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SPATIAL AND VERTICAL VARIATION OF RHIZOSPHERE SOIL PHYSICOCHEMICAL AND BIOLOGICAL PROPERTIES IN DECIDUOUS FORESTS OF BANGLADESH

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Abstract

Soil physicochemical and biological properties are key determinants of ecosystem functioning in tropical deciduous forests. This study investigated the spatial and vertical variability of soil characteristics and microbial activity in the Bhawal (BNP) and Madhupur (MNP) Sal forests of Bangladesh. Rhizosphere soil samples were collected from two depths to analyze physicochemical and biological parameters. Two-way ANOVA, correlation, regression, and NMDS analyses were applied to evaluate variation and interrelationships. Significant spatial and vertical differences ($p < 0.001$) were observed, where Madhupur exhibiting higher OC, TN, CEC, and microbial activity than Bhawal. Upper soil layers showed greater microbial abundance and respiration, while deeper layers had higher sodium but lower biological activity. Regression analysis identified OC, TP, and Na as key positive predictors of soil respiration, whereas CEC had a negative influence. NMDS ordination distinguished samples by site and depth, emphasizing the dominant role of nutrient and microbial factors in soil variability. Overall, soil heterogeneity in the Sal forests is primarily governed by organic matter and microbial processes, with anthropogenic disturbance reducing fertility in Bhawal.

Introduction

Soil physicochemical and biological properties are fundamental determinants of forest ecosystem productivity, nutrient cycling, and carbon sequestration (Ataullah *et al.* 2017, Khan *et al.* 2025). In terrestrial ecosystems, these properties regulate essential biogeochemical processes, including organic matter decomposition, nutrient mineralization, and soil respiration, which collectively sustain vegetation growth and influence the global carbon balance (Khan *et al.* 2025). The spatial and vertical variability of soil characteristics is driven by a combination of climatic conditions, vegetation type, topography, and anthropogenic influences (Ataullah *et al.* 2018, Liu *et al.* 2023). Understanding these variations is crucial for assessing soil fertility, ecosystem health, and the sustainability of forest management practices, particularly in deciduous systems where environmental gradients and seasonal dynamics strongly affect soil function.

The Sal (*Shorea robusta* Roxb. Ex. Gaertn. F.) forest ecosystem, locally known as Shalban, represents the most extensive tropical deciduous forest type in Bangladesh. The two major remnants of this forest, the Bhawal and Madhupur Sal forests, constitute ecologically significant but highly fragmented landscapes that differ in geomorphology, vegetation structure, and land-use history (Akter *et al.* 2025). Although both forests occur under similar climatic regimes, they exhibit marked heterogeneity in soil texture, moisture availability, and organic matter content due to differences in elevation, drainage, and anthropogenic pressure. These edaphic variations are

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likely to influence microbial community structure, nutrient dynamics, and soil respiration, yet the extent and pattern of these influences remain insufficiently understood. Previous studies in the Sal forests have primarily focused on vegetation composition, regeneration potential, and forest degradation, whereas systematic evaluations of soil physicochemical and biological properties across spatial and vertical gradients are limited (Rahman and Vacik 2010, Propa *et al.* 2021, Rahman *et al.* 2022).

Microbial communities, particularly bacteria and fungi, play a pivotal role in decomposing organic residues and driving nutrient transformations (Khan *et al.* 2025). Their abundance and activity are closely linked to soil organic carbon, nitrogen, and moisture content, and they directly contribute to soil respiration (Rs) a key indicator of belowground carbon flux (Alam *et al.* 2024). The relationships among these microbial and physicochemical parameters, however, are often site-specific and depth-dependent. Surface soils generally exhibit higher microbial activity and nutrient concentrations due to litter deposition and root interactions, while subsurface soils tend to accumulate soluble ions and exhibit lower biological activity (Naylor *et al.* 2022). Therefore, evaluating how soil properties and microbial parameters vary between forest sites and soil depths provides valuable insights into ecosystem functioning and carbon dynamics.

