PLANT SCIENCE TODAY
Vol 12(sp4): 1-7
https://doi.org/10.14719/pst.10142

eISSN 2348-1900

RESEARCH ARTICLE

The role of *Moringa oleifera* in soil microbial dynamics and environmental sustainability

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Received: 19 June 2025; Accepted: 23 July 2025; Available online: Version 1.0: 23 October 2025

Cite this article: Sahil T, Anjali C, Rajesh K, Ruchi T, Rahul P. The role of *Moringa oleifera* in soil microbial dynamics and environmental sustainability. Plant Science Today. 2025; 12(sp4): 1-7. https://doi.org/10.14719/pst.10142

Abstract

Soil microbial communities and enzymatic activities play a crucial role in maintaining soil fertility, nutrient cycling and overall ecosystem health. This study investigates the microbial and enzymatic properties of soils across four distinct blocks: Dharampur, Kunihar, Solan and Nalagarh. Key parameters assessed include microbial biomass carbon, bacterial, fungal and Actinomycetes populations, along with urease and dehydrogenase enzymatic activities, which serve as indicators of soil biological health. The results reveal substantial spatial variability in microbial and biochemical attributes, with Kunihar soils exhibiting the highest microbial biomass, microbial populations and enzymatic activities, suggesting favorable biological conditions. Conversely, Nalagarh soils consistently recorded the lowest values, indicative of diminished microbial activity and suboptimal soil conditions. These findings underscore the significant impact of environmental and management factors on shaping soil microbial dynamics, highlighting the importance of adopting site-specific strategies for soil enhancement. Future research should focus on identifying key drivers of microbial health and support the development of sustainable land management interventions, particularly for ecologically vulnerable or degraded regions.

Keywords: actinomycetes; enzyme activity; microbial biomass carbon; moringa; soil health

Introduction

Soil is a complex and dynamic environment that serves as a natural habitat for diverse communities of microorganisms. These microbial populations-comprising bacteria, fungi, Actinomycetes, Protozoa and Archaea play a critical role in regulating ecosystem functioning, including organic matter decomposition, nutrient mineralization, nitrogen fixation and the suppression of plant pathogens (1, 2). Soil properties, climate, vegetation and land management practices play a crucial role in shaping the structure and function of microbial communities. In forest ecosystems, the kind of vegetation and the quality of litter input from trees greatly affect the abundance, diversity and activity of soil microbes (2). *Moringa oleifera*, commonly known as drumstick or "miracle tree," *Moringa oleifera* is valued for its fast growth, adaptability to different climatic conditions and rich nutritional and medicinal properties. Indigenous to parts of India, it is now cultivated worldwide for its edible leaves, pods and seeds. In recent years, *Moringa oleifera* has been increasingly integrated into agroforestry and reforestation projects due to its ecological benefits. It is known to contribute to soil fertility through nutrient-rich litter fall, improve organic matter content and support soil conservation through its root system (3). The high nitrogen and calcium content in its biomass, especially the leaf litter, provides a favorable substrate for microbial growth and enzymatic activity. The rhizosphere, narrow zone of soil influenced by root secretions is a hot spot for microbial activity. Plants like *Moringa oleifera*, which have vigorous root systems and high root exudate release, can significantly influence the microbial community structure in the rhizosphere (4). These root exudates serve as a source of carbon and energy for microbes, thereby affecting their abundance and diversity. The antimicrobial and phytochemical properties of Moringa are mainly due to compounds like quercetin, kaempferol, chlorogenic acid and isothiocyanates. These phytochemicals can selectively stimulate or suppress specific microbial groups (5). The introduction of *Moringa oleifera* into forest ecosystems may have far reaching impacts on microbial community dynamics. These effects can include changes in microbial biomass, enzymatic activity, species composition and functional diversity.

