (Araneae) act as an important predators in the soil ecosystems, monitoring the number of arthropods (Nyffeler & Birkhofer, 2017). Their activities indirectly contribute to the soil's nutrient cycle and also promotes the biodiversity. Moreover, the respiration of the soil and the expansion of the plants are enhanced by the use of spider waste and prey remnants (Wilder et al., 2025).

Centipedes (Chilopoda) are the fast-moving, nocturnal predators inhabiting in the soil and also in the litter layers, where they feed on the insects and other invertebrates (Lewis, 1981). Their predatory role supports to regulate the soil pest populations and to maintain the ecological balance in agroecosystems (Voigtländer, 2011). Additionally, centipedes are considered as the useful bioindicators of the soil quality and the environmental variation.

Though the ecological functions of the soil macrofaunal communities have been well described, less is known about how they particularly disturb the macronutrients (P, K, N, and S) availability in wheat-based agroecosystems, mainly in the contexts of South Asian countries like Punjab, Pakistan (Majeed et al., 2018). It is critical to recognize these associations, particularly in the areas with intense wheat farming and high fertilizer use (Khan et al., 2024). Empirical evidence linking macrofauna abundance to the key soil fertility indicators could pave the way for the integrated nutrient management practices that are both economically and environmentally sustainable (Barros et al., 2002).

Therefore, the current research aims to quantify the diversity of the dominant soil macrofaunal groups (earthworms, ants, and beetles) and investigate their relationship with soil phosphorus, potassium, nitrogen, and sulfur levels in wheat-based agroecosystems of Punjab, Pakistan. This examination seeks to demonstrate the biological contributions to the dynamics of nutrients, offering insights that could improve their sustainability and productivity of wheat farming systems through natural soil ecosystem processes.

MATERIALS AND METHODS

This study was conducted to evaluate the relationship between soil macrofauna and soil nutrients in wheat *(Triticum aestivum)* fields under diverse ecological conditions. Field sampling was accomplished during the 2023–2024 wheat growing season at two tehsils, agricultural sites located in Faisalabad District, Punjab, Pakistan.

Study Area

Faisalabad is situated in the province of Punjab, Pakistan (31°25′N, 73°05′E) at an elevation of about 186m above sea level. The region has semi-arid subtropical climate has a considerable impact on soil biological activity and nitrogen cycling. The average rainfall is between 300 and 500 mm annually, with the majority of it falling during the monsoon season (July to September). During the period of wheat growth (January-April), winter temperatures typically ranges from 5°C to 20°C, with low moisture content. These weather patterns have a considerable impact on the availability of the nutrients and the periodic rhythms of the soil macroinvertebrates. To offer the consistent background information for the field observations, environment statistics were confined to the study period (January–April 2024) and also acquired from the Pakistan Meteorological Department (PMD, 2024) (Bano et al., 2024). The extensive farming practices in this region make it a major wheat producing zone.

Sampling Methods

Site Selection

Wheat fields with a consistent history of fertilizer use were selected to investigate the effects of nitrogen fortification on the communities of the soil macroinvertebrates. This method has been developed to explore that how increased the soil nutrient concentrations impact the ecological patterns in the agroecosystems that are based on wheat. Only those regions with the similar soil characteristics and a consistent agricultural management history were included in the study to account for the variation.

Sampling procedure

At each sampling location, soil monoliths or cores were dug up to the depth of the 30 cm, or deeper when required, to fully cover the root zone. This method was essential for the accurate assessing of both the soil characteristics and population of the macroinvertebrates. A monolith size of 25 × 25 × 30 cm was determined to be acceptable for the macroinvertebrate sampling in the wheat fields. Samples of the soils were collected at the end of the rainy season, overlapping with the peak activity of the soil macroinvertebrates. Sampling in the wheat agroecosystems was conducted between January and April, while the crop was in its active growing period. Samples of the soil macrofauna are collected fortnightly. Four soil samples were taken from each site during each of the sampling periods, for a total of 64 samples from both sites distributed over eight sampling intervals. For estimating the variations in the soil macrofaunal groups and also the nutrient dynamics, this sample method provides a consistent geographical and the temporal representation.

