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Soil Fertility, Acidity and Microbial Indicators across the Agro-Ecological Zones of Bangladesh

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Abstract

Aims: Soil properties and microbial communities jointly influence nutrient cycling, soil fertility, and agricultural sustainability, yet their distribution across the agro-ecological zones (AEZs) of Bangladesh remains insufficiently characterized at the national scale. This study aimed to evaluate soil physicochemical properties, culturable microbial abundance, and previously published bacterial community data to establish a national baseline for soil health assessment.

Methods: A total of 62 composite topsoil samples collected through the semi-detailed soil survey program of the Soil Resource Development Institute represented 27 of Bangladesh's 30 AEZs and were classified into seven broad physiographic units. Soil texture, pH, organic matter (OM), total nitrogen (N), available phosphorus (P), potassium (K), sulfur (S), boron (B), zinc (Zn), and the trace metals lead (Pb), cadmium (Cd), nickel (Ni), and chromium (Cr) were assessed using standard analytical methods. Culturable bacteria, fungi, and rhizobia were enumerated in 42 samples from 22 AEZs, while bacterial richness and community composition were examined using previously published 16S rRNA gene amplicon sequencing data covering 14 AEZs. Spearman correlation and Kruskal–Wallis tests were used to assess associations and differences among groups.

Results: The soils were predominantly silt loams, with a median pH of 5.2 and median OM content of 1.9%; 58% of samples had pH values below 5.5. Total N was classified as low in 95% of samples, while K and B were classified as low in 71%; approximately one-quarter of samples had very high available P. Cd and Cr concentrations were below the applicable maximum allowable limits, whereas Ni exceeded 20 mg kg^{-1} in 38 of 51 samples and was positively associated with clay content (Spearman's $\rho = 0.62$), suggesting a potential influence of soil mineralogy and parent material. Culturable bacterial counts ranged from 2.1×10^6 to 1.85×10^9 CFU g^{-1}, fungal counts from 1.0×10^5 to 7.0×10^7 CFU g^{-1}, and rhizobial counts from 1.0×10^5 to 1.7×10^8 CFU g^{-1}. Culturable microbial counts did not differ significantly among physiographic groups and showed weak associations with the measured soil

properties. The published 16S rRNA dataset contained 544 operational taxonomic units (OTUs) assigned to 341 genera, with reported dominant groups including *Planctomycetota*, *Anaerolinea*, and *Clostridium*. Bacterial richness ranged from 208 to 445 OTUs, with the highest richness near pH 6 and lower richness in strongly acidic Madhupur and hill soils. These patterns identify soil acidity as a candidate environmental correlate of bacterial richness, although the observational design does not establish causation.

Conclusions: Overall, the findings reveal widespread soil acidity and nutrient limitations alongside substantial variation in culturable microbial abundance and reported bacterial richness across Bangladesh's AEZs. The results provide a basis for prioritizing soil-acidity management, organic-matter restoration, balanced nutrient management, and replicated soil-biological monitoring. Further standardized sampling and molecular analyses across all AEZs are needed to establish more robust links between soil properties, microbial community structure, and soil functions.

Keywords: Agro-ecological zones; soil microbial ecology; soil fertility; rhizobia; 16S rRNA gene sequencing; soil acidity; Bangladesh.

1. Introduction

Bangladesh feeds more than 170 million people from about 8.5 million hectares of cultivable land, with one of the highest cropping intensities in the world. Much of this land is cropped two or three times a year and receives heavy doses of urea, triple superphosphate and muriate of potash, while organic inputs have declined as crop residues and cow-dung are diverted to fuel and fodder. Declining soil organic matter, nutrient imbalance, acidification of terrace and piedmont soils, and coastal salinity are widely recognized threats to soil productivity.

Soil fertility, however, is not only a matter of chemistry. A single gram of fertile soil can contain around 10^9 bacterial cells belonging to thousands of genomically distinct types (Torsvik and Øvreås, 2002), together with fungal hyphae, protozoa and viruses. These organisms decompose organic matter, cycle carbon, nitrogen, phosphorus and sulphur, form symbioses with roots, suppress pathogens and regulate greenhouse-gas fluxes (Bardgett and van der Putten, 2014; Fierer, 2017). Soil pH is the single strongest predictor of bacterial community composition and diversity at regional to continental scales (Fierer and Jackson, 2006; Lauber et al., 2009; Rousk et al., 2010).

Bangladesh is divided into 30 agro-ecological zones (AEZs) that integrate physiography, soils, flooding regime and agro-climate (FAO/UNDP, 1988). The AEZ framework already underpins

crop zoning, the Fertilizer Recommendation Guide (BARC, 2018) and the Upazila land and soil resource guides, but these tools describe soils almost exclusively in physical and chemical terms. If microbial communities differ systematically among AEZs, soil-biological management — liming, organic amendments and biofertilizers — could be targeted by zone in the same way as mineral fertilizers. Previous Bangladeshi studies have examined individual environments, such as Sundarbans mangrove soils (Das et al., 2024), arsenic-affected soils (Sanyal et al., 2016) and arbuscular mycorrhizal fungi in Dinajpur (Mridha and Dhar, 2007), and a first sequencing survey of the soil bacteriome of 14 AEZs has recently been published (Islam et al., 2025). A national, AEZ-wise synthesis linking microbial abundance and diversity to soil properties is still lacking.

The objectives of this study were therefore (i) to characterise the physical and chemical properties, including heavy metals, of topsoils sampled across the AEZs of Bangladesh; (ii) to quantify the abundance of culturable bacteria, fungi and rhizobia and the fungal-to-bacterial balance; (iii) to relate microbial abundance and bacterial richness to soil properties and physiographic setting; and (iv) to derive implications for soil-health management and future monitoring.

2. Materials and Methods

2.1 Study area and physiographic grouping

The Bangladesh AEZ system comprises 30 agro-ecological regions, 88 sub-regions and 535 agro-ecological units, derived by overlaying physiography, soils, depth and duration of seasonal flooding, length of the rainfed growing period, pre-kharif rainfall reliability, length of the cool winter period and the frequency of temperature extremes (FAO/UNDP, 1988). Zones range in area from St. Martin's Coral Island (about 8 km²) to the Northern and Eastern Hills (about 18,172 km²). For analysis, the 30 AEZs were grouped into seven physiographic groups that share parent material, relief and hydrology: Piedmont (AEZ-1, 22), Active floodplain (2, 7, 10), Meander floodplain (3, 4, 8, 9, 11, 12, 16, 17, 20), Basin/Bil (5, 6, 14, 15, 21), Tidal/Estuarine (13, 18, 19, 23, 24), Terrace (25–28, 30) and Hills (29). Sampling sites are shown in Fig. 1.