The Nahan Forest Circle, situated in the southern part of Himachal Pradesh, forms part of the outer Himalayan range known as the Shivaliks. This region is ecologically significant due to its rich biodiversity, varying altitudinal gradients and

subtropical climatic conditions. The forests in this area include a mix of broadleaf and coniferous species, along with scattered agroforestry plantations. This ecosystem variability creates a range of habitats and organic inputs, which can enhance the diversity and functional complexity of soil microbial communities. The soil microbial communities in such ecosystems are susceptible to changes in vegetation patterns and land-use practices (6). Government agencies, state authorities and NGOs are actively promoting *Moringa oleifera* in India for its economic, nutritional and ecological benefits. These organizations support its cultivation to improve rural livelihoods, combat malnutrition and enhance environmental sustainability. Despite the growing body of research on the nutritional, medicinal and agronomic aspects of *Moringa oleifera*, limited studies have been conducted on its ecological interactions, especially concerning soil microbiology in forested landscapes. Most existing literature focuses on its effects in agricultural soils or arid regions. However, forest soils in humid-subtropical zones such as Nahan have unique microbial assemblages and nutrient cycles that respond differently to plant inputs. Therefore, an investigation into how *Moringa oleifera* influences the soil microbial community structure in such a region can provide valuable insights into its role in forest ecosystem sustainability (7).

This study aims to bridge the knowledge gap by evaluating the impact of *Moringa oleifera* on the microbial community structure in forest soils of the Nahan Forest Circle. It aims to assess changes in microbial biomass, community composition (using indicators such as microbial biomass carbon and count) and functional diversity (through soil enzymatic activities). The outcomes of this research will help in understanding whether Moringa plantations support or disrupt native microbial communities, thereby guiding forest managers and policymakers in making informed decisions about the species' ecological suitability in Himachal Pradesh. “This study is timely and essential given the global focus on sustainable reforestation and land management”. Given its wide ranging potential and current popularity, evaluating its influence on soil microbial health, especially in sensitive forest ecosystems such as those of the Nahan Forest Circle, is a timely and necessary endeavour.

Materials and Methods

Study area selection

The investigation was conducted in the Nahan Forest Circle of Himachal Pradesh, which spans a broad elevation range from 300 to 3000 m above mean sea level (AMSL) as depicted in Fig. 1. The disturbance level in forest patches was determined by assessing indicators such as cut stems, presence of trails and canopy openness during field surveys. Additionally, remote sensing data like NDVI values and distance from roads were used to confirm minimal anthropogenic disturbance. The region covers diverse topographical and climatic zones extending from the outer Shiwalik Hills to the Middle Himalayan ranges, with selected locations situated within the 500 to 2500 m altitudinal belt. The geological parent material in the study area comprises sandstone, conglomerates, boulders, dolomite and calcareous formations. These materials give rise to soils primarily classified as Inceptisols and Typic Eutrochrepts, with textures varying from loam to clay loam. These soil types are generally fertile and support a diverse range of vegetation, including *Moringa oleifera* plantations.

Sampling design and site selection

A stratified random sampling approach was used to ensure ecological representation across the study area. The sampling design involved selecting four main forest blocks within the Nahan Forest Circle. Each block was further divided into three sub-blocks, chosen based on accessibility, the distribution of *Moringa oleifera and* representativeness of the terrain. In each block three further sub-blocks were selected as mentioned in Table 1. All sub-blocks were geo-referenced using GPS to ensure spatial accuracy during sampling and mapping. Each sub-block was geo-referenced using GPS to maintain spatial accuracy during sampling and mapping.

Tree selection and soil sampling

From each sub-block, ten healthy and mature trees of *Moringa oleifera* were randomly selected. Care was taken to maintain uniformity in tree age and size across sites to reduce biological variability. Soil samples were collected from the rhizosphere zone (0-15 cm depth) at four points around each tree and then composited to form a representative sample per tree. Samples were collected during September - October using sterilized augers and stored in clean, labelled polyethylene bags. All samples were immediately transported to the laboratory in iceboxes to preserve microbial integrity and stored at 4 °C until analysis of parameters, including microbial biomass carbon, microbial count and enzyme activities.