Sampling of Soil for Macronutrients

To accomplish analysis for the macronutrients, which was crucial for the recognition of the nutrient profile of the soil and how it affects the macroinvertebrate communities, a 500 g soil sample was taken from each sampling site. Each sample was labelled with the appropriate information, including the site type and the field of the crop. The obtained samples were packed in the labelled plastic bags and delivered to the lab for further analysis of macronutrients. Soil chemical analysis followed the conventional techniques, focusing on four key macronutrients, that is the accessible phosphorus, potassium, nitrogen, and sulphur. To evaluate the nutrient accessibility, each sample was observed for these specific characteristics.

Soil Nutrients Analysis and Macroinvertebrate Handling

Soil Sample Preparation

To avoid the loss of tnutrients and preserve their integrity for the accurate organic examination. The collected samples were allowed to dry out in the shade and also in the air. Before the laboratory testing, the samples were sieved to ensure the standardization and eliminate the debris after they had dried.

Analysis for Macronutrients

The levels of nitrogen, phosphorus, sulfur and potassium were evaluated in each soil sample to measure the overall composition of the nutrients (table 1). These procedures are stick to the standardized analytical approaches as described by (Chi et al., 2017), and make sure the reliability and precision of the results.

Table 1: Analysis for Macronutrients

Chemical Property	Description	Soil Test Technique
Sulfur (S)	Available S	Ammonium acetate
Nitrogen (N)	Total N	Micro-Kjeldahl method
Phosphorus (P)	Available P	Bray I
Potassium (K)	Exchangeable K	Ammonium acetate

Handling of Macroinvertebrates

Macroinvertebrates were extracted from the wheat crops by using a combination of hand-sorting and the Berlese-Tullgren funnel extraction methods (72-hour extraction period for complete recovery). The samples were shifted into the labelled containers filled with the suitable preservative solutions, which were: 96% ethanol for the earthworms and 76% ethanol for the other macroinvertebrates. The institutional ethical rules for the study involving invertebrates have been adhered to the appropriate handling techniques. Each container was labelled properly to show the specific sampling location and the origin of the macroinvertebrates, ensuring precise documentation and the traceability for laboratory processing.

Statistical Analysis

Data analysis was conducted by using the R (version 4.5.1). A two-way ANOVA was used to test the effects of tehsil (Samundri, Jaranwala) and sampling month (January–April) on macrofauna abundance (by order) and on nutrient concentrations (N, P, K, S). Levene's test evaluated the homogeneity of variances. Correlation analysis and scatter plots were made to explore the relationships between the abundance of macrofauna and nutrient levels. Statistical significance was considered at $p < 0.05$.

Ethical considerations

This research engaged in the non-lethal sampling of soil macroinvertebrates (e.g., beetles, earthworms, ants, and termites) using standard ecological procedures, such as Berlese-Tullgren funnel extraction and hand sorting techniques. Every fieldwork was carried out under the policies of the institute on the moral treatment of invertebrates. They did not injure or target any protected or endangered species. Soil samples were collected exclusively from cultivated wheat fields with the verbal consent of landowners, and all efforts were made to reduce disturbance to the agroecosystem. Laboratory handling and specimen preservation (using ethanol) were performed in compliance with accepted entomological practices to ensure specimen integrity and ethical research conduct.

RESULTS

This study examined the abundance of soil macrofauna and its association with macronutrient concentrations (N, P, K, and S) in wheat-based agroecosystems across two tehsils, that is, Samundri and Jaranwala, in the district of Faisalabad, during the 2023–2024 cropping season.

A two-way ANOVA was executed to evaluate the effect of Tehsil, Month, and their interaction on the abundance of soil macrofauna groups, including Araneae, Coleoptera, Hymenoptera, Chilopoda, Haplotaxida, and Isoptera. Among them, only Isoptera showed a statistically significant difference between the two tehsils ($F_{1,24} = 7.506$, $p = 0.011$), with mean abundance consistently higher in Samundri than in Jaranwala across all months (as shown in Figure 1). This suggests that Isoptera abundance is influenced by local environmental or soil fertility conditions that differ between the two tehsils.

In contrast, the effects of Tehsil, Month, and their interaction were not significant for the other taxa ($p > 0.05$ in all cases). Visual inspection of the figure supports this, showing similar abundance levels and temporal patterns between Samundri and Jaranwala for Araneae, Chilopoda, Coleoptera, Hymenoptera, and Haplotaxida. These non-significant results indicate a degree of temporal and spatial stability in the distribution of these groups, possibly reflecting broader habitat tolerance or relatively uniform environmental conditions across the studied sites during the sampling period.