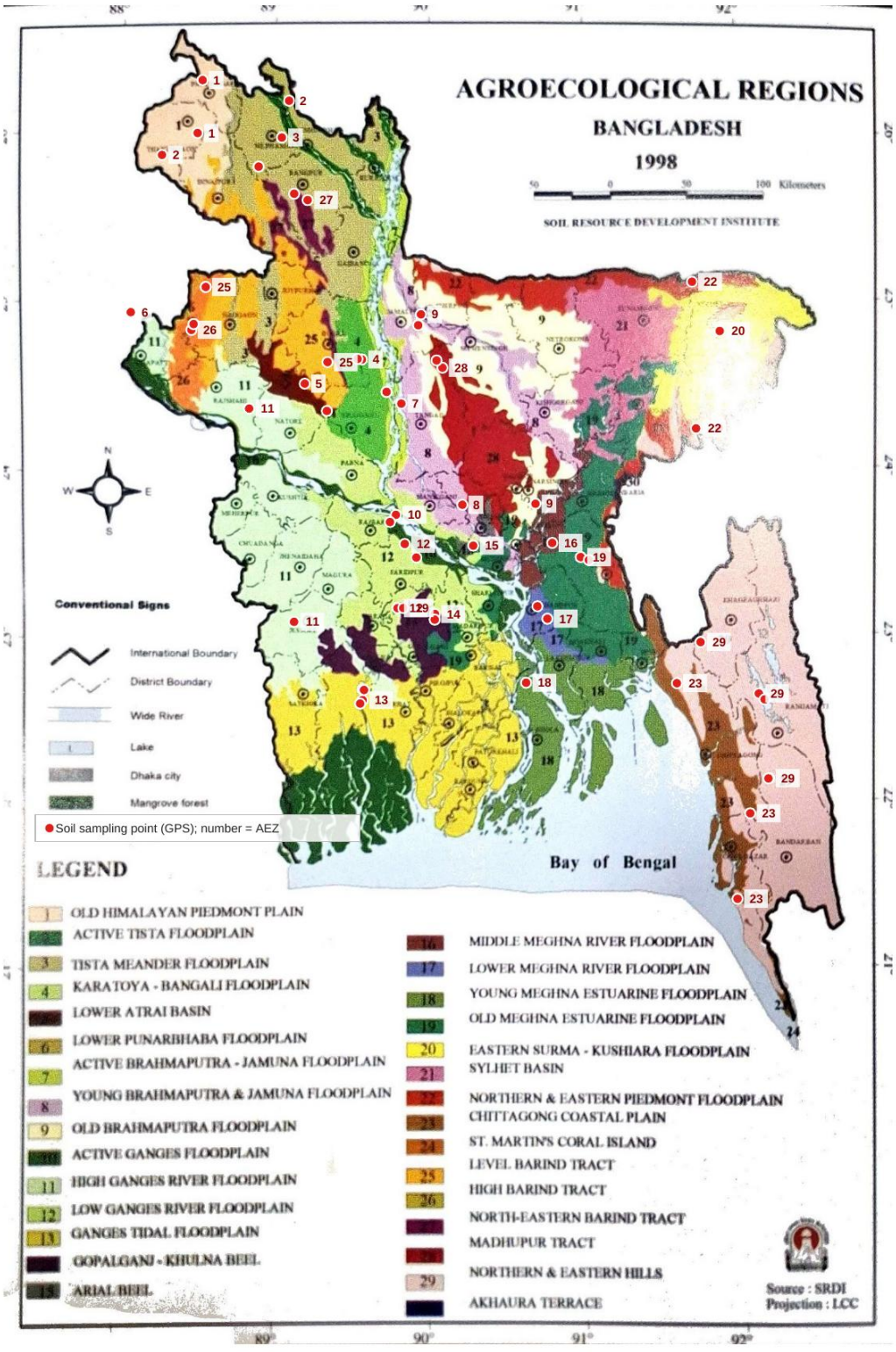


Figure 1. Location of the 62 soil sampling sites (red dots; number = AEZ) on the Agroecological Regions map of Bangladesh. Base map: SRDI (1998); sampling points plotted from GPS coordinates.

2.2 Soil survey and sampling

Soils were sampled through the semi-detailed soil survey programme of the Soil Resource

Development Institute (SRDI), which updates the Upazila Land and Soil Resource Utilization Guides. Upazila soil and landform maps (1:50,000), aerial photographs, topographic and mouza maps served as base materials, and one composite sample was collected for approximately every 200 ha, with GPS readings, land type, inundation depth and cropping pattern recorded at each site (Fig. 2). Each composite topsoil sample was made from at least nine sub-samples taken 1.0–1.5 m inside the field boundaries, cut as a 7–8 cm slice down to the plough pan, mixed and quartered to 400–500 g. In total, 62 sample records were obtained from 27 AEZs; no samples were available from AEZ-21 (Sylhet Basin), AEZ-24 (St. Martin's Coral Island) or AEZ-30 (Akhaura Terrace).

2.3 Physical and chemical analyses

Particle-size distribution was determined by the hydrometer method and texture classes assigned from the USDA triangle (Bouyoucos, 1962; Gee and Bauder, 1986). Soil pH was measured with a glass electrode at a soil:water ratio of 1:2.5 (Jackson, 1973); organic carbon by wet oxidation (Walkley and Black, 1934), converted to organic matter (OM) with a factor of 1.73; total N by micro-Kjeldahl digestion (Bremner and Mulvaney, 1982); available P by the Bray–Kurtz method for pH < 7 and the Olsen method for pH ≥ 7 (Bray and Kurtz, 1945; Olsen et al., 1954); exchangeable K by 1 M NH_4OAc extraction; available S by 0.15% $CaCl_2$ extraction and turbidimetry (Tabatabai, 1996); available B by hot-water extraction and azomethine-H colorimetry (Berger and Truog, 1939); and available Zn by DTPA extraction (Lindsay and Norvell, 1978). Pb, Cd, Ni and Cr were determined by acid digestion and atomic absorption spectrometry. Nutrient status was interpreted using the critical levels of the Fertilizer Recommendation Guide (BARC, 2018): total N 0.12%, P 8 μg g^{-1}, K 0.12 meq 100 g^{-1}, S 10 μg g^{-1}, B 0.2 μg g^{-1} and Zn 0.6 μg g^{-1}. Heavy metals were compared with the maximum allowable concentration (MAC) ranges for agricultural soils given by Kabata-Pendias (2011).

2.4 Enumeration of culturable micro-organisms

Culturable micro-organisms were enumerated by serial dilution and plate counting. Ten grams of fresh soil were shaken in 90 mL sterile saline and diluted ten-fold in series; aliquots were spread on nutrient agar for total heterotrophic bacteria, rose-bengal or potato-dextrose agar for total fungi (Martin, 1950) and yeast-extract mannitol agar with Congo red for rhizobia (Vincent, 1970). After incubation at 28–30 °C, colonies were counted and expressed as colony-forming units per gram of soil (cfu g^{-1}). Colonies on YEMA are regarded as rhizobia-like, because

confirmation requires nodulation of a host plant.

2.5 Bacterial community data

Bacterial community composition and richness were taken from the companion study of Islam et al. (2025), who sampled the 0–5 cm layer of 14 AEZs (AEZ-2, 4, 5, 10, 12, 13, 16, 17, 19, 25, 26, 27, 28 and 29) between March and August 2024, pooling three cores per AEZ. DNA was extracted with the Qiagen DNeasy PowerSoil kit, the V3–V4 region of the 16S rRNA gene was amplified with primers 341F/806R and sequenced on an Illumina MiSeq (2 × 300 bp). Reads were quality-checked (FastQC, Trimmomatic) and processed in QIIME 2; sequences with ≥ 98% similarity were clustered into OTUs and classified against SILVA v138. Raw data are available under NCBI BioProject PRJNA1181648.

2.6 Data analysis

Microbial counts were $\log_{10}$-transformed before analysis and summarised by median and geometric mean. The fungal-to-bacterial ratio (F:B) was calculated from cfu counts as a rough index of the relative importance of fungal and bacterial energy channels (Strickland and Rousk, 2010). Relationships between microbial counts and soil properties were tested with Spearman rank correlation (ρ), and differences among physiographic groups with the Kruskal–Wallis test, both of which are robust to small and uneven sample sizes. Records whose sand, silt and clay fractions did not sum to 100 ± 6%, and microbial records in which rhizobia exceeded total bacteria, were retained in the dataset but excluded from the analyses that depend on them.

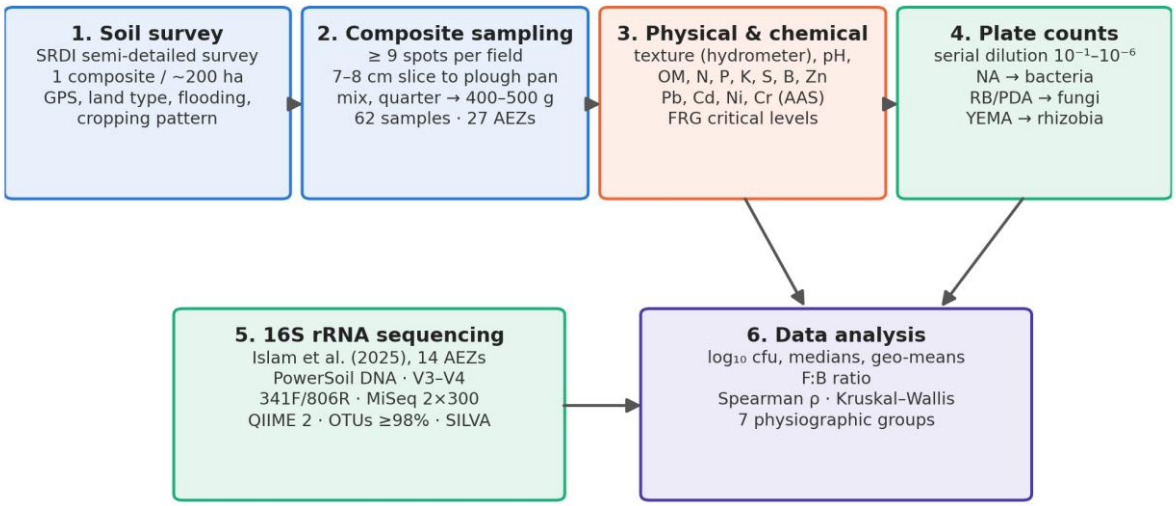


Figure 2. Schematic overview of the experimental workflow used for soil sampling, physicochemical characterization, microbial enumeration, 16S rRNA gene sequencing, and statistical analysis. The workflow

comprised (1) a semi-detailed soil survey and site characterization; (2) composite soil sampling from multiple spots within each field; (3) determination of soil physical and chemical properties; (4) culture-based enumeration of bacteria, fungi, and rhizobia using serial dilution and selective media; (5) 16S rRNA gene sequencing of selected samples; and (6) statistical analysis of microbial abundance and its relationships with soil and physiographic variables.