Microbial biomass carbon (MB-C)

MB-C was determined using the soil fumigation extraction method, as detailed elsewhere (8). In this method, 20 g of soil was fumigated with 50 mL chloroform in vacuum desiccator for 24 hrs in dark and other 20 g soil sample was refrigerated, then both the samples (fumigated and un-fumigated) were extracted with 80 mL of 0.5 M K_2SO_4, for half an hour and filtered through Whatman no.1 filter paper. Then add 2 mL of 66.5 mM $K_2Cr_2O_7$ and 5 mL digestion mixture containing H_2SO_4 and ortho-phosphoric acid (2:1) to 8 mL filtrate and heated on a hot plate at 120 ºC for 30 min. After that made, the final volume was made to 250 mL with distilled water and 2-3 drops of ferroin indicator were added and titrated against 0.005 N ferrous ammonium sulphate (FAS).

$$\text{MB - C } (\mu g\ g^{-1}\ \text{soil}) = \frac{\text{EC (F) - EC (UF)}}{K} \qquad \text{(Eqn. 1)}$$

Where,

K = 0.25 ± 0.05 (factor which represents the efficiency of extraction of microbial biomass carbon)

Table 1. Forest blocks and sub-blocks

Main block	Sub-block 1	Sub-block 2	Sub-block 3
Dharampur	Koti (L_1)	Chamgah (L_2)	Sari (L_3)
Solan	Rawala (L_4)	Kharyana (L_5)	Ghatti (L_6)
Kunihar	Kotla (L_7)	Kaonta Warla (L_8)	Kansal (L_9)
Nalagarh	Kirpalpur (L_{10})	Dhadi Kanyan (L_{11})	Nikulwal (L_{12})