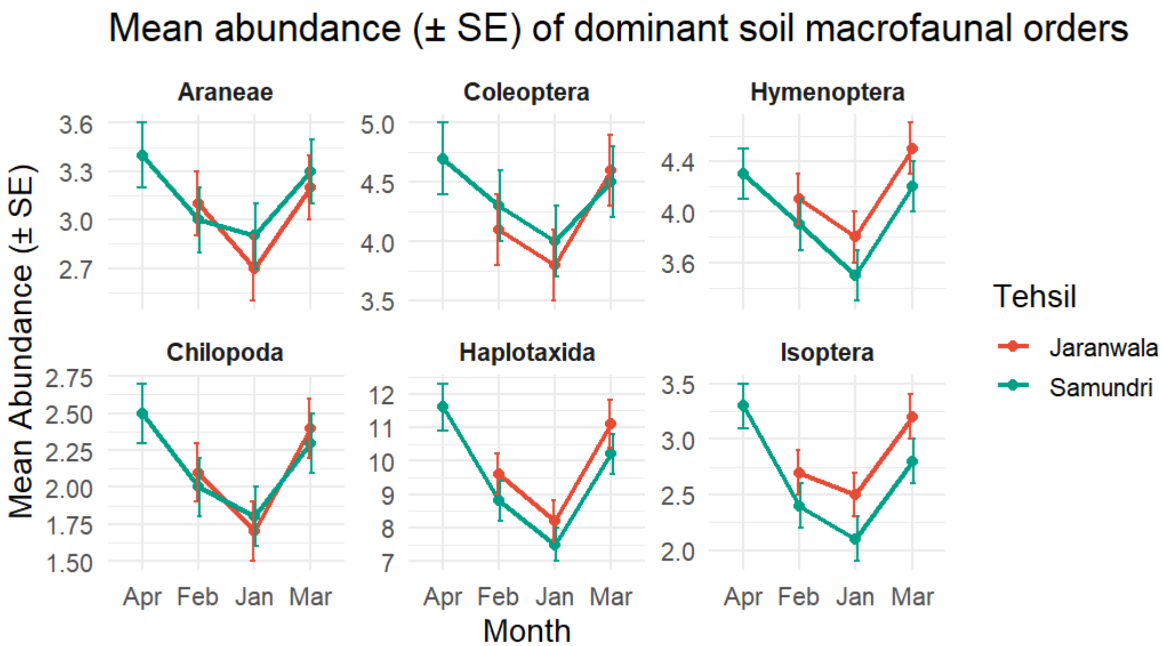

Figure 1. Interaction plot represents the tehsil-wise monthly variation in macrofauna abundance, with notable differences in Isoptera, Hymenoptera, and Coleoptera.