In this context, the present study investigates the spatial and vertical variation of soil physicochemical and microbial properties in the Bhawal and Madhupur Sal forests of Bangladesh. Specifically, the study aims to characterize the differences in soil physicochemical and biological attributes between the two forests and between upper and lower soil layers, identify the key soil factors that predict soil respiration and evaluate the overall structure of soil variability using multivariate ordination techniques.

Materials and Methods

The study was carried out in Bhawal National Park (BNP), Gazipur and Madhupur National Park (MNP), Tangail. Both sites lie within the Madhupur Tract, a Pleistocene terrace dominated by reddish-brown clay loam to sandy loam soils (Rahman and Vacik 2010). The region experiences a tropical monsoonal climate with mean annual rainfall of approximately 2,000-2,200 mm and an average temperature ranging from 12°C in winter to 33°C in summer. Although both forests share similar climatic conditions, but differ markedly in geomorphology, vegetation structure, and human disturbance levels. Madhupur represents a comparatively intact forest with richer vegetation cover and minimal anthropogenic intrusion, whereas Bhawal has undergone substantial degradation due to industrialization, urbanization, and deforestation.

Soil samples were collected from the rhizosphere of *S. robusta* trees in both forests. In each forest, ten representative plots (20 m × 20 m) were established within relatively undisturbed areas to encompass spatial variability. From each plot, samples were taken at two depths: the upper layer (0-15 cm), representing the biologically active and litter-decomposition zone, and the lower layer (15-30 cm), representing the underlying subsurface soil. A total of 40 samples were collected, sealed in sterile polyethylene bags, and transported to the laboratory in iceboxes to preserve microbial integrity.

In the laboratory, soil samples were air-dried, gently crushed, and sieved through a 2 mm mesh for physicochemical analyses. Soil pH and electrical conductivity (EC) were measured in a 1:2.5 soil-water suspension using digital meters. Soil texture (sand, silt, and clay fractions) was determined using the hydrometer method (Bouyoucos 1962). Organic carbon (OC) was analyzed following the Walkley-Black dichromate oxidation method, while total nitrogen (TN) was determined by the Kjeldahl digestion method (Walkley and Black 1934). Total phosphorus (TP) was measured colorimetrically after acid digestion according to Olsen *et al.* (1954). Exchangeable sodium (Na) and cation exchange capacity (CEC) were analyzed using 1N ammonium acetate extraction followed by flame photometry. Soil moisture content (MC) was determined gravimetrically by oven-drying at 105°C to a constant weight.

Fresh, non-dried soil was used for microbial and respiration analyses. Bacterial (BC) and fungal counts (FC) were determined using the serial dilution and spread plate technique on nutrient agar and potato dextrose agar (PDA), respectively. The plates were incubated at 28 ± 2°C for 48-72 hrs, and colony-forming units (CFU) were expressed as CFU $\times 10^6$ g^{-1} for bacteria and CFU $\times 10^4$ g^{-1} for fungi. Soil respiration (Rs) was measured by the alkali absorption method (FAO 2023).

Data were checked for normality and homogeneity of variance before statistical analysis. Two-way analysis of variance (ANOVA) was applied to assess the effects of forest location and soil depth on soil variables. Pearson's correlation analysis was performed to examine interrelationships among physicochemical and biological properties. Multiple linear regression was used to identify the key predictors of Rs. To assess multivariate differences in overall soil composition, permutational multivariate analysis of variance (PERMANOVA) based on Bray-Curtis dissimilarity was applied. Non-metric multidimensional scaling (NMDS) was then used to visualize the spatial and vertical separation of samples, and vector fitting identified variables significantly influencing the ordination patterns. All statistical analyses were performed in R (version 4.3.1) using the packages vegan, ggplot2, and dplyr, with statistical significance considered at $p < 0.05$.