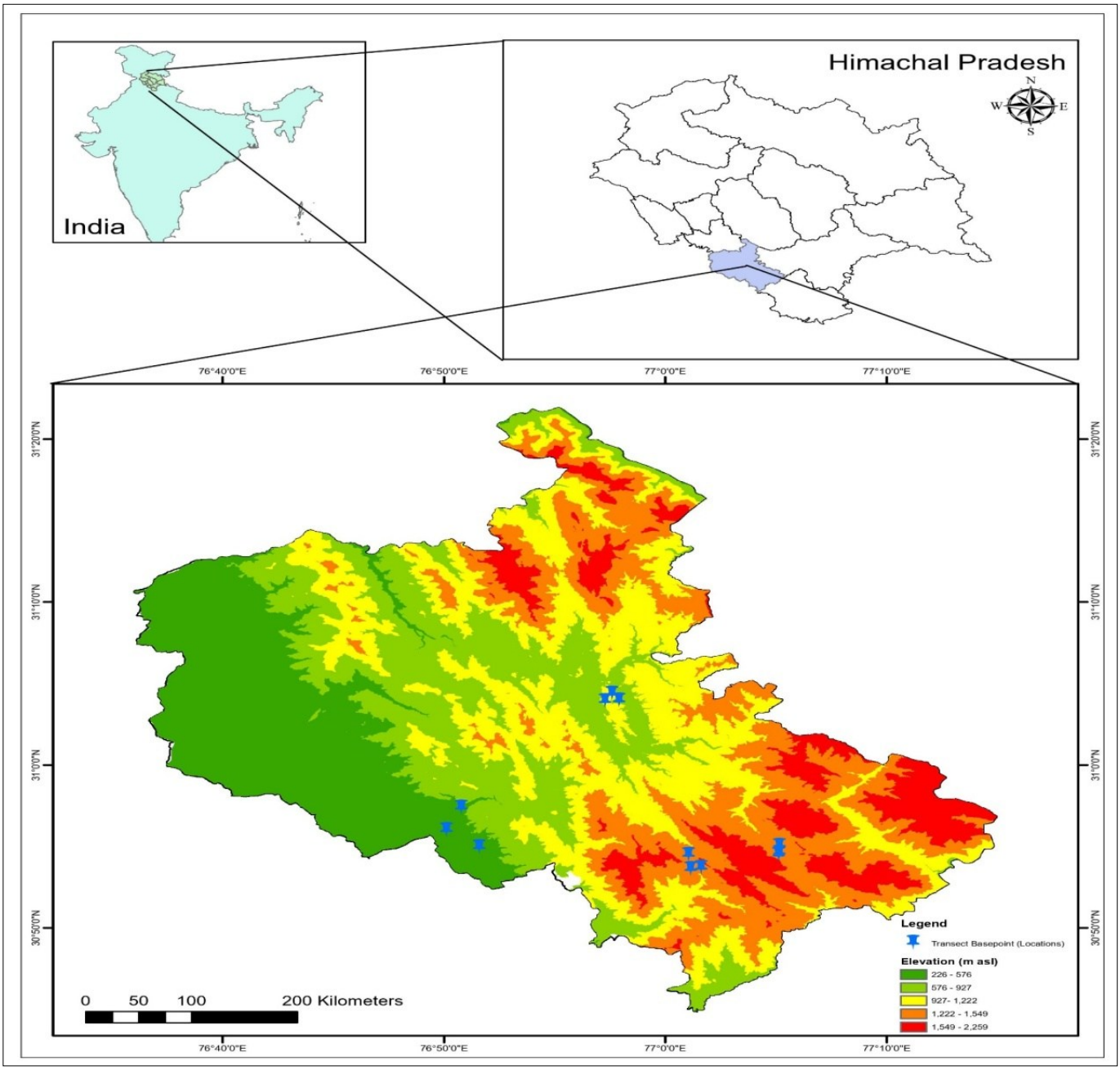

Fig. 1. Geospatial representation across different locations of the Nahan forest circle of Himachal Pradesh.

EC (F) = Total amount of extractable carbon in fumigated soil samples

EC (UF) = Total amount of extractable carbon in un-fumigated soil samples

Total viable microbial count (Bacteria, Fungi and Actinomycetes)

The enumeration of Total viable bacteria, actinomycetes and fungi was carried out using serial dilution and pour plate techniques (9). Specific media were used for each group of microorganisms: nutrient agar (NA) for bacteria, potato dextrose agar (PDA) for fungi and Kenknight and Munaier's medium (KNM) for actinomycetes. 1 g of sieved soil (< 2 mm) was added to 9 mL of sterile water in a test tube and shaken thoroughly for 15-20 min. Serial dilutions ranging from 10^{-2} to 10^{-8} were prepared. From the appropriate dilutions, 0.1 mL aliquots were pipetted into sterile Petri plates. Subsequently, molten agar medium at approximately 45 °C was poured into the plates. The plates were gently rotated in both clockwise and counterclockwise directions to ensure uniform distribution of the inoculum and the medium was allowed to solidify. Once solidified, the plates were incubated in an inverted position at 28 °C for 1 to 3 days. After incubation, colony growth was observed and visible colonies were counted using standard methods. Results were expressed as colony-forming units (CFU) per gram of soil (Fig. 2).

Soil enzymes

Urease

Urease activity was measured spectrophotometrically using a UV-Visible spectrophotometer. 10 g of soil sample was incubated for 15 min with 15 mL of toluene, 10 mL of urea solution and 20 mL of citrate buffer were added, mixed and incubated for 3 hrs at 37 ° C, then diluted to 100 mL with water, mixed and filtered. 1 mL of filtrate was pipetted out, added 9 mL of water, 4 mL phenate solution and 3 mL of sodium hypochlorite solution. It was mixed and allowed to stand for 20 min until the maximum colour was developed. It was then diluted to 50 mL with water, mixed well and the transmittance absorbance was read at 630 nm using red filter against the water blank. The standard curve was prepared

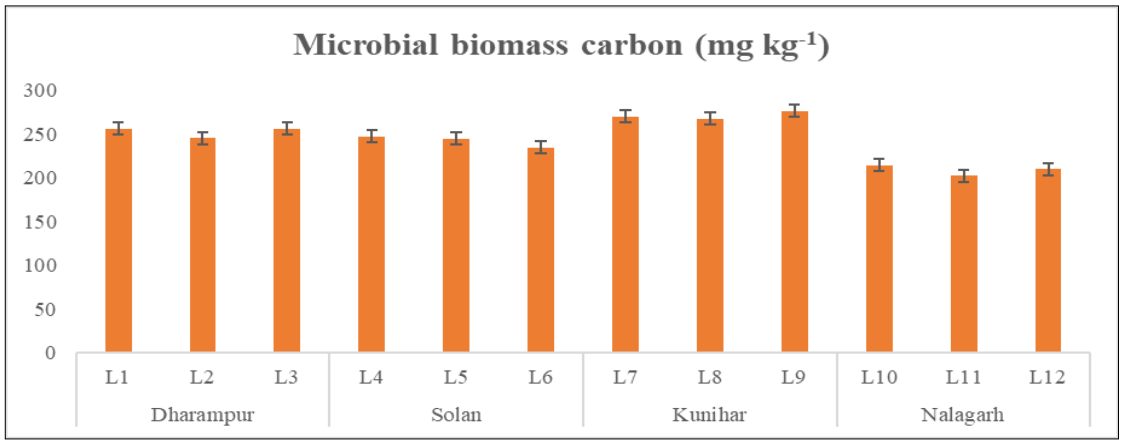

Fig. 2. Microbial biomass carbon (mg kg^{-1}) in soils under *Moringa oleifera* across different locations in the Nahan Forest Circle, Himachal Pradesh.

from ammonium sulphate solution. Results were expressed as (mg $NH_4^+ g^{-1}$ soil) to get urease number. Urease number was multiplied by 0.32 to obtain urease units as described previously (10).