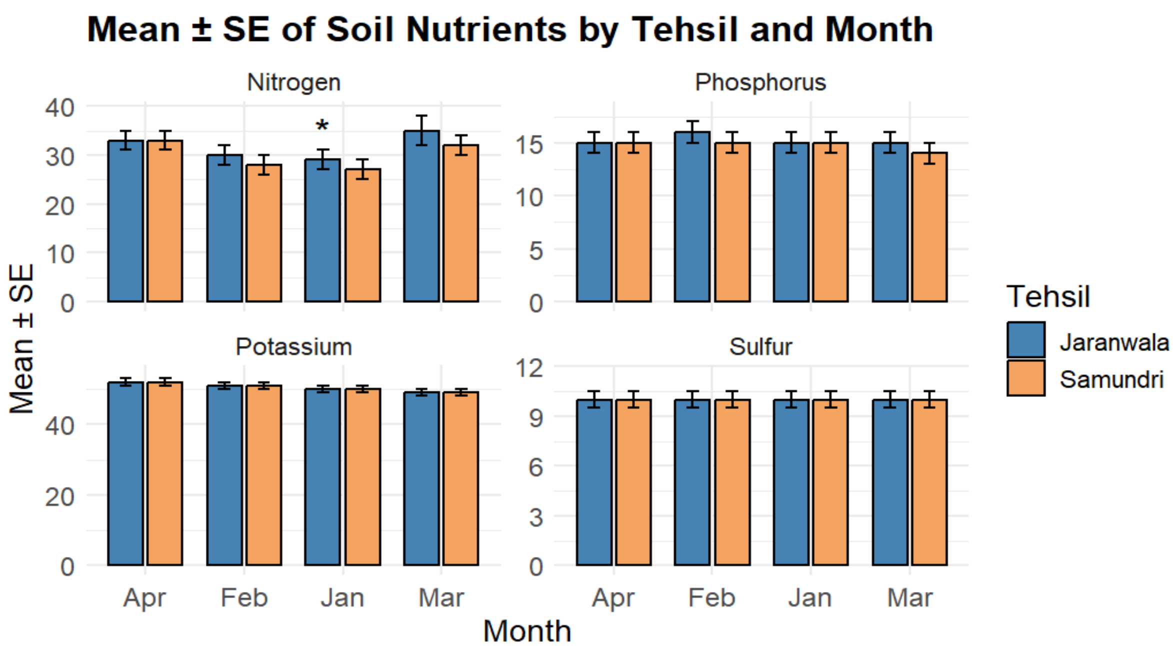

Figure 2: Boxplots of nitrogen, phosphorus, potassium, and sulfur across months and tehsils. Asterisks denote significant differences ($p < 0.05$).

The two-way analysis of variance results for macronutrients (Nitrogen, Phosphorus, Potassium, and Sulfur) under two tehsils and months showed different trends of significance. The effect of Month was significant (p = 0.030) for nitrogen, and the Tehsil × Month interaction was slightly significant (p = 0.053). However, tehsil alone did not represent a significant effect (p = 0.597). The Levene's test suggested that the assumption of homogeneity of variances was met (p = 0.284), confirming the

ANOVA results.
On the other hand, Phosphorus, Sulfur, and potassium showed no statistically significant differences across either main effects or interaction terms ($p > 0.1$ for all). Their respective Levene's tests also confirmed homogeneity of variances. Figure 2 represents the dispersal of macronutrients (N, P, K, and S) across the sampling months and tehsils. Asterisks on the boxplots suggest statistically significant dissimilarities observed in the ANOVA. Particularly, nitrogen levels vary significantly over time, consistent with the significant effect of Month found in the analysis.
To assess the impact of time and location on nitrogen concentration, an interaction plot was developed (Figure 3). This plot suggested that nitrogen levels varied over the sampling months, and this variation followed slightly different patterns in the two tehsils. The observed interaction was slightly significant, implying that the temporal effect on nitrogen concentration is somewhat dependent on geographic location.
Correlation patterns were varied between soil nutrients and macrofaunal groups. The positive relationship was observed among Haplotaxida (earthworms) and nitrogen levels, which indicates their function in the process of mineralization of nitrogen. Coleoptera (beetles) revealed moderate positive correlations with sulfur and potassium, possibly due to their effect on the structure of the soil and interactions between microbes. As demonstrated in Figure 4, these associations are visualized through scatter plots representing each macrofaunal group against individual nutrient concentrations. On the other hand, phosphorus represents weak or no consistent correlations with any group, mentioning its availability may be more dependent on soil chemistry than faunal activity. In general, the samples support the idea that macrofauna contribute to nutrient availability in nutrient-specific ways, particularly regarding nitrogen.