Results and Discussion

Soil physicochemical and biological properties exhibited pronounced spatial and vertical variability between the Bhawal (BNP) and Madhupur (MNP) Sal forests (Figs 1 and 2, Table 1). The two-way ANOVA revealed that both forest location and soil depth significantly influenced most measured variables ($p < 0.001$), whereas their interaction effects were generally weak, except for soil respiration (Rs) and fungal count (FC), which responded differently to depth across sites ($p < 0.05$) (Table 1). Overall, the results demonstrate strong edaphic heterogeneity between the forest ecosystems, with Madhupur showing superior soil fertility, moisture retention, and microbial activity compared with Bhawal.

Among the parameters, location exerted the most dominant effect, as indicated by high F-values for FC (F = 162), Rs (F = 147), bacterial count (BC; F = 121), sodium (Na; F = 112), and cation exchange capacity (CEC; F = 120). These differences underline the distinct microbial and physicochemical environments of the two sites. Madhupur soils were generally richer and more biologically active, as evidenced by higher Rs (83.94 ± 20.26 µg CO_2 h^{-1} g^{-1} soil) compared to Bhawal (54.97 ± 13.74 µg CO_2 h^{-1} g^{-1} soil), reflecting enhanced microbial and root respiration under the relatively moist and organic-rich conditions of Madhupur (Fig. 1). Similarly,the significantly greater CEC (22.04 ± 1.37 meq/100 g) and clay content (34.6 ± 3.47%) in Madhupur indicate higher nutrient-holding capacity and finer texture, consistent with superior soil structure and fertility (Masha *et al.* 2023).

The higher OC (1.51 ± 0.23%) and TN (0.343 ± 0.062%) contents in Madhupur soils further support this interpretation, suggesting greater litter decomposition and microbial turnover. This aligns with previous findings that fine-textured and humid forest soils retain more organic matter and sustain higher microbial activity (Xia *et al.* 2020). By contrast, the coarser texture and significantly higher sand fraction in Bhawal (38.1 ± 11.51%) imply lower water-holding capacity and nutrient retention. Such edaphic limitations likely stem from anthropogenic disturbance, as Bhawal is located closer to urbanized and industrial regions, experiencing greater deforestation, soil compaction, and topsoil erosion (Rahman *et al.* 2022). The lower microbial abundance (BC and FC) and reduced Rs in Bhawal further confirm the consequences of this degradation on biological functioning (Solanki *et al.* 2024).