3. Results

3.1 Physical properties

Texture was determined for 51 samples, of which 44 passed the particle-size check. The soils were dominated by silt, with a median composition of about 22% sand, 55% silt and 16% clay; silt loam was by far the most common texture class, followed by loam and silty clay loam (Fig. 3). The Old Himalayan Piedmont (AEZ-1: 49–61% sand) and Hill soils (AEZ-29: 37–66% sand) were the coarsest, and a Jamuna char soil (AEZ-7) was almost pure sand (92%). At the other extreme, soils of the Lower Atrai Basin (AEZ-5: 39–42% clay) and the Ganges Tidal Floodplain (AEZ-13: up to 35% clay) were the heaviest. Bulk density, measured on a single soil (Gangachara series, AEZ-3), was 1.47 g cm^{-3} with 41.4% porosity, indicating moderate compaction consistent with a plough pan beneath puddled rice soils.

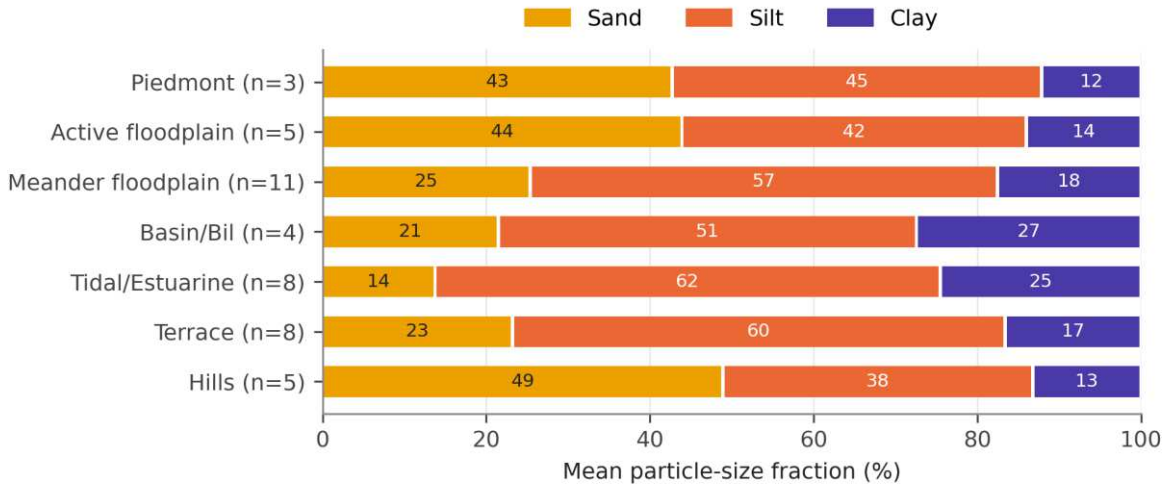


Figure 3. Mean sand, silt and clay fractions by physiographic group (44 samples with valid particle-size data; fractions normalized to 100%).

3.2 Chemical properties and heavy metals

Soil pH ranged from 3.4 (Tejgaon series, Madhupur Tract, AEZ-28) to 7.4, with a median of 5.2 (Fig. 4a). More than half of the samples (34 of 59) were strongly or very strongly acidic

(pH < 5.5) and 10 were very strongly acidic (pH < 4.5). Acid soils were concentrated in the Old Himalayan Piedmont Plain, the Tista floodplains, the Old Brahmaputra Floodplain, the Surma–Kushiyara Floodplain, the Madhupur Tract and the Northern and Eastern Hills, whereas neutral to slightly alkaline soils occurred in the Ganges floodplains and the Ganges Tidal Floodplain. Organic matter ranged from 0.07% in a fresh Jamuna char sand to 20.1% in a peat soil of the Gopalganj–Khulna Bils (AEZ-14), with a median of 1.9% (Fig. 4b); about one-third of samples (21 of 59) had low or very low OM (< 1.7%).

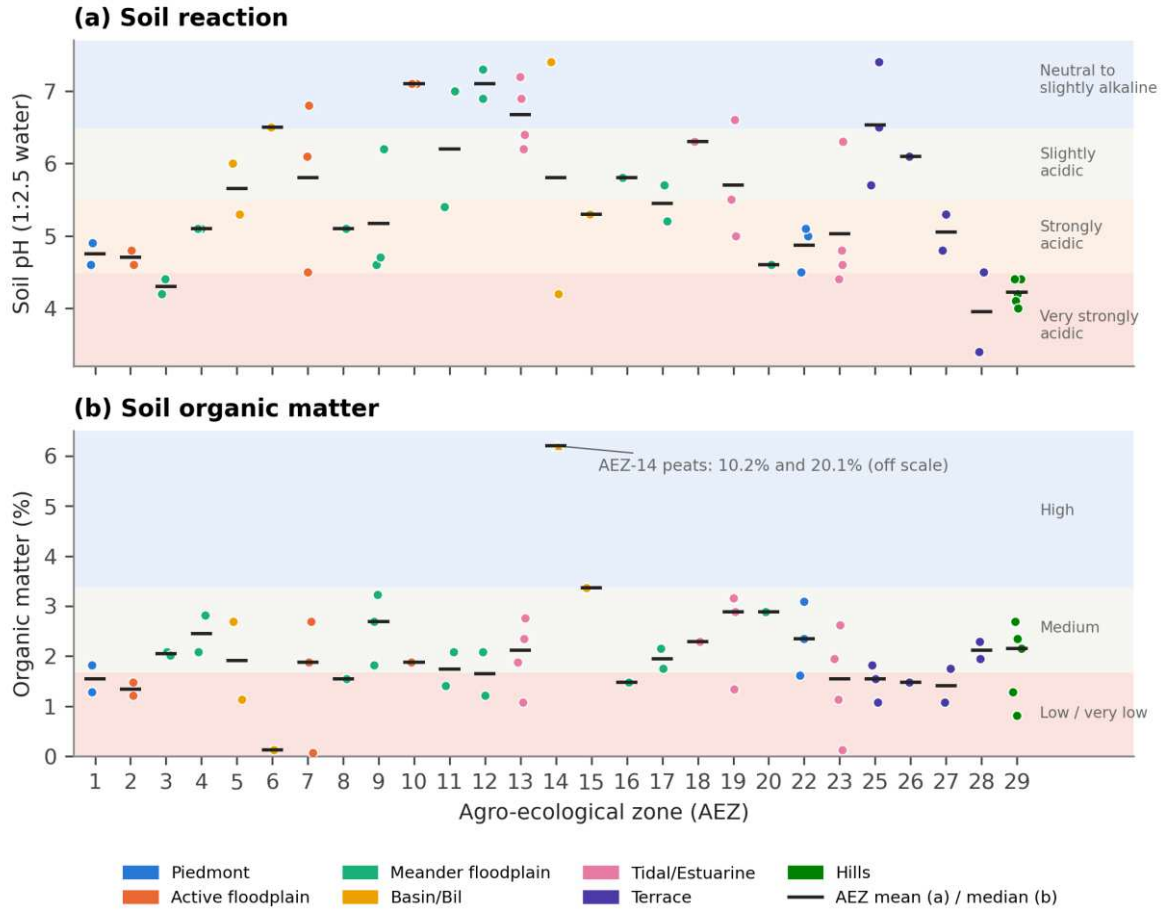


Figure 4. (a) Soil pH and **(b)** organic matter of individual samples by AEZ, colored by physiographic group, against standard interpretation classes. Horizontal bars show AEZ means (pH) and medians (OM).