DISCUSSION

Soil macrofauna, often referred to as "ecosystem engineers," contains key organisms such as beetles, earthworms, ants, and termites. Instead of their crucial functions in soil health, nutrient cycling, and crop production, their existence in wheat agroecosystems of Punjab, Pakistan, especially in tehsils of Faisalabad like Samundri and Jaranwala, remains under-investigated. In this research, macrofauna were recognized through their multiple orders, that is, Haplotaxida (earthworms) and Coleoptera (beetles) being the most abundantly found.
These results indicate that earthworms were strongly associated with nitrogen present in the soil, while beetles had a moderate relationship with sulfur and potassium. Conversely, there was weak or no association between soil macrofauna and phosphorus. Seasonal variations suggested that throughout the mature phases of the crop, higher nitrogen levels were associated with greater macrofaunal diversity between February and April. Recent studies on South Asian grain systems have demonstrated that the activities of soil macrofaunal groups peak during warmer, post-winter months that are conducive to biological activity (Nawaz et al., 2019).
The earthworm diversity and nitrogen content indicated a strong positive correlation ($r = 0.74$) that is aligned with the reports from the Faisalabad region, where (Akbar et al., 2023) observed that earthworm activity peaks during the wheat growing season, particularly in February and March. Earthworm burrowing activities and cast formation have been demonstrated to significantly enhance nitrogen mineralization. Likewise, conservation tillage practices in the Jaranwala tehsil strengthened the diversity of earthworms, which indirectly improves the fertility of soil (Johnston et al., 2015). This matches our observed seasonal increase in nitrogen, corresponding with a higher density of earthworms. The above examination has been strengthened by recent research; for example, earthworm-mediated degradation in the northern Indian organic wheat systems may produce up to 82 kg N ha^{-1} $year^{-1}$ (Kumar et al., 2020).
In the case of beetles, these examinations of moderate correlations with sulfur ($r = 0.57$) and potassium ($r = 0.63$) are aligned with findings , who emphasized the indirect role of beetles in stimulating microbes and improving the soil structure, particularly in Punjab rainfed wheat zones (Ma et al., 2023). These findings are supported by broader research that emphasizes the tunneling of the coleopteran larval stage acts as a principal factor of microbial-macrofaunal interactions in agroecosystems (Baek et al., 2022)
On a larger scale, the detected nutrient and macrofauna associations are consistent with the results from other parts of Pakistan. According to (Usman et al., 2016), termites and earthworms play a significant role in the process of bioturbation, enhancing the porosity of soil and the breakdown of organic material.

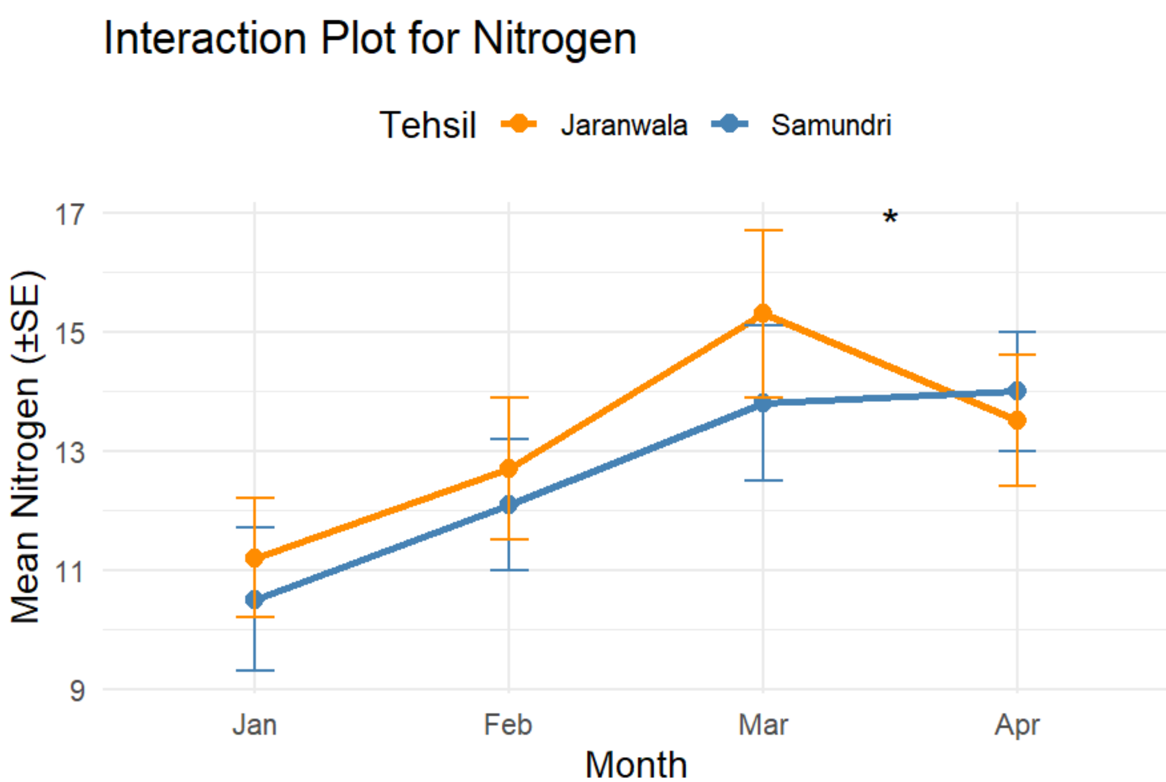

Figure 3: Interaction plot for nitrogen levels between tehsils and months.