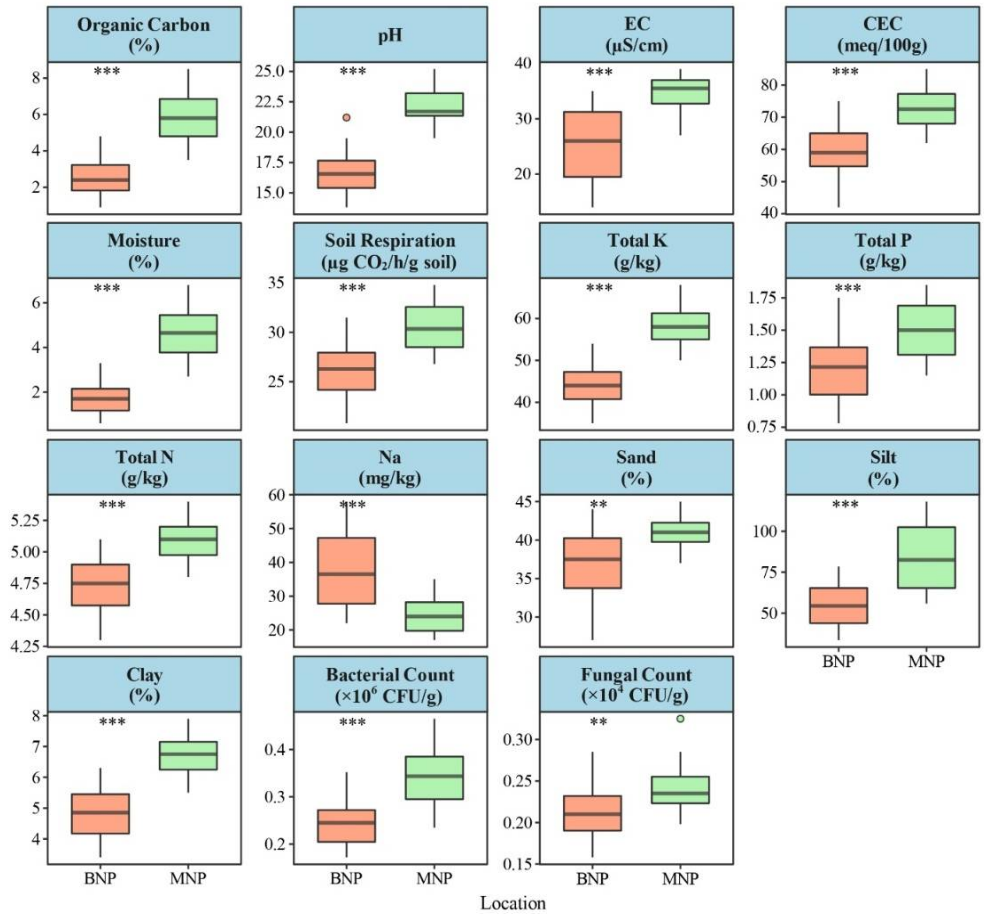

Fig. 1. Boxplots comparing soil physicochemical properties and respiration between the Bhawal National Forest (BNP) and Madhupur National Forest (MNP) of Bangladesh. Significance levels are indicated by asterisks ($p < 0.05$ = *, $p < 0.01$ = **, $p < 0.001$ = ***).

Vertical stratification also influenced several parameters, with OC, TN, Rs, BC, and FC all significantly higher in the upper soil layer ($p < 0.001$), confirming the biological richness of surface soils (Fig. 2, Table 1). This surface enrichment results from Sal leaf litter decomposition, root exudation, and active microbial recycling (Propa *et al.* 2021). Conversely, the lower layer exhibited increased Na ($p < 0.001$) but decreased EC ($p < 0.01$), suggesting differential ionic redistribution or localized leaching with depth (Ahmed *et al.* 2010). Soil pH and texture fractions (sand, silt, clay) remained largely unchanged across depths, indicating a relatively uniform parent material influence within the top 30 cm of the profile (Zhou *et al.* 2020). These patterns collectively indicate that vertical gradients in biological and nutrient variables are mainly driven by organic inputs and microbial processes rather than by mineralogical or textural differences (Naylor *et al.* 2022).

Table 1. Two-way ANOVA results for soil properties across locations and soil depths.

Variable	Location		Layer		Location × Layer	
	F-value	P-value	F-value	P-value	F-value	P-value
OC	46.8	<0.001	78.5	<0.001	0.0126	0.911
pH	35	<0.001	2.55	0.119	6.91E-30	1
EC	39.2	<0.001	9.65	0.00368	0.00215	0.963
CEC	120	<0.001	9.49	0.00394	0.0128	0.91
MC	33.7	<0.001	5.46	0.0251	0.0697	0.793
Rs	147	<0.001	158	<0.001	5.86	0.0207
TK	61.2	<0.001	1.73	0.197	0.00392	0.95
TP	8.06	0.00739	0.196	0.661	0.0217	0.884
TN	63.5	<0.001	40.9	<0.001	0.62	0.436
Na	112	<0.001	13.1	<0.001	0.504	0.482
Sand	21.1	<0.001	0.118	0.734	9.28E-31	1
Silt	12.6	0.00109	0.682	0.414	4.84E-31	1
Clay	29.8	<0.001	1.35	0.253	1.12E-30	1
BC	121	<0.001	32.1	<0.001	3.04	0.0899
FC	162	<0.001	30.7	<0.001	5.37	0.0263

OC: Organic carbon, EC: Electrical conductivity, CEC: Cation exchange capacity, MC: Moisture content, Rs: Soil respiration, TK: Total potassium, TP: Total phosphorus, TN: Total nitrogen, Na: Sodium, BC: Bacterial count, FC: Fungal count.

