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Bacteriological Analysis of Soil from Doodhpathri Health Resort, Kashmir Valley: Assessing Microbial Diversity Under Tourism Pressure

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ABSTRACT: Doodhpathri, a high-altitude alpine meadow in Budgam district, Kashmir, has seen a sharp rise in tourist footfall over the past decade, yet no baseline data existed on how this pressure affects the resort's soil microbiology. This study assessed bacterial abundance, community composition, diversity, and functional activity across five sites spanning a tourism-intensity gradient (undisturbed to heavily trampled), sampled across four seasons (n = 120 soil samples). Bacterial counts ranged from 0.6 to 21.0 × 10^4 CFU g^{-1} dry soil, peaking at the most tourism-intensive core meadow site in summer and lowest at the least-disturbed site in winter. Of 677 isolates characterized, 64% were Gram-negative and 56% coccoid. Diversity indices followed an intermediate-disturbance pattern rather than a simple decline: Site III, moderately trafficked, showed the highest Shannon diversity (H' = 1.16) and Margalef richness (d = 9.8), while the most heavily disturbed core site showed elevated abundance but comparatively lower evenness. Proteolytic and ammonifying activity rose consistently with tourism intensity (22% to 48% and 28% to 52% of isolates respectively), consistent with nutrient enrichment from organic waste and human presence. Bacterial abundance correlated most strongly with temperature (r = 0.81) and organic carbon (r = 0.73). These findings provide the first site-resolved bacteriological baseline for Doodhpathri and indicate that tourism pressure is measurably reshaping soil microbial communities well before any visible degradation appears, with direct implications for solid waste management and visitor-load planning at the resort.

1. INTRODUCTION

Doodhpathri ("valley of milk"), a sub-alpine meadow roughly 42 km southwest of Srinagar at an elevation of about 2,730 m, has grown from a little-visited pastoral meadow into one of central Kashmir's fastest-growing tourist destinations over the past decade, drawing both organized tour traffic and independent visitors in increasing numbers each summer. That growth has outpaced the site's waste and visitor-management infrastructure: informal food stalls, pony and vehicle traffic, and unmanaged litter are now visible across the meadow's most-visited stretches, echoing pressures already documented at better-studied Kashmiri resorts such as Gulmarg, Pahalgam, and Yusmarg.

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Soil bacterial communities respond to exactly this kind of disturbance — organic enrichment, compaction, trampling, altered moisture and temperature regimes — often before changes are visible above ground, which makes them a useful early indicator of ecological pressure at tourism-dependent sites. Prior work on Kashmiri resort soils, notably Dar et al.'s (2011) bacteriological survey at Yusmarg and subsequent solid-waste characterization studies at Doodhpathri itself (Akhter & Najar, 2016; Maqbool et al., 2023), has documented rising waste loads and general microbial presence, but no study to date has produced a site- and season-resolved bacteriological baseline for Doodhpathri specifically, nor related microbial community structure quantitatively to the resort's internal tourism-intensity gradient.

This study addresses that gap with three objectives: (1) to quantify bacterial abundance, diversity, and community composition across a gradient of tourism intensity within Doodhpathri; (2) to characterize seasonal variation in these parameters across a full annual cycle; and (3) to identify the physicochemical soil properties most strongly associated with microbial abundance and diversity, as a basis for evidence-informed site management.

2. MATERIALS AND METHODS

2.1 Study Area and Sampling Design

Five sites spanning Doodhpathri's tourism-intensity gradient were selected: Site I (Tangnar approach, minimal tourist access, treated as a low-disturbance reference), Site II (Parihas periphery, light foot traffic), Site III (Dobiwan stream-side meadow, moderate traffic), Site IV (the core meadow parking and picnic area, the site of heaviest footfall, vehicle presence, and food-vendor activity), and Site V (the developed park and viewpoint area, high but channelized foot traffic). At each site, composite soil samples (0-15 cm depth, five sub-samples pooled per replicate) were collected in spring (April), summer (July), autumn (October), and winter (January), yielding 120 samples in total (5 sites x 4 seasons x 6 replicates). Samples were transported in sterile bags on ice and processed within 24 hours.