Nitrogen was the most widespread limitation (Fig. 5): 95% of samples were low or very low in total N (0.01–0.20%, median 0.11%). Potassium (0.05–0.83 meq 100 g^{-1}) and boron were each low or very low in 71% of samples. Phosphorus was highly variable (0.04–189 µg g^{-1}): nearly half the samples were low or very low, but a quarter were very high, reflecting long-term P fertilization. Sulphur was medium or better in about two-thirds of samples, with very high values in the Ganges Tidal and High Ganges floodplains. Median heavy-metal concentrations (n = 51; Fig. 6) were Pb 15.1, Cd 0.20, Ni 37.0 and Cr 20.8 mg kg^{-1}. All Cd and Cr values were

below the lower MAC bounds; Pb exceeded the lower bound (20 mg kg^{-1}) in 10 samples but none approached the upper bound. Nickel exceeded 20 mg kg^{-1} in 38 of 51 samples and 60 mg kg^{-1} in three, and was strongly correlated with clay content ($\rho = +0.62$, $p < 0.001$), suggesting a largely geogenic origin. Median properties by physiographic group are summarised in Table 1.

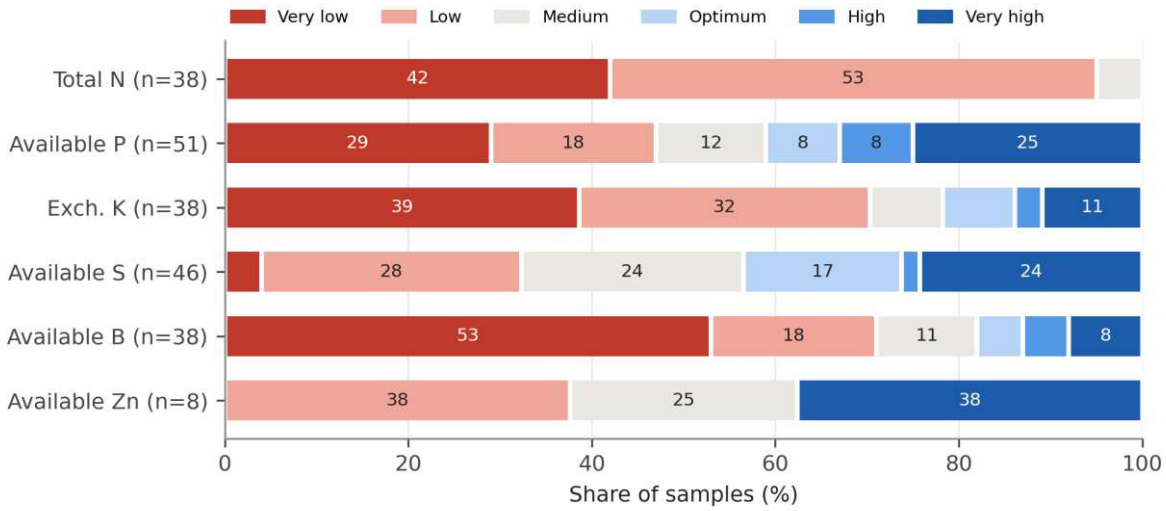


Figure 5. Distribution of samples among nutrient status classes of the Fertilizer Recommendation Guide (BARC, 2018). Numbers inside bars are percentages of samples.

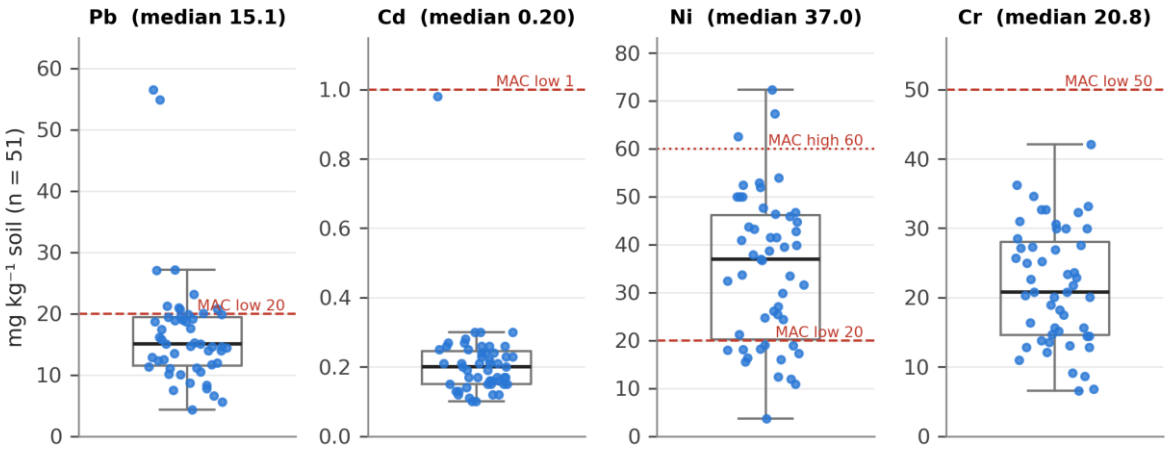


Figure 6. Distribution of heavy metal concentrations in soil samples. Box-and-jitter plots show the concentrations of lead (Pb), cadmium (Cd), nickel (Ni), and chromium (Cr) in soil samples (n = 51), expressed as mg kg^{-1} soil. Individual points represent individual soil samples, the horizontal line within each box indicates the median, boxes represent the interquartile range, and whiskers show the distribution of

observations. Median concentrations were 15.1 mg kg^{-1} for Pb, 0.20 mg kg^{-1} for Cd, 37.0 mg kg^{-1} for Ni, and 20.8 mg kg^{-1} for Cr. Red dashed/dotted horizontal lines indicate the corresponding maximum allowable concentrations (MACs) or reference thresholds for agricultural soils (Kabata-Pendias, 2011); the dotted line marks the upper Ni bound.

Table 1. Median soil properties by physiographic group.

Group	n	pH	OM (%)	Clay (%)	N (%)	P (µg g^{-1})	K (meq 100 g^{-1})	S (µg g^{-1})	Ni (mg kg^{-1})
Piedmont	5	4.9	1.82	10.5	0.08	22.9	0.12	13.1	16.0
Active floodplain	7	6.1	1.88	9.2	0.12	7.2	0.13	18.7	27.1
Meander floodplain	18	5.2	2.08	16.6	0.11	12.2	0.10	23.6	45.3
Basin/Bil	6	5.7	3.02	29.9	0.08	4.7	0.10	24.1	42.6
Tidal/Estuarine	12	6.2	2.12	21.4	0.10	8.9	0.13	19.6	42.3
Terrace	9	5.5	1.65	15.9	0.14	28.4	0.65	26.8	19.0
Hills	5	4.2	2.15	13.3	–	16.6	–	–	33.5

Clay medians include all samples with texture data; the Hills group consists of AEZ-29 only; "–" = not determined.

3.3 Abundance of culturable bacteria, fungi and rhizobia

Microbial counts were available for 42 samples from 22 AEZs (Table 2; Fig. 7). Total bacteria spanned almost three orders of magnitude, from 2.1×10^{6} to 1.85×10^{9} cfu g^{-1}, with a median of 2.2×10^{8} and a geometric mean of 1.5×10^{8} cfu g^{-1}. Four samples exceeded 10^{9} cfu g^{-1} — two from the Tista Meander Floodplain (AEZ-3), a clay of the Low Ganges River Floodplain (AEZ-12) and Chandina (AEZ-19) — while six had fewer than 10^{7} cfu g^{-1}; several of the latter combined very low OM, coarse texture or strong acidity. Total fungi ranged from 1.0×10^{5} to 7.0×10^{7} cfu g^{-1} (median 1.0×10^{7}) and rhizobia from 1.0×10^{5} to 1.7×10^{8} cfu g^{-1} (median 1.0×10^{7}). The rhizobial distribution was bimodal: 27 samples had $\geq 10^{7}$ cfu g^{-1} and 15 had $< 10^{6}$ cfu g^{-1}.