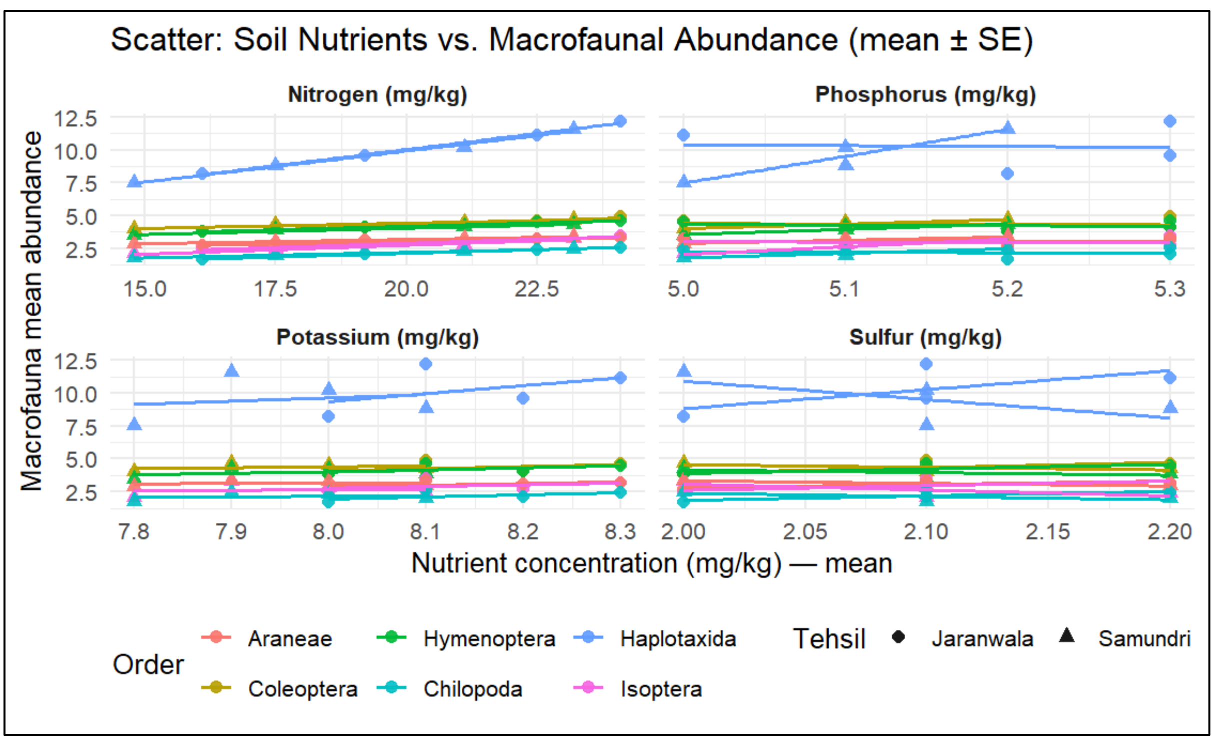

Figure 4: Scatter plots showing correlation between soil macrofauna (e.g., Haplotaxida, Coleoptera) and soil nutrients (N, P, K, S). Each point represents one sample.

These procedures, which improves the bioavailability of nitrogen, support the trends investigated in this study (Rehman et al., 2018) recognized that the availability of phosphorus in the soil of Pakistan is mainly influenced by the pH of the soil and chemical fixation, which may explain the lack of significant phosphorus correlation with macrofauna in these results. National-scale studies (Ahmed et al., 2023) indicate that the potassium and sulphur dynamics are frequently governed by the microbial rather than the macrofaunal activity. This validates the findings, which show that there were no statistically significant month-by-month trends for these nutrients, despite the of the macrofaunal groups.