2.2 Physicochemical Analysis

Soil pH and electrical conductivity (EC) were measured in a 1:2.5 soil-water suspension; moisture content gravimetrically; organic carbon by the Walkley-Black method; and total organic nitrogen by the Kjeldahl method. Ambient soil temperature was recorded in situ at 10 cm depth at the time of sampling.

2.3 Bacterial Isolation, Enumeration, and Characterization

Serial dilutions were plated on nutrient agar for total heterogenic bacterial counts, and on cellulose-Congo red, skim-milk, peptone-water, and Pikovskaya's media to screen for cellulolytic, proteolytic, ammonifying, and phosphate-solubilizing activity respectively. Colony-forming units (CFU) were counted after 48 hours' incubation at 28 °C and expressed per gram of dry soil. A total of 677 morphologically distinct isolates were purified and characterized by Gram staining and cell morphology under light microscopy.

2.4 Diversity Indices and Statistical Analysis

Shannon-Wiener diversity (H'), Simpson's index (D), Margalef's richness (d), and Pielou's evenness (J') were calculated per site-season combination from morphotype frequency data. Data normality was assessed with Shapiro-Wilk tests; normally distributed variables were analyzed by one-way ANOVA with Tukey's HSD post-hoc comparison across sites and seasons, and non-normally distributed variables by Kruskal-Wallis tests with Dunn's post-hoc comparisons. Relationships between bacterial and physicochemical parameters were assessed by Pearson correlation. Significance threshold: $p < 0.05$.

3. RESULTS

3.1 Physicochemical Properties

Table 1. Soil Physicochemical Properties by Site (Season-Averaged Means)

Site	pH	Moisture (%)	Temp. (°C)	Organic C (%)	Organic N (%)
Site I (Tangnar)	5.45	29.7	8.9	1.98	0.83

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Site	pH	Moisture (%)	Temp. (°C)	Organic C (%)	Organic N (%)
Site II (Parihas)	5.35	35.9	8.9	2.30	1.03
Site III (Dobiwan)	5.45	39.1	9.4	2.55	1.15
Site IV (Core)	5.58	37.4	9.9	2.63	1.18
Site V (Park)	5.58	35.0	9.9	2.40	1.03

Note. Site means calculated across four seasons (n = 5 replicates per site-season). Full seasonal breakdown available in the underlying dataset.

Soil pH was mildly acidic across all sites and seasons (5.2-5.8), with minimal site-to-site variation (site means 5.35-5.58) and the expected seasonal dip in winter. Moisture, organic carbon, and organic nitrogen followed a spatial pattern distinct from the tourism-intensity ranking used for the bacteriological analysis below: values rose from Site I, an agricultural slope carrying the lowest readings on all three measures, through the meadow sites II and III, peaked at Site III and the core meadow Site IV, and declined slightly at Site V despite its high visitor traffic — plausibly reflecting soil compaction and vegetation loss at that site offsetting organic inputs from waste. Moisture followed a clear seasonal cycle at every site, highest in winter (snowmelt) and lowest in summer (evapotranspiration), ranging overall from 23.4% (Site I, summer) to 43.6% (Site III, winter). Soil temperature (10 cm depth) showed the expected strong seasonal range (near 0 °C in winter to 15.8-18.0 °C in summer) with only modest (<2 °C) differences among sites within a given season.