Table 2. Summary of culturable microbial populations (cfu g^{-1} soil; n = 42 samples from 22 AEZs).

Group	Minimum	Maximum	Median	Geometric mean
Total bacteria	2.1×10^{6}	1.85×10^{9}	2.2×10^{8}	1.5×10^{8}
Total fungi	1.0×10^{5}	7.0×10^{7}	1.0×10^{7}	6.4×10^{6}
Rhizobia (YEMA)	1.0×10^{5}	1.7×10^{8}	1.0×10^{7}	3.4×10^{6}

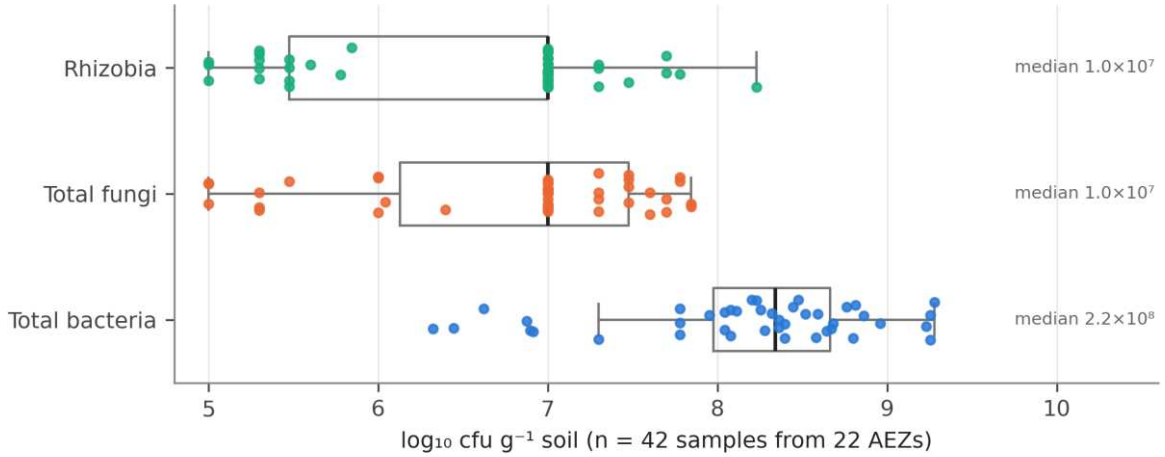


Figure 7. Culturable microbial abundance in soil samples. Box-and-jitter plots show the abundance of rhizobia, total fungi, and total bacteria across 42 soil samples from 22 Agro-Ecological Zones (AEZs). Microbial abundance is expressed as $\log_{10}$ CFU g^{-1} soil. Individual points represent samples, boxes indicate the interquartile range, horizontal lines within boxes indicate medians, and whiskers represent the distribution of observations. Median abundances were 1.0×10^7 CFU g^{-1} for rhizobia and total fungi and 2.2×10^8 CFU g^{-1} for total bacteria.

On the basis of AEZ means, the silt-rich, seasonally flooded meander-floodplain and basin soils of the north-west — AEZ-3, 4, 5, 11 and 12 — hosted the largest bacterial populations (4.7×10^8 to about 10^9 cfu g^{-1}), whereas the Old and Young Brahmaputra floodplains (AEZ-8, 9) showed low counts of all three groups (Fig. 8; Table 3). Fungi were most abundant where soils remain wet for long periods and are rich in clay or organic matter (AEZ-5, 13, 14) and in the acidic hill soils of AEZ-29.

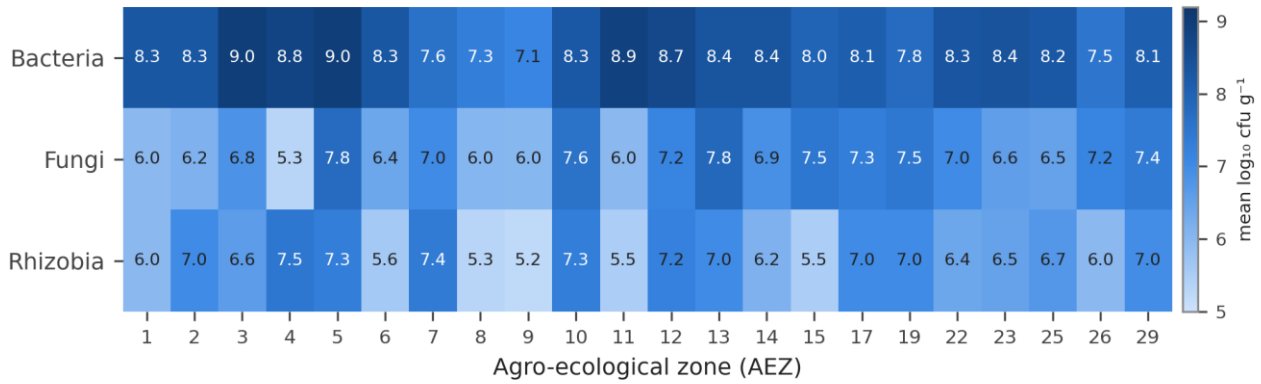


Figure 8. Distribution of culturable bacteria, fungi, and rhizobia across agro-ecological zones (AEZs). The heatmap presents the mean microbial abundance in soil samples from different AEZs, expressed as $\log_{10}$ CFU g^{-1} soil. Each cell shows the mean abundance of bacteria, fungi, or rhizobia within the corresponding

AEZ, with darker shading indicating higher microbial abundance. Bacterial abundance was consistently higher than fungal and rhizobial abundance across most AEZs, whereas rhizobial abundance showed greater spatial variation.

Table 3. Microbial "hotspots" and "cold spots" among AEZs, based on AEZ mean counts (cfu g^{-1}).

Group	Highest AEZ means	Lowest AEZ means
Total bacteria	AEZ-3 Tista Meander FP and AEZ-5 Lower Atrai Basin (≈10^9); AEZ-11 High Ganges FP (7.3×10^8); AEZ-4 Karatoya-Bangali FP (6.5×10^8); AEZ-12 Low Ganges FP (4.7×10^8)	AEZ-9 Old Brahmaputra FP (1.3×10^7); AEZ-8 Young Brahmaputra FP (2.0×10^7); AEZ-26 High Barind (3.2×10^7); AEZ-7 Active Brahmaputra-Jamuna FP (4.1×10^7)
Total fungi	AEZ-13 Ganges Tidal FP (7.0×10^7); AEZ-5 Lower Atrai Basin (6.0×10^7); AEZ-10 Active Ganges FP (4.0×10^7); AEZ-29 Hills (2.4×10^7)	AEZ-4 (2.0×10^5); AEZ-1 Old Himalayan Piedmont (1.0×10^6); AEZ-8, 9 and 11 (≈10^6)
Rhizobia	AEZ-4 (3.0×10^7); AEZ-7 (2.4×10^7); AEZ-5 and AEZ-10 (2.0×10^7); AEZ-12 (1.7×10^7)	AEZ-9 (1.6×10^5); AEZ-8 (2.0×10^5); AEZ-11 and AEZ-15 (3.0×10^5); AEZ-6 (4.0×10^5)

FP = floodplain. The AEZ-7 rhizobia mean is inflated by one questionable record in which rhizobia exceeded total bacteria.

3.4 Physiographic groups, fungal-to-bacterial balance and soil properties

Differences among physiographic groups were small relative to within-group variation and not statistically significant (Kruskal–Wallis: bacteria $p = 0.96$, fungi $p = 0.33$, rhizobia $p = 0.46$). The Basin/Bil, Tidal/Estuarine and Hills groups had somewhat higher mean fungal counts (≈10^7 cfu g^{-1}) than the Piedmont and Meander floodplain groups (≈3×10^6 cfu g^{-1}). The median F:B ratio was 0.038 (Fig. 9) — roughly one fungal colony for every 26 bacterial colonies — and 10 samples had F:B below 0.01, half of them meander-floodplain soils under intensive rice-based cropping (AEZ-3, 4, 9, 11). Five samples had F:B above 1, in most cases because the bacterial count was unusually low rather than because fungi were unusually abundant.