Dehydrogenase

Dehydrogenase activity was measured spectrophotometrically using a UV-Visible spectrophotometer. The assay was based on the reduction of NAD+ to NADH, which was monitored by measuring the increase in absorbance at 480 nm. The dehydrogenase enzyme estimation was carried out by standard method (11). 1 g of soil was incubated for 12 hrs with 1mL of TTC (Tri-phenyl tetrazolium chloride) and 0.5 mL of 1 % glucose. After incubation 10 mL of methanol was added. Then the test tube was shaken and allowed to stand in dark for 24 hrs. Supernatant was withdrawn and colour intensity was measured using blue filter at 485 nm wavelength. The amount of formazan from standard curve prepared from TPF (Triphenyl formazan) was in the range of 0.04 to 0.5 mg/mL. The results were expressed in the terms of TPF per hour per gram of soil (µg TPF $h^{-1} g^{-1}$ soil).

Statistical analysis

The data obtained from microbiological properties were subjected to descriptive statistics, ANOVA (one-way to determine significant differences among treatment groups. Statistical significance was considered at $p < 0.05$) and correlation analysis using standard statistical software (OP STAT).

Critical difference (CD) =

SE (d) × t (5 %) value at error degrees of freedom

$CD_{0.05}$ = Critical difference at 5 % level of significance

Results and Discussion

Microbial biomass carbon (mg kg^{-1})

The microbial characteristics of soils varied significantly across the different locations and blocks studied. Among all the blocks, Kunihar recorded the highest microbial biomass carbon values, with the maximum being 276.46 mg kg^{-1} at location L_9, followed closely by L_7 and L_8. In contrast, the Nalagarh block exhibited the lowest microbial biomass carbon, with the minimum observed at L_{11} (202.14 mg kg^{-1}) (Fig. 3).

Viable microbial count (bacteria, fungi and actinomycetes)

The bacterial population also followed a similar trend, being highest in Kunihar, particularly at L_9 (98.75 $\times 10^5$ cfu g^{-1} soil) and lowest in Nalagarh at L_{11} (83.88 $\times 10^5$ cfu g^{-1} soil). Higher fungal populations in Dharampur are likely due to a combination of abundant leaf litter, shaded and moist soil conditions and minimal disturbance each of which creates an optimal environment for fungal growth and activity. The Solan and Nalagarh blocks showed comparatively lower fungal counts. Notably, the highest actinomycetes population was observed in