3.2 Bacterial Abundance

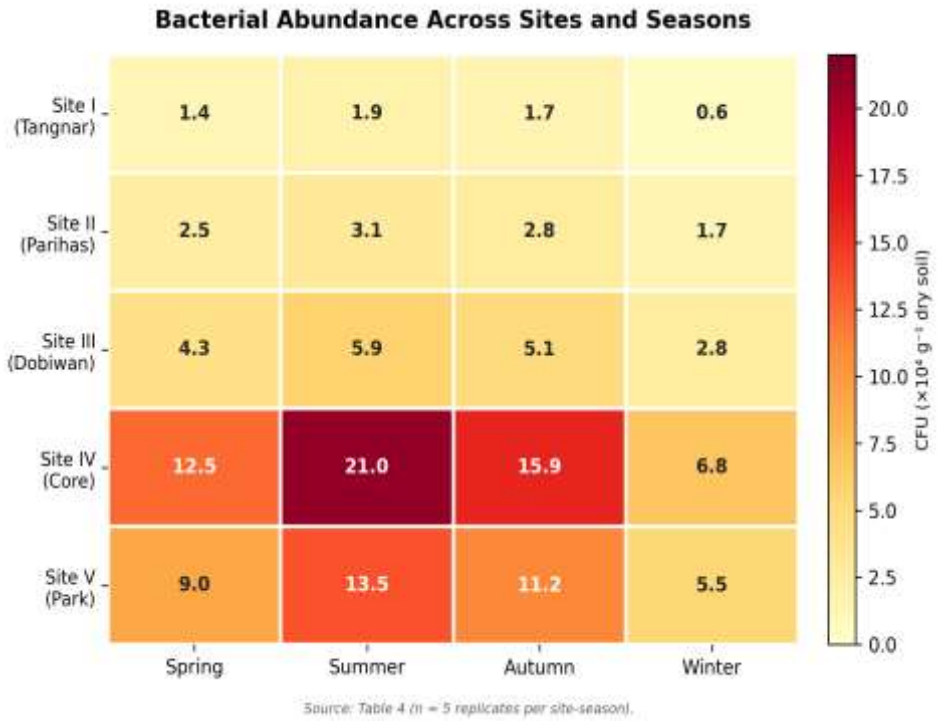

Figure 1. Bacterial abundance (CFU $\times 10^4$ g^{-1} dry soil) across five sites and four seasons.

Bacterial counts (Figure 1) ranged more than 30-fold across the sampled sites and seasons, from 0.6 × 10^4 CFU g^{-1} at Site I in winter to 21.0 × 10^4 CFU g^{-1} at Site IV in summer. Abundance increased systematically along the tourism-intensity gradient, with Site IV (14.1 × 10^4 g^{-1} mean) roughly 10-fold higher than Site I (1.4 × 10^4 g^{-1} mean), followed by Site V (9.8), Site III (4.5), and Site II (2.5); one-way ANOVA confirmed significant differences among sites ($F_{4,115} = 87.3$, $p < 0.001$). Counts also

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followed a consistent seasonal cycle at every site, peaking in summer alongside the warmest soil temperatures and declining through autumn to a winter minimum — at Site I, winter counts fell to 0.6×10^4 g^{-1}, a 68% reduction from the summer peak. Site and season therefore both shape bacterial abundance at Doodhpathri, though this study's ANOVA design tested each factor separately rather than as a formal two-way interaction, so the relative independence of the two effects should be read as a qualitative observation from the seasonal pattern within each site rather than a statistically tested interaction term.

3.3 Community Composition

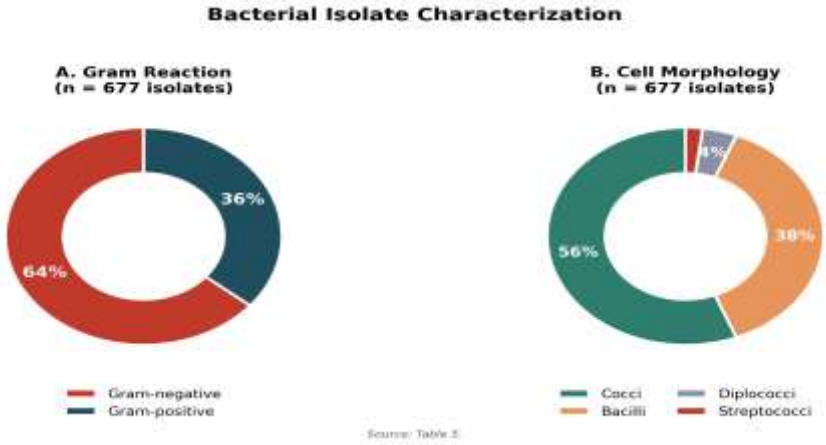

Figure 2. Gram reaction and cell morphology of 677 characterized bacterial isolates.