Relationships between culturable counts and soil properties were weak (Fig. 10). No correlation between pH or OM and any of the three microbial groups was significant ($|\rho| \leq 0.25$). The strongest associations were a negative tendency of bacteria with exchangeable K ($\rho = -0.37$, $p = 0.08$, $n = 24$) and positive tendencies of fungi with clay ($\rho = +0.30$) and S ($\rho = +0.27$). Rhizobial counts tended to decline with Ni ($\rho = -0.23$), Cr (-0.20) and Pb (-0.17), although none of these relationships was significant.

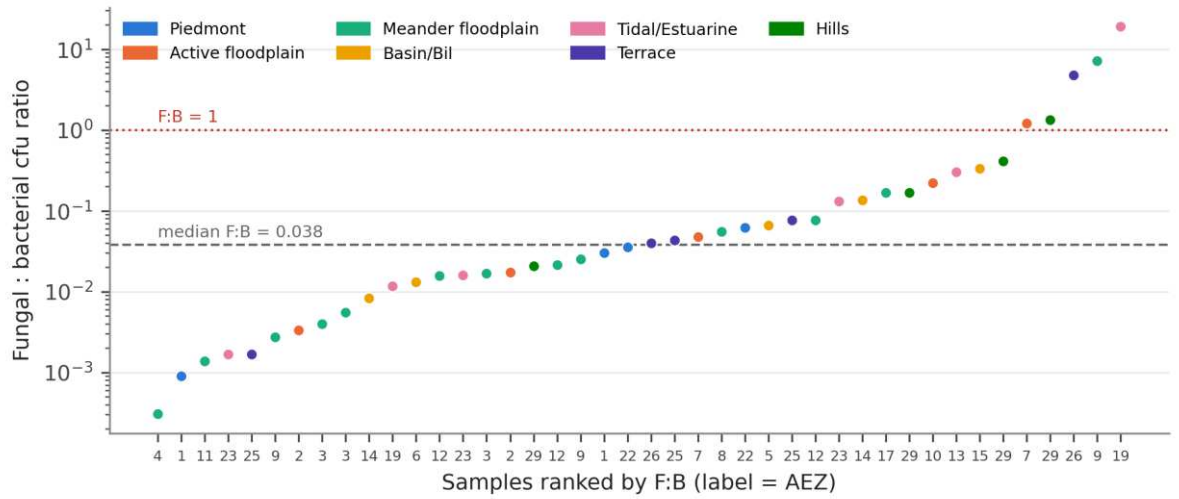


Figure 9. Fungal-to-bacterial cfu ratio (log scale) of each sample, ranked from lowest to highest and coloured by physiographic group.

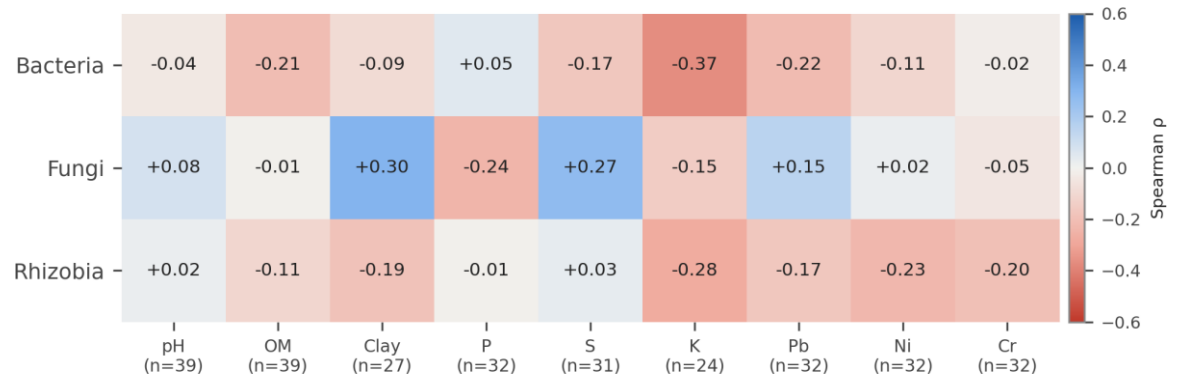


Figure 10. Spearman rank correlations (ρ) between microbial counts and soil properties; n = number of paired samples. Blue = positive, red = negative.

3.5 Bacterial community composition and richness

In the 16S rRNA dataset of Islam et al. (2025), 343,166 quality reads (from 7.2 million raw reads) were assigned to 544 OTUs belonging to 16 phyla, 45 classes, 77 orders, 151 families and 341 genera. The community was dominated by Planctomycetota — *Gemmata* (12.1%), *Planctomyces* (8.8%) and *Pirellula* (3.5%) together accounted for about a quarter of classified reads — followed by *Nocardioides* (Actinomycetota, 6.1%), *Anaerolinea* (Chloroflexota, 5.3%), *Solibacter* (Acidobacteriota, 4.3%), *Bacillus* (3.7%), *Clostridium* (3.6%) and *Rhodoplanes* (Pseudomonadota, 3.5%) (Fig. 11a).

Observed richness varied twofold among AEZs (Fig. 11b), from 208 OTUs (125 genera) in the

very strongly acidic Tejgaon soil of the Madhupur Tract (AEZ-28, pH 3.4) and 226 OTUs in the Hill soil (AEZ-29, pH 4.1) to 444–445 OTUs (280–290 genera) in the Karatoya-Bangali Floodplain (AEZ-4, pH 5.1) and the High Barind Tract (AEZ-26, pH 6.0). Richness rose from very acidic soils to a maximum at around pH 6, following a quadratic trend. OTU numbers were, however, strongly correlated with sequencing depth ($\rho = 0.90$). AEZ-4 combined high culturable bacteria (6.5×10^{8} cfu g^{-1}) with the highest OTU richness, whereas the acidic AEZ-28 and AEZ-29 soils had the lowest richness; elsewhere, culturable counts and sequence richness agreed poorly.

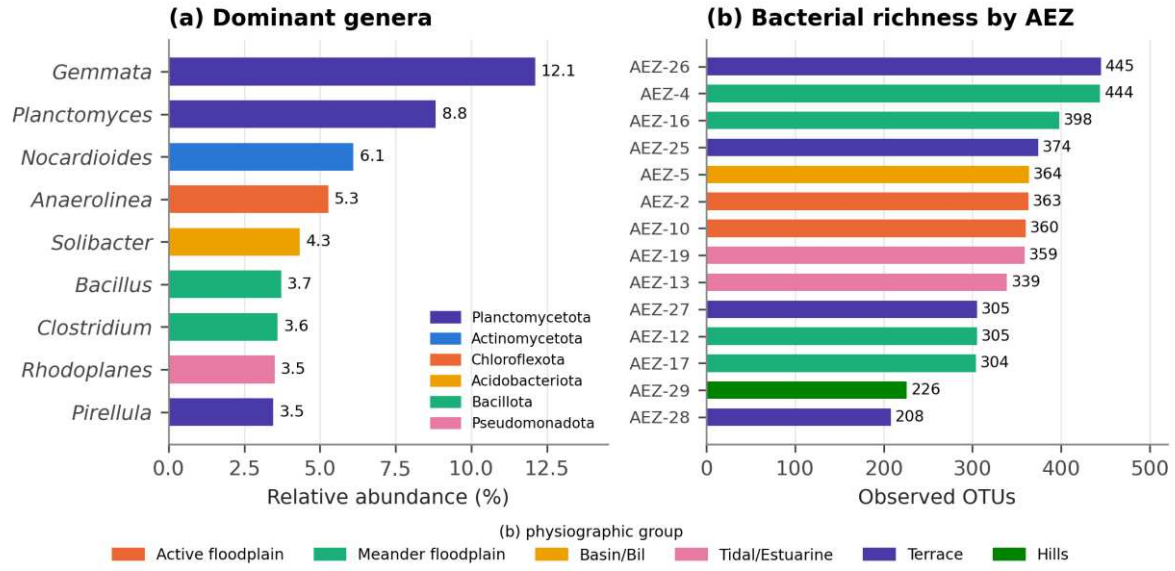


Figure 11. Bacterial community of 14 AEZ soils (16S rRNA data from Islam et al., 2025): (a) the most abundant genera, coloured by phylum; (b) observed operational taxonomic units (OTUs) richness by AEZ, colored by physiographic group.