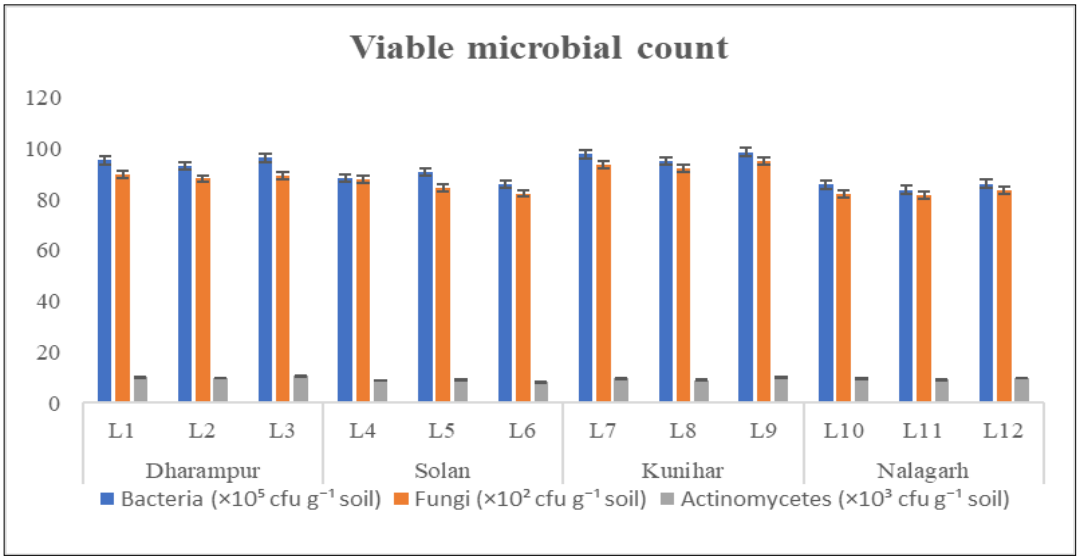

Fig. 3. Total viable microbial count (cfu g^{-1} soil) in various soils of Moringa trees of Nahan Forest Circle, Himachal Pradesh.

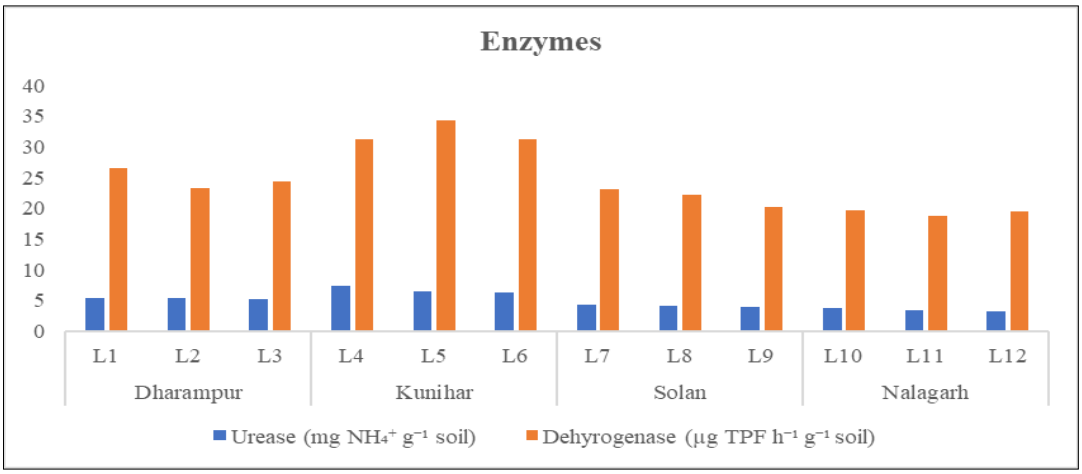

Fig. 4. Enzyme activity in soils under Moringa trees of Nahan Forest Circle, Himachal Pradesh.

Dharampur at location L_3 (10.53 ×10^3 cfu g^{-1} soil), while the lowest was recorded in Solan at L_6 (8.12 ×10^3 cfu g^{-1} soil). Overall, the soils from Kunihar showed superior microbial health, reflected in higher microbial biomass and microbial populations, while Nalagarh consistently recorded the lowest values across most parameters, suggesting less favourable microbial conditions (Fig. 4).

The superior microbial health in the soils of Kunihar can be attributed to favorable edaphic and environmental conditions, such as higher organic matter content, optimal soil moisture and balanced pH levels, which collectively support microbial growth and activity. Additionally, Kunihar may have less anthropogenic stress (e.g., reduced industrial pollution or chemical overuse) and better vegetative cover, which contribute to improved microbial biomass and diversity. On the other hand, Nalagarh consistently exhibited poor microbial indicators, likely due to industrialization, soil degradation, or lower organic inputs, which adversely affect soil micro-biota. Poor soil aeration, compaction, or nutrient imbalances might also limit microbial proliferation in this region. These differences underscore the critical influence of local land use, soil management and environmental conditions on soil microbial health. The findings emphasize the need for region-specific soil conservation and organic matter management strategies to restore and enhance microbial activity, particularly in regions like Nalagarh. The results are consistent with the findings of studies (12-14), which reported a similar range of microbial counts in the soils of the mid hills of Himachal Pradesh. Likewise, studies (15-17) observed comparable levels of microbial biomass in the Himalayan region.