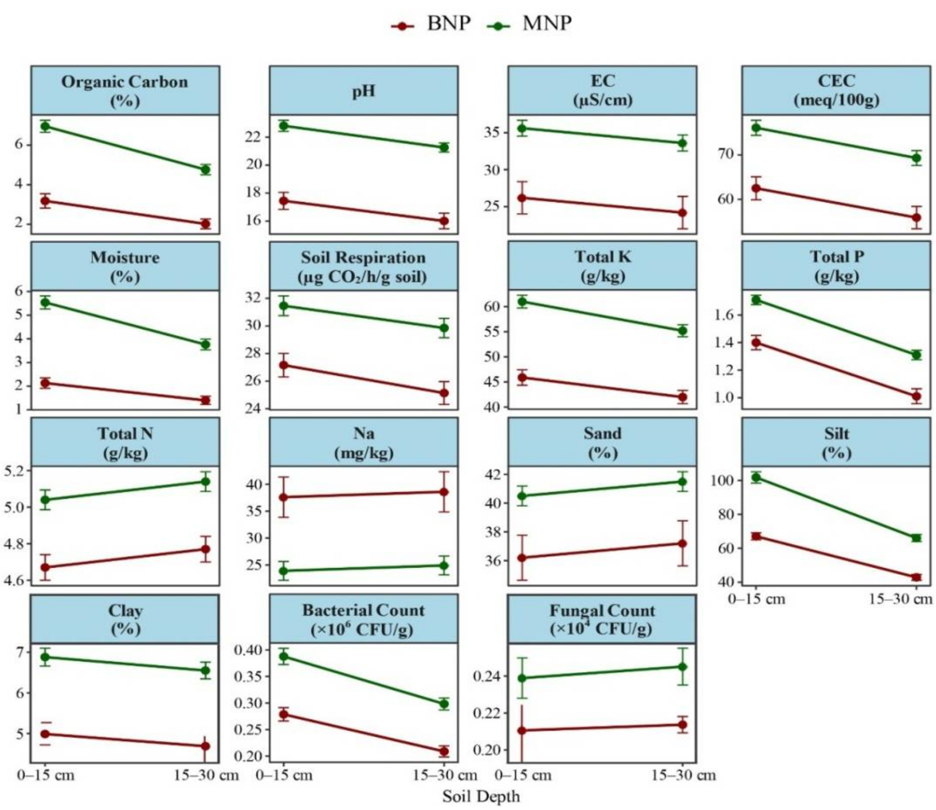

Fig. 2. Line graph depicting the variation in soil physicochemical properties and respiration between the upper (0-15 cm) and lower (15-30 cm) soil depths.

The radar plot visually supported these trends, showing higher values of OC, TN, CEC, EC, Rs, BC, FC, and MC in the upper layer and consistently greater mean values across nearly all parameters in Madhupur (Fig. 3). The only notable exception was sand, which was more abundant in Bhawal soils, reflecting their coarser texture and reduced fertility potential. Thus, both the spatial and vertical patterns highlight a strong linkage between soil texture, organic matter accumulation, and microbial processes that shape ecosystem productivity in these tropical deciduous forests (Jiang *et al.* 2015, Rodrigues *et al.* 2021).