4. Discussion

4.1 Acidity as the main constraint on bacterial diversity

The clearest biological signal in this national dataset is the depression of bacterial richness in very strongly acidic soils. The two poorest communities came from the Madhupur Tract and the Northern and Eastern Hills, at pH 3.4 and 4.1, and richness peaked near pH 6. This pattern matches continental and field-scale evidence that pH is the dominant control on bacterial diversity, with maxima in near-neutral soils (Fierer and Jackson, 2006; Lauber et al., 2009; Rousk et al., 2010). Because more than half of the samples were strongly or very strongly acidic, and acidity was universal in terrace, piedmont and hill soils, the potential gain in soil-biological function from liming is large. The absence of a pH signal in plate counts is not

contradictory: culturable counts capture a small, fast-growing fraction of the community, and two soils can hold similar numbers of culturable bacteria but very different communities.

4.2 Flooded rice soils and the dominant bacterial taxa

The prominence of Planctomycetota, *Anaerolinea* and *Clostridium* is a molecular fingerprint of the alternating aerobic and anaerobic conditions of seasonally flooded, rice-dominated landscapes. Planctomycetes are slow-growing bacteria of wet, periodically anoxic soils and sediments, some capable of anaerobic ammonium oxidation, while *Anaerolinea* and *Clostridium* are fermenters that feed methanogenic archaea. Upon flooding, soil microbes use successive electron acceptors — oxygen, nitrate, manganese and iron oxides, sulphate and finally carbon dioxide (Fig. 12) — with direct agronomic consequences: loss of nitrate by denitrification, release of phosphate and ferrous iron from iron oxides, precipitation of zinc as sulphide, and methane emission. These processes support the practical recommendations of split or deep-placed urea application and alternate wetting and drying in Boro rice. The low F:B ratios of intensively puddled meander-floodplain soils are likewise consistent with the disruption of fungal hyphae by puddling, flooding and frequent tillage.

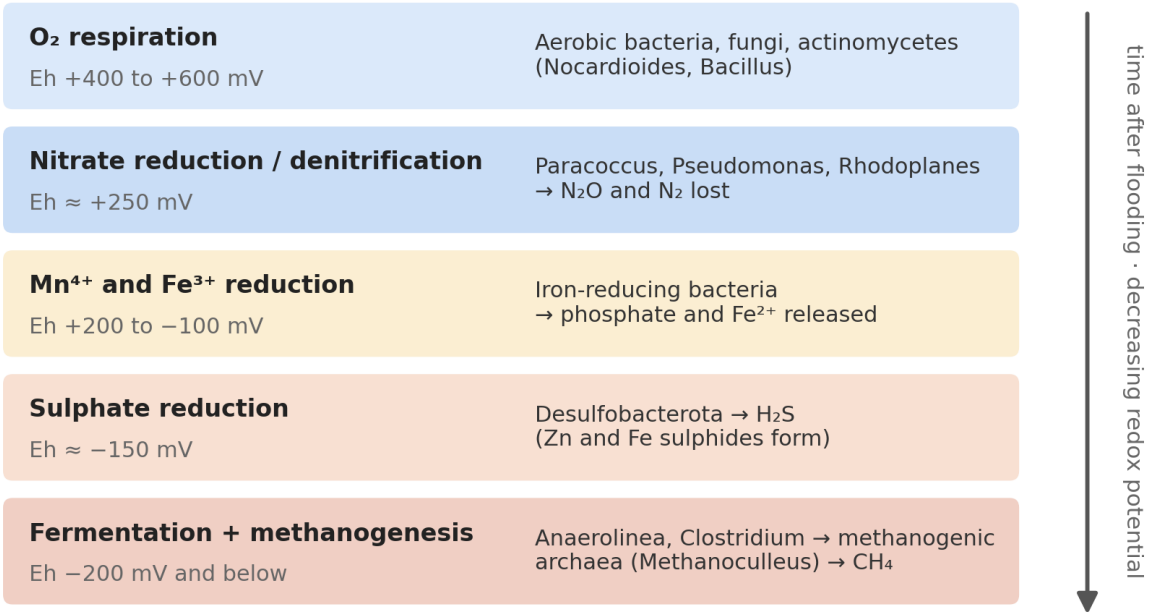


Figure 12. Sequence of microbial processes in a flooded rice soil ("redox ladder"), with approximate redox potentials and representative organisms, including genera detected in Bangladesh AEZ soils.

4.3 Microbial abundance, rhizobia and nitrogen fixation

Bacterial counts were high by international standards (10^6–10^9 cfu g^{-1} is typical of agricultural soils; Alexander, 1977), indicating very active heterotrophic populations in seasonally flooded, silt-rich soils. Fungal counts were at the upper end of the usual range for fungal propagules, which may reflect abundant sporulating saprotrophs such as *Aspergillus*, *Penicillium* and *Trichoderma* in these warm soils but also points to the need to standardise media and dilutions. Rhizobia-like counts (10^5–10^8 cfu g^{-1}) exceeded the 10^3–10^4 cells g^{-1} usually considered sufficient for nodulation, but YEMA also supports non-rhizobial bacteria, so these values are upper estimates. The persistently low counts in the Old and Young Brahmaputra floodplains, the High Ganges Floodplain — a major pulse-growing zone — the Arial Bil and the Lower Punarbhaba Floodplain identify soils where seed inoculation with effective strains is most likely to give a response. Low N status in 95% of samples further underlines the value of biological nitrogen fixation in rice-based rotations.

4.4 Implications for soil-health management

Four management priorities follow from the results (Fig. 13). First, liming with dolomite, according to soil test, is warranted in the very acidic AEZ-1, 3, 9, 20, 22, 28 and 29 and acidic parts of the Barind, with priority for legumes, maize, wheat and vegetables. Second, returning crop residues and applying compost, farmyard or poultry manure and green manures (*Sesbania*, sunnhemp) would raise OM in the third of soils where it is low and improve microbial habitat. Third, AEZ-targeted biofertilizers are indicated: *Rhizobium* inoculants for pulses in AEZ-6, 8, 9, 11 and 15 and the Barind, and phosphate-solubilizing bacteria and arbuscular mycorrhizal fungi in acidic, P-fixing terrace and hill soils; plant-growth-promoting bacteria such as *Paraburkholderia* and *Delftia* have already improved rice growth and yield in Bangladesh (Khan et al., 2017; Islam et al., 2023). Fourth, balanced fertilization is itself a soil-biology measure: correcting K, S, B and Zn deficiencies supports root exudation, whereas excess N and P can suppress symbiotic fixation and mycorrhizas, and P rates should be reduced where available P is already very high. Sites with elevated Ni or Pb should be monitored and protected from untreated sludge and contaminated irrigation water.

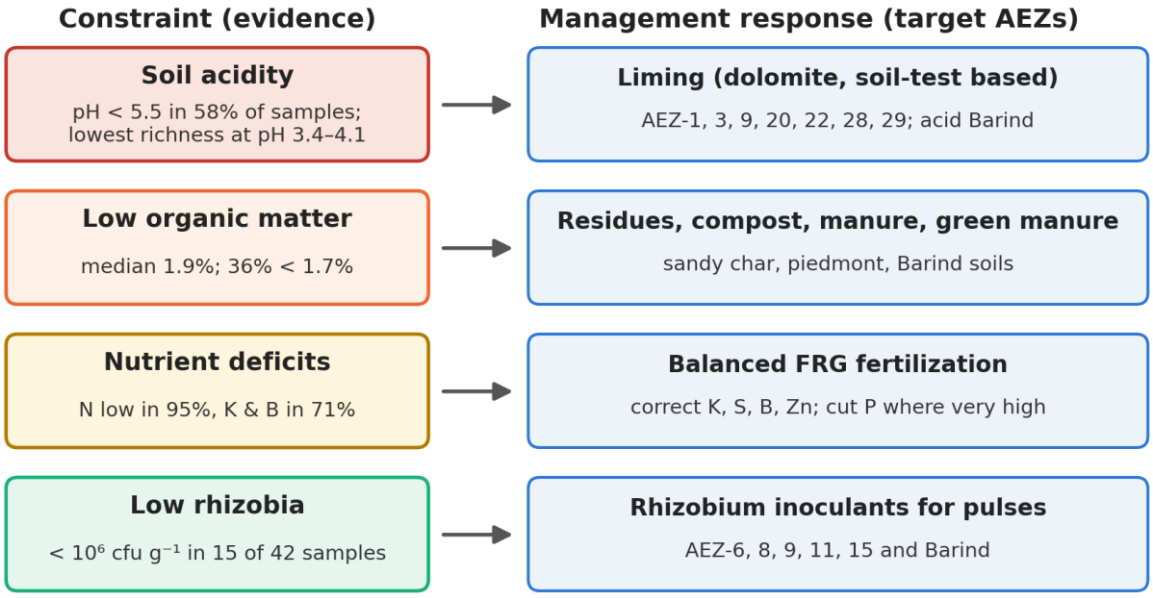


Figure 13. Conceptual framework linking the main soil constraints identified in this study to AEZ-targeted management responses and monitoring.