Enzyme activity

Urease

The urease activity in soils across different blocks showed considerable variation. Among all the blocks, Kunihar recorded the highest urease activity, with a peak value of 7.34 mg NH_4^+ g^{-1} soil at location L_4, followed by L_5 (6.45 mg NH_4^+ g^{-1} soil) and L_6 (6.33 mg NH_4^+ g^{-1} soil). Dharampur also exhibited intermediate urease activity, ranging from 5.12 to 5.45 mg NH_4^+ g^{-1} soil.

The lowest urease activity was observed in the Nalagarh block, with values ranging from 3.24 mg NH_4^+ g^{-1} soil at L_{12} to 3.67 mg NH_4^+ g^{-1} soil at L_{10}, likely reflecting reduced microbial biomass and organic matter (18). In contrast, the Solan block showed intermediate activity levels, between 4.01 and 4.23 mg $NH_4^+g^{-1}$ soil, consistent with studies showing that soils with lower organic inputs and microbial populations exhibit diminished urease activity (19). The spatial variability of urease activity highlights the significance of localized soil properties in microbial enzyme regulation. Variables such as organic carbon, total nitrogen and clay content notably affect urease activity, whereas elevated $CaCO_3$ levels and salinity can inhibit it. These findings are essential for enhancing nitrogen management practices, especially in areas utilizing urea-based fertilizers (Fig. 5).

Dehydrogenase

Dehydrogenase activity was highest in Kunihar, particularly at L_5 (34.23 µg TPF $h^{-1}g^{-1}$soil)), indicating elevated microbial oxidative activity. Dharampur showed moderate dehydrogenase activity, ranging from 23.34 to 26.56 µg TPF $h^{-1}g^{-1}$soil, Solan and Nalagarh exhibited the lowest dehydrogenase activity, with minimum values recorded at L5 (20.97 µg TPF $h^{-1}g^{-1}$soil) and L_{11} (18.77 µg TPF $h^{-1}g^{-1}$soil), indicating diminished microbial respiration and overall biological activity in these locations. Overall, the results indicate that Kunihar soils possess the most favorable biological activity in terms of both urease and dehydrogenase enzyme levels, while Nalagarh exhibits the least, reflecting possible limitations in organic matter content, microbial activity, or environmental conditions. The significant variation in urease and dehydrogenase activity across the different blocks suggests that local environmental and soil conditions play a crucial role in regulating soil enzyme functions. Soils from Kunihar consistently exhibited the highest levels of both urease and dehydrogenase activities, indicating a biologically active soil environment. This may be due to the presence of higher organic matter content, balanced soil pH, sufficient soil moisture and favorable aeration factors that support microbial growth and enzymatic functioning. The elevated dehydrogenase activity in Kunihar also reflects increased microbial respiration and overall metabolic activity of the soil micro biome. In contrast, Nalagarh soils showed the lowest enzyme activity values, which may point to several constraints such as reduced microbial biomass, lower organic matter, or environmental stress caused by industrialization or land degradation. The lower dehydrogenase activity particularly suggests poor microbial respiration and oxidative metabolism, highlighting unfavourable biological conditions in the soil (18-20). The results emphasize that soil enzyme activities are sensitive indicators of soil health and fertility. Differences in land use practices, vegetation cover and anthropogenic influences likely contribute to the observed spatial variation. These findings underline the importance of adopting sustainable land