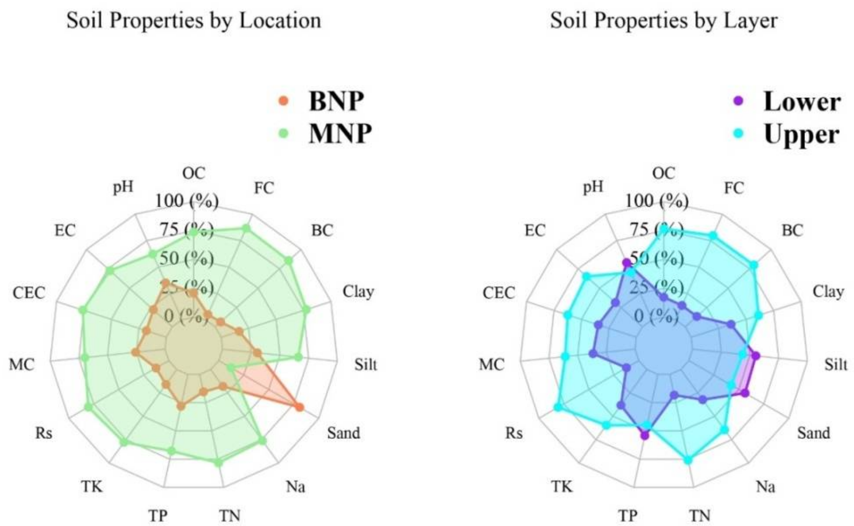

Fig. 3. Radar plots showing multivariate soil property profiles across (A) locations (BNP and MNP) and (B) soil layers (Upper and Lower).

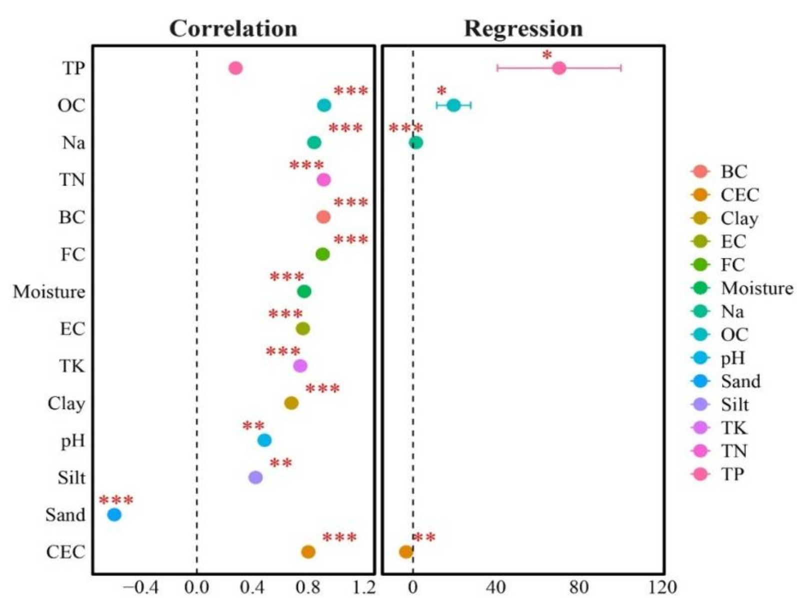

Fig. 4. Correlation and regression analyses among soil properties and predictors of soil respiration (Rs), where significance levels are indicated by asterisks ($p < 0.05$ = *, $p < 0.01$ = **, $p < 0.001$ = ***).

Correlation and regression analyses revealed tight interconnections among the soil properties and identified the main drivers of Rs (Fig. 4). Positive associations were observed among OC, TN, TP, BC, FC, and MC, indicating that nutrient-rich and biologically active soils promote higher CO_2 efflux (Fig. 4). Clay content and EC, however, showed negative correlations with Rs and other fertility indicators, implying that finer texture and salinity may restrict oxygen diffusion and thereby limit microbial activity (Vieira *et al.* 2025). Regression models confirmed that OC, TP, and Na were the most influential positive predictors of Rs (β = 21, 72, and 4, respectively) (Fig. 4), demonstrating that substrate and nutrient availability predominantly control soil respiration (Wu *et al.* 2020). Conversely, the negative effect of CEC (β = -8, $p < 0.01$) suggests that although higher nutrient-retention capacity benefits long-term fertility, it may reduce immediate CO_2 fluxes by stabilizing organic matter and limiting decomposition (Fontaine *et al.* 2024).