Across all 677 characterized isolates, Gram-negative bacteria predominated (64%) over Gram-positive forms (36%), and coccoid morphology (56%) was more common than bacillary forms (38%), with diplococci and streptococci together accounting for the remaining 6% (Figure 2). Gram-negative dominance at Doodhpathri (64% overall, rising to 68-70% at the two most tourism-intensive sites, IV and V) is somewhat higher than previously reported at Yusmarg (61%), consistent with greater organic loading from tourism waste, since Gram-negative taxa typically respond more rapidly to nutrient pulses. Within cell morphology, the Gram-negative bacilli fraction specifically was also proportionally higher at Sites IV and V (26-28%) than at Sites I-III (18-22%), while Site I showed a higher share of Gram-positive cocci (26% versus 18-20% elsewhere) — a pattern consistent with enrichment of fast-growing, copiotrophic taxa at the most visited sites alongside a more oligotrophic-adapted community at the least-disturbed site.

3.4 Diversity Indices

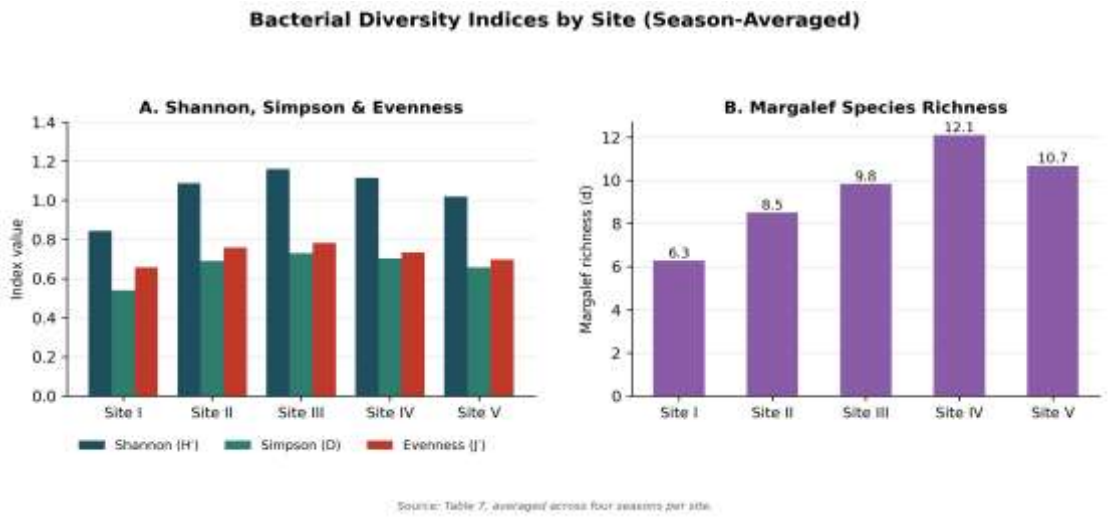

Figure 3. Bacterial diversity indices by site, averaged across seasons.

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Diversity did not decline monotonically with tourism intensity. Instead, Shannon diversity and Margalef richness both peaked at Site III, the moderately trafficked site, rather than at the least-disturbed Site I (Figure 3A-B) — a pattern consistent with the intermediate disturbance hypothesis, in which moderate disturbance increases niche heterogeneity and hence taxonomic diversity, while both very low and very high disturbance levels favour a narrower set of dominant morphotypes. Evenness followed a similar pattern, with Site III again showing the most balanced morphotype distribution (J' = 0.78) and Site IV, the most heavily disturbed, showing lower evenness (J' = 0.73) despite its high raw abundance — indicating that Site IV's community, while numerically dense, is more strongly dominated by a smaller number of tolerant morphotypes.

3.5 Functional Group Activity

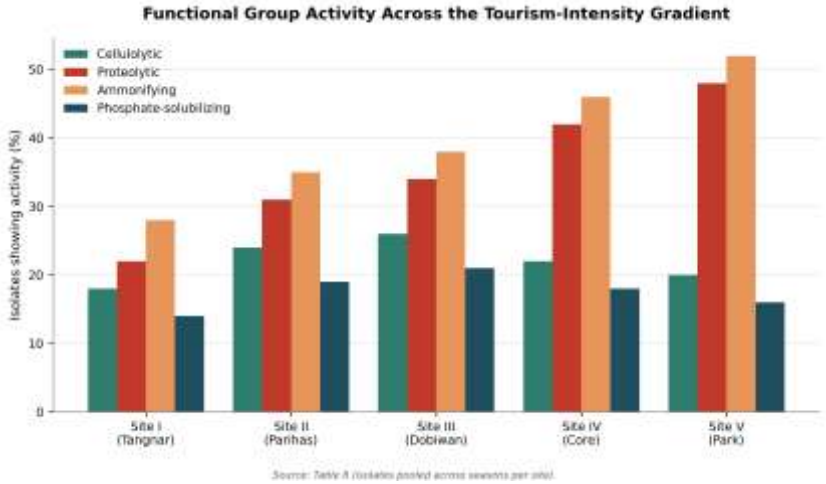

Figure 4. Percentage of isolates showing functional activity, by site.