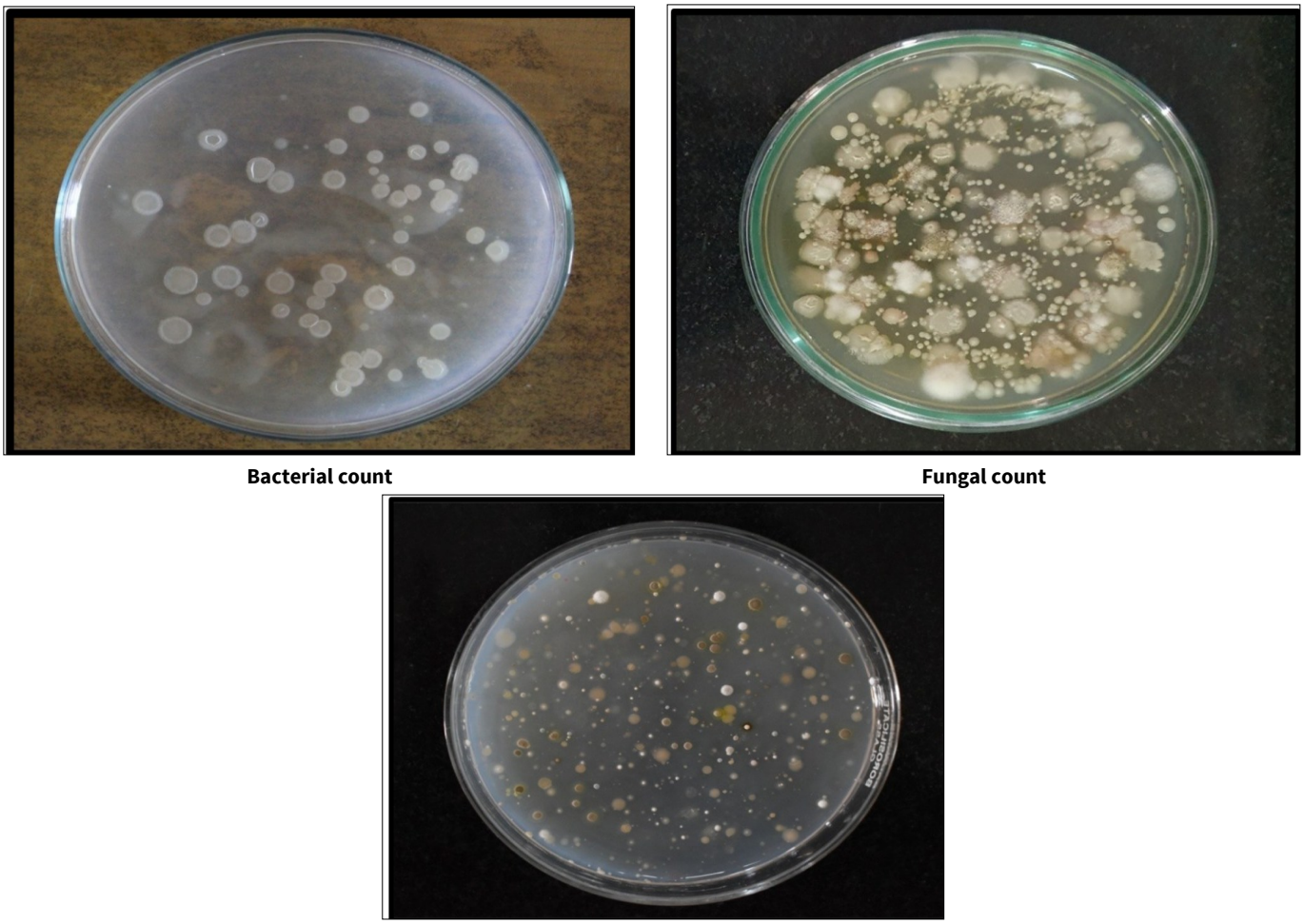

Fig. 5. Total viable microbial count in soils of Moringa trees of Nahan Forest Circle, Himachal Pradesh.

management strategies tailored to local conditions, particularly in low activity regions like Nalagarh, to restore microbial balance and improve soil biological health. Microbes in the soil may be stimulated to produce more urease and dehydrogenase, which results in higher enzyme activities (21-23) (Fig. 5).

Conclusion

Biological indicators such as microbial biomass, populations and enzyme activities revealed significant variation across the Nahan Forest circle. Kunihar block showed the highest soil biological activity, likely due to favorable management and environmental conditions, while Nalagarh showed the lowest, indicating potential soil degradation. These results emphasize the value of biological metrics in assessing soil health and the need for targeted, site-specific management practices to support sustainable land use.

Acknowledgements

The authors would be thankful to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Himachal Pradesh, precisely the Department of Soil Science and Water Management and Horticulture for providing the farm as well as lab facilities for conducting the trial.

Authors' contributions

ST, AC and RK contributed to the conceptualization of the study. The methodology was developed by ST, AC and RK. Software support was provided and validation was conducted by ST, AC, RK, RT and RP. Formal analysis was performed by ST, AC and RP. The investigation was carried out by ST, AC, RK, RT and RP. The original draft was prepared by ST and RP. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

The authors acknowledge the use of AI-assisted technologies in the preparation of this manuscript. Generative AI tools were employed to improve language clarity and structure; however, all scientific content, data interpretation and conclusions were developed and verified by the authors.

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Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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