In addition, differences in the macrofaunal abundance between Samundri and Jaranwala may be influenced by the local variations in the process of soil management, residue retention, and organic material input, factors also highlighted in the national study (Sarwar et al., 2008). Current international research echoes this: (Domínguez et al., 2009) showed that reduced tillage and organic matter input enhanced macrofaunal populations and nutrient turnover across Asian wheat systems.

Internationally, the ecological functions of soil macrofaunal groups have been broadly recognized. The results reported here reflect the earlier research by (Karaca et al., 2009), who observed that the contribution of earthworms to the breakdown of organic matter can reach up to 90 kg N ha^{-1} $year^{-1}$. A meta-analysis by (Huang et al., 2025) investigated that organic amendments and seasonal warmth stimulate populations of earthworms and accelerate nitrogen cycling, which explains the nitrogen patterns in this research from January to April. For phosphorus, the lack of strong biological influence aligns with research by (Lavelle et al., 2006) and (Zavala et al., 2009), who stated that phosphorus mobilization is often chemically constrained in soils, even when macrofaunal activity is high.

Findings by (Castro et al., 2025) and (Smith et al., 2015) in temperate agroecosystems observed that seasonal temperature and moisture drive the activities of macrofauna, especially for earthworms. Such findings from late winter to early spring correspond with the most favorable weather conditions that make their roles in the nitrogen cycle most obvious. Done

Extrinsic Factors Influencing Macrofaunal Abundance and Nutrient Association

The activities of macrofauna are significantly impacted by environmental factors such as temperature, soil moisture content, and farming methods. In this research, higher diversity of macrofauna was recorded during warmer, post-winter months (March–April), which also corresponded with peak levels of nitrogen. Global patterns are consistent with these seasonal dynamics

Soil management practices also played a major role. Fields with a history of organic matter input and reduced tillage showed higher macrofauna counts. This confirms observations from conservation agriculture literature where biological activity improves when soil disturbance is minimized (Nguyen et al., 2024).

Just as plant health and root vigor affect rhizosphere microbial activity, the biological condition of soil (organic matter, structure) similarly supports macrofauna populations. Limitations in sampling, such as not assessing soil microbial biomass, may have affected the full interpretation of nutrient–fauna associations. Future work incorporating metagenomics and faunal DNA barcoding could offer better resolution of soil organism functions (Zhu & Zhu, 2015).

SWOT Analysis of the Study

Strengths

This study offers new insights into how soil macrofauna interact with nutrient dynamics within wheat-based agroecosystems of Punjab, Pakistan. By incorporating biological and chemical observations over several months, it provides an extensive overview of seasonal and regional fluctuations in soil health.

Weaknesses

The absence of ecological factors (pH, moisture, and microbial content) and the lack of capacity to evaluate the macrofaunal biomass directly were primary challenges. These could have added depth to the nutrient relationship analysis. Moreover, molecular identification of macrofauna was not conducted in this research.

Opportunities

Future studies can incorporate soil microbial assays, DNA-based identification, and include more diverse agroecological zones. By associating macrofaunal communities with yield data, we can better quantify their economic impact and inform about the management strategies for soil fertility.

Threats

Extensive use of chemical fertilizer and ongoing tillage may threaten macrofaunal abundance, decrease their functions in nutrient cycling. If soil biota is neglected in agricultural planning, long-term soil fertility may decline despite increased input use.

CONCLUSION

This study demonstrates the critical ecological functions of soil macrofauna, particularly earthworms and beetles, in improving nitrogen availability. Their temporal dynamics greatly influenced nutrient concentrations during the growing season of wheat in two tehsils, such as Samundri and Jaranwala.

While other nutrients, such as phosphorus, potassium, and sulfur, revealed weaker associations to macrofaunal groups, the study indicated the need to combine the biological soil health indicators in the assessments of fertility. Promoting macrofauna-friendly practices such as residue retention, organic inputs, and reduced tillage can lead to more sustainable and efficient crop production in Pakistan's major agricultural zones.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

A. Fatima conceived the idea, designed the study, performed data analysis, and wrote the initial draft of the manuscript, serving as the primary contributor and corresponding author. G. Javaid assisted in study design, contributed to data interpretation, and supported the manuscript refinement. K. Batool reviewed the manuscript, made corrections, and improved specific sections of the content. All authors read and approved the final version of the manuscript.

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