The multivariate analysis further supported the spatial and vertical heterogeneity of soil properties across the two Sal forests. PERMANOVA results (Table 2) indicated that forest location had a highly significant effect on overall soil physicochemical characteristics (F = 5513.5, R^2 = 0.993, p = 0.001), explaining nearly 99% of the total variation. Although soil depth alone did not exert a statistically significant influence (p = 0.348), the interaction between location and depth was also significant (F = 1204.3, R^2 = 0.990, p = 0.002), suggesting that vertical variation patterns differed between sites. The NMDS ordination (stress = 0.191, Fig. 5a) corroborated these results by showing clear clustering of samples based on both forest location and soil depth, indicating strong spatial and vertical structuring of soil composition. Vector fitting identified FC (R^2 = 0.6399, $p < 0.001$), Rs (R^2 = 0.5765, $p < 0.001$), and BC (R^2 = 0.5536, $p < 0.001$) as the dominant variables shaping the ordination structure, followed by nutrient-related parameters such as TN, OC, EC, and CEC (Fig. 5b). These patterns highlight that biological activity and nutrient gradients are the key determinants of soil variability within and between the two forests.

Table 2. PERMANOVA results for overall soil physicochemical properties across geographic locations and soil depths.

Factor	DF	SS	R^2	F	P-value
Location	1	11611	0.993	5513.5	0.001
Layer	1	8043.0	0.688	83.785	0.348
Location × Layer	3	11575.5	0.990	1204.3	0.002

DF: degrees of freedom, SS: sum of squares, R^2: variance explained. Analysis based on 999 permutations.

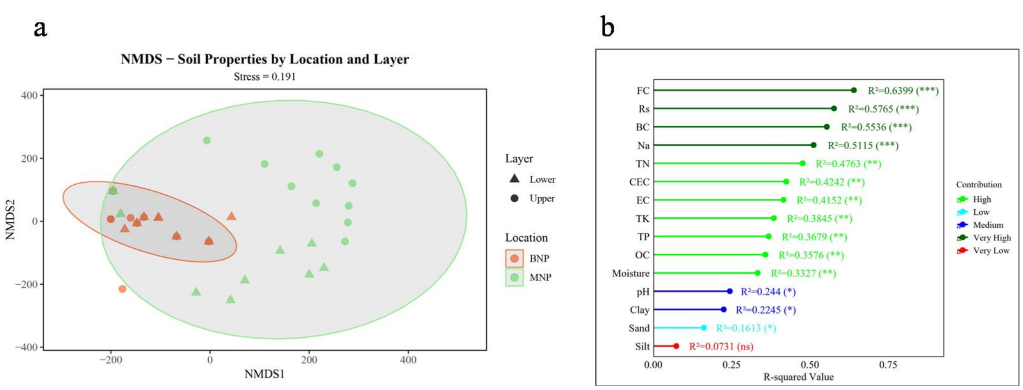

Fig. 5. NMDS analysis of soil properties showing (a) ordination plot, and (B) variable contributions ranked by R-squared values with significance levels (***p <0.001, **p <0.01, *p <0.05, ns = not significant).

In conclusion, Madhupur soils are characterized by higher organic matter, nutrient concentrations, and microbial activity compared to Bhawal, reflecting their finer texture, higher moisture content, and better preservation status. Bhawal's coarser, sandier soils exhibit lower fertility and microbial potential, likely due to more intense anthropogenic disturbance and topsoil loss. Vertically, the upper soil layer in both the forests supports higher organic matter and microbial populations, driving greater respiration and nutrient cycling. Thus, soil heterogeneity in the Sal forests of Bangladesh is governed primarily by organic matter dynamics and microbial processes, with texture and ionic composition modulating their intensity. These results underscore the need for conservation and management strategies that protect surface soil integrity and sustain microbial-driven nutrient processes, particularly in the more degraded Bhawal ecosystem.

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