4.5 Limitations

The dataset has important limitations. Most AEZs are represented by one to five samples, collected in different seasons, and three AEZs have no data, so the power to detect differences among zones is low. Nineteen rhizobia and fourteen fungal counts were reported as exactly 1.0×10^{7} cfu g^{-1}, typical of counts read from a single dilution, and should be interpreted only on a log scale. Several records had texture fractions that did not sum to 100%, rhizobia exceeding total bacteria, or GPS coordinates requiring verification; these were excluded from the relevant analyses. Physical properties were measured on only one soil and Zn on eight. Finally, OTU richness was closely tied to sequencing depth, so rarefied richness and diversity indices (Shannon, Simpson, Chao1) from the deposited reads are needed for a firmer comparison among AEZs.

5. Conclusions

This study provides the first AEZ-wise synthesis of the physical, chemical and microbiological properties of Bangladesh soils. The soils are predominantly silt loams that are acidic, low in organic matter and widely deficient in N, K and B, yet they support large culturable microbial populations, with bacteria dominating over fungi. Culturable counts varied more within than

among physiographic groups, whereas bacterial richness responded clearly to soil acidity, reaching its lowest values in very strongly acidic terrace and hill soils. Liming, organic-matter management and AEZ-targeted biofertilizers are therefore the main levers for protecting and using the biological capital of these soils. We recommend that SRDI's routine surveys adopt a small set of biological indicators — microbial biomass carbon, soil respiration, enzyme activities, MPN counts of rhizobia and 16S/ITS amplicon sequencing — with at least three to five replicated sites per AEZ, to establish a national soil-biodiversity monitoring baseline.

CRediT authorship contribution statement

Md. Nurul Islam: Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization; **Md. Sabbir Hossen:** Writing – review & editing, Software, Resources, Formal analysis; **Md. Mehedi Hasan**: Writing – review & editing, Writing – original draft; **Muhammad Fazle Rabbee**: Writing – review & editing, Writing – original draft, Supervision; and **Kwang-Hyun Baek**: Writing – review & editing, Writing – original draft, and Supervision

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Data availability

16S rRNA amplicon data are available in the NCBI Sequence Read Archive under BioProject PRJNA1181648. The AEZ-wise soil dataset is available from the corresponding author on reasonable request.

Conflict of interest

The authors declare no conflict of interest.

References

Alexander, M. (1977). *Introduction to Soil Microbiology*, 2nd edn. John Wiley & Sons, New York.

Bardgett, R.D. and van der Putten, W.H. (2014). Belowground biodiversity and ecosystem functioning. *Nature* 515: 505–511.

BARC (2018). *Fertilizer Recommendation Guide-2018*. Bangladesh Agricultural Research Council, Dhaka.

Berger, K.C. and Truog, E. (1939). Boron determination in soils and plants. *Industrial and Engineering Chemistry, Analytical Edition* 11: 540–545.

Bouyoucos, G.J. (1962). Hydrometer method improved for making particle size analyses of soils. *Agronomy Journal* 54: 464–465.

Bray, R.H. and Kurtz, L.T. (1945). Determination of total, organic and available forms of phosphorus in soils. *Soil Science* 59: 39–45.

Bremner, J.M. and Mulvaney, C.S. (1982). Nitrogen – total. In: Page, A.L. et al. (eds) *Methods of Soil Analysis, Part 2*, 2nd edn. ASA-SSSA, Madison, pp. 595–624.

Das et al. (2024). Comparative metagenomic analysis from Sundarbans ecosystems advances our understanding of microbial communities and their functional roles. *Scientific Reports* 14: 16218.

FAO/UNDP (1988). *Land Resources Appraisal of Bangladesh for Agricultural Development. Report 2: Agro-ecological Regions of Bangladesh*. FAO, Rome.

Fierer, N. (2017). Embracing the unknown: disentangling the complexities of the soil microbiome. *Nature Reviews Microbiology* 15: 579–590.

Fierer, N. and Jackson, R.B. (2006). The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences USA* 103: 626–631.

Gee, G.W. and Bauder, J.W. (1986). Particle-size analysis. In: Klute, A. (ed.) *Methods of Soil Analysis, Part 1*, 2nd edn. ASA-SSSA, Madison, pp. 383–411.

Islam, N., Hoque, M.N., Hossen, S., Gilman, A.A. and Islam, T. (2025). Microbiome diversity in soils of the agro-ecological zones of Bangladesh. *Microbiology Resource Announcements* 14(4): e01237-24. https://doi.org/10.1128/mra.01237-24

Islam, T., Fatema, Hoque, M.N., Gupta, D.R., Mahmud, N.U., Sakif, T.I. and Sharpe, A.G. (2023). Improvement of growth, yield and associated bacteriome of rice by the application of probiotic *Paraburkholderia* and *Delftia*. *Frontiers in Microbiology* 14: 1212505.

Jackson, M.L. (1973). *Soil Chemical Analysis*. Prentice Hall of India, New Delhi.

Kabata-Pendias, A. (2011). *Trace Elements in Soils and Plants*, 4th edn. CRC Press, Boca Raton.

Khan, M.M.A., Haque, E., Paul, N.C., Khaleque, M.A., Al-Garni, S.M.S., Rahman, M. and Islam, M.T. (2017). Enhancement of growth and grain yield of rice in nutrient deficient soils by rice probiotic bacteria. *Rice Science* 24: 264–273.

Lauber, C.L., Hamady, M., Knight, R. and Fierer, N. (2009). Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology* 75: 5111–5120.

Lindsay, W.L. and Norvell, W.A. (1978). Development of a DTPA soil test for zinc, iron, manganese and copper. *Soil Science Society of America Journal* 42: 421–428.

Martin, J.P. (1950). Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. *Soil Science* 69: 215–232.

Mridha, M.A.U. and Dhar, P.P. (2007). Biodiversity of arbuscular mycorrhizal colonization and spore population in different agroforestry trees and crop species growing in Dinajpur, Bangladesh. *Journal of Forestry Research* 18(2): 91–96.

Olsen, S.R., Cole, C.V., Watanabe, F.S. and Dean, L.A. (1954). *Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate*. USDA Circular 939.

Rousk, J., Bååth, E., Brookes, P.C. et al. (2010). Soil bacterial and fungal communities across a pH gradient in an arable soil. *The ISME Journal* 4: 1340–1351.

Sanyal et al. (2016). Diversity of arsenite oxidase gene and arsenotrophic bacteria in arsenic affected Bangladesh soils. *AMB Express* 6: 21.

Strickland, M.S. and Rousk, J. (2010). Considering fungal:bacterial dominance in soils – methods, controls, and ecosystem implications. *Soil Biology and Biochemistry* 42: 1385–1395.

Tabatabai, M.A. (1996). Sulfur. In: Sparks, D.L. (ed.) *Methods of Soil Analysis, Part 3*. SSSA, Madison, pp. 921–960.

Torsvik, V. and Øvreås, L. (2002). Microbial diversity and function in soil: from genes to ecosystems. *Current Opinion in Microbiology* 5: 240–245.

Vincent, J.M. (1970). *A Manual for the Practical Study of Root-Nodule Bacteria*. IBP Handbook No. 15. Blackwell, Oxford.

Walkley, A. and Black, I.A. (1934). An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Science* 37: 29–38.