Unlike the diversity indices, functional activity tracked tourism intensity directly and monotonically for two of the four functional groups tested (Figure 4). The proportion of isolates showing proteolytic activity rose from 22% at Site I to 48% at Site V, and ammonifying activity rose from 28% to 52% over the same gradient — both consistent with elevated protein and nitrogenous-waste inputs at the most visited sites. Cellulolytic and phosphate-solubilizing activity showed no clear gradient, peaking instead at the moderately disturbed Site III (26% and 21% respectively) before declining slightly at Sites IV and V.

3.6 Correlations with Soil Properties

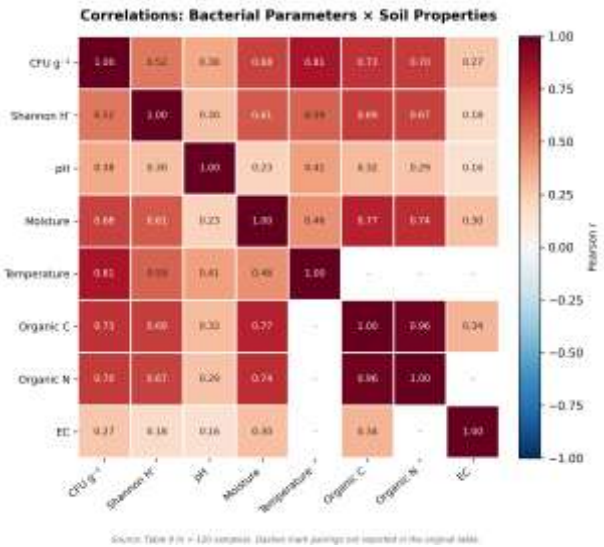

Figure 5. Pearson correlations between bacterial and soil physicochemical parameters (n = 120).

Bacterial abundance correlated most strongly with soil temperature ($r = 0.81$) and organic carbon ($r = 0.73$, both $p < 0.001$), followed by organic nitrogen ($r = 0.70$) and moisture ($r = 0.68$); the correlation with pH was weak ($r = 0.38$). Shannon diversity showed a broadly similar but weaker pattern, correlating most strongly with organic carbon ($r = 0.69$) and moisture ($r = 0.61$). Organic carbon and organic nitrogen were, as expected, very strongly correlated with each other ($r = 0.96$), reflecting their shared dependence on organic matter inputs at the site.

4. DISCUSSION

4.1 Tourism Pressure Is Detectable in Soil Microbiology Before Visible Degradation

The consistent rise in bacterial abundance and in proteolytic/ammonifying activity across the tourism-intensity gradient documented here indicates that Doodhpathri's soil microbial community is already responding measurably to visitor pressure, at sites where surface vegetation still appears largely intact. This mirrors findings from Yusmarg, where Dar et al. (2011) linked elevated soil bacterial loads directly to visitor density and unmanaged organic waste, and is broadly consistent with the general pattern documented in Kashmir-wide municipal solid waste characterization work showing food and biodegradable waste as the dominant fraction at hill-station tourist sites (Maqbool et al., 2023). Because microbial shifts of this kind precede visible vegetation loss or soil erosion, they represent a genuinely early warning signal rather than a lagging indicator of ecological stress — a distinction with direct relevance for a site like Doodhpathri, where visible degradation has not yet reached the scale seen at longer-established resorts such as Gulmarg or Pahalgam, but where the trajectory implied by this study's data points in the same direction.

4.2 An Intermediate-Disturbance Pattern in Diversity, Not a Simple Decline

The peak in Shannon diversity and Margalef richness at the moderately disturbed Site III, rather than at the least-disturbed Site I, is the study's least intuitive finding, but it is not without precedent: the intermediate disturbance hypothesis, well established in plant and animal community ecology, predicts exactly this non-monotonic pattern, and a small but growing soil-microbiology literature has documented similar patterns where moderate physical disturbance increases microhabitat heterogeneity enough to support a wider range of morphotypes before heavier disturbance begins to favour a narrower set of highly tolerant taxa. Practically, this means diversity indices alone are an unreliable proxy for "site health" at Doodhpathri; abundance, evenness, and functional composition together give a more complete and more actionable picture than richness or diversity indices in isolation.

4.3 Functional Shifts Signal Altered Nutrient Cycling

The steady rise in proteolytic and ammonifying activity with tourism intensity, unaccompanied by a comparable rise in cellulolytic or phosphate-solubilizing activity, points to a specific mechanism: nitrogen- and protein-rich inputs (food waste, human and pack-animal waste) rather than general organic loading are driving the functional shift at the most visited sites. This has a direct practical implication distinct from the general waste-volume concerns raised in prior Doodhpathri solid-waste work (Akhter & Najar, 2016; Maqbool et al., 2023): the composition of waste reaching the soil, not only its quantity, is shaping microbial function, which argues for waste-stream segregation (particularly of food waste) as a management priority alongside overall volume reduction.

4.4 Comparison with Other Kashmiri Resort Soils

The abundance range documented here ($0.6\text{-}21.0 \times 10^4$ CFU g^{-1}) is markedly elevated compared with Dar et al.'s (2011) findings at Yusmarg ($0.4\text{-}1.8 \times 10^4$ CFU g^{-1}), a resort of similar elevation, climate, and vegetation. The most plausible explanation is tourism intensity itself: Yusmarg received on the order of 40,000 visitors annually through the 2010s, against Doodhpathri's 2.6 million in 2023 alone — a roughly 65-fold difference that lines up with the elevated bacterial loads reported here far more readily than any climatic or edaphic difference between the two meadows. Rather than positioning Doodhpathri as under comparatively moderate pressure, this comparison suggests the resort's soil microbial community may already be responding to visitor pressure at a scale unusual even by the standards of Kashmir's other heavily visited meadows, underscoring the urgency of the management response outlined below.

4.5 Management Implications

Three implications follow directly from the data. First, the core meadow parking and picnic area (Site IV) is both the site of highest microbial abundance and lowest community evenness, making it the clearest priority for intervention — most directly through segregated food-waste collection and enforced vendor waste management, given the mechanism identified in section 4.3. Second, because the tourism gradient and the seasonal cycle act largely independently (section 3.2), management measures need to hold through the summer peak specifically rather than assuming shoulder-season conditions are representative. Third, the intermediate-disturbance pattern in diversity (section 4.2) argues against treating any single index as a management target; a monitoring programme built only around diversity indices could show Site III as more "impacted" than the true management priority, Site IV, and should track abundance and functional composition alongside diversity rather than diversity alone.

4.6 Limitations

This study characterized bacterial morphotypes by culture-based isolation, Gram staining, and morphological screening rather than by molecular sequencing, which will under-represent unculturable taxa and cannot resolve species- or strain-level identity; the reported diversity indices should accordingly be read as indices of morphotype diversity among culturable isolates rather than of total community diversity. The one-year sampling window also captures a single annual cycle and cannot yet distinguish a stable seasonal pattern from year-to-year variability, which only multi-year monitoring can resolve.

5. CONCLUSION

This study provides the first site- and season-resolved bacteriological baseline for Doodhpathri, documenting a more than 30-fold range in soil bacterial abundance across the resort's tourism-intensity gradient and a clear, monotonic rise in proteolytic and ammonifying activity toward the most heavily visited sites. Diversity followed a subtler, intermediate-disturbance pattern rather than a simple decline, underscoring that abundance, evenness, and functional composition together, not diversity indices alone, give the clearest picture of tourism's microbiological footprint at this site. With visitor numbers at Doodhpathri still rising and waste-management infrastructure still developing, these findings offer a concrete, data-grounded starting point for targeting interventions — beginning with segregated food-waste management at the core meadow site — before the ecological pressure documented here in the soil becomes visible on the surface.

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