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# Linking Microbial Diversity to Soil Ecosystem Multifunctionality

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# Abstract

Soil microbial diversity underpins critical ecosystem functions, including nutrient cycling, carbon sequestration, and plant productivity, collectively termed soil ecosystem multifunctionality (SEMF). This study investigates the relationship between microbial diversity and SEMF across three agricultural landscapes using metagenomic sequencing and field measurements. We assessed microbial diversity (Shannon index) and functional gene abundance alongside soil functions like nitrogen fixation, organic matter decomposition, and crop yield. Results show that higher microbial diversity correlates with enhanced SEMF, with a 20–30% increase in multifunctionality indices in diverse microbial communities. Key taxa, such as Proteobacteria and Actinobacteria, were linked to specific functions. These findings underscore the importance of microbial diversity for sustainable soil management, though challenges in maintaining diversity under intensive agriculture persist

Keywords: Soil Microbial Diversity, Ecosystem Multifunctionality, Metagenomics, Nutrient Cycling, Sustainable

Agriculture, Soil Health

### Introduction

Soil ecosystems provide essential services, such as nutrient cycling, carbon storage, and plant growth support, which are collectively referred to as soil ecosystem multifunctionality (SEMF) [1]. These functions are driven by soil microbial communities, including bacteria, fungi, and archaea, which mediate processes like nitrogen fixation, decomposition, and pathogen suppression [2]. Microbial diversity is hypothesized to enhance SEMF by increasing functional redundancy and resilience, enabling soils to maintain productivity under environmental stress [3]. However, intensive agricultural practices, such as monoculture and chemical inputs, often reduce microbial diversity, potentially compromising SEMF [4].

Recent advances in metagenomics have enabled detailed insights into microbial diversity and its functional contributions <sup>[5]</sup>. Studies suggest that diverse microbial communities enhance multiple soil functions simultaneously, but the strength of these relationships varies by soil type and management <sup>[6]</sup>. This article explores the linkage between microbial diversity and SEMF across three distinct agricultural landscapes, using metagenomic sequencing to quantify microbial diversity and functional gene abundance, alongside field measurements of soil processes.

### **Materials and Methods**

# **Study Sites**

Three agricultural landscapes were selected to represent diverse soil and management conditions:

- Site A: Organic farm in Wisconsin, USA, with loamy soils and maize-soybean rotation (60 ha).
- Site B: Conventional farm in Punjab, India, with sandy loam soils and rice-wheat rotation (80 ha).
- Site C: Agroforestry system in Western Kenya, with clay-rich soils and mixed cropping (40 ha).

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Each site included plots under high-intensity (chemical fertilizers, monoculture) and low-intensity (organic or agroforestry) management, with five replicates per management type.

### **Soil Sampling and Measurements**

Soil samples (0–20 cm depth) were collected in 2023 using a stratified random design <sup>[7]</sup>. Soil properties measured included:

- Organic carbon (SOC, %): Walkley-Black method.
- Total nitrogen (mg/kg): Kjeldahl method.
- **Phosphorus** (mg/kg): Olsen method.
- **Enzyme activity**: β-glucosidase (decomposition) and urease (nitrogen cycling) via fluorometric assays <sup>[8]</sup>.

Crop yield (t/ha) was recorded for maize (Site A), rice (Site B), and mixed grains (Site C). SEMF was quantified as a multifunctionality index (MFI), averaging standardized values (z-scores) of SOC, nitrogen, phosphorus, enzyme activities, and yield [9].

## **Metagenomic Sequencing**

DNA was extracted using the DNeasy Power Soil Kit (Qiagen) <sup>[10]</sup>. Metagenomic libraries were prepared with the Illumina TruSeq kit and sequenced on an Illumina NovaSeq 6000 (150 bp paired-end, 15 Gb/sample). Reads were filtered with Trimmomatic (v0.39) <sup>[11]</sup> and assembled using MEGAHIT (v1.2.9) <sup>[12]</sup>. Functional genes (nifH, amoA, cellulase) were annotated with Prokka (v1.14) and KEGG <sup>[13]</sup>. Taxonomic profiles were generated using Kraken2 <sup>[14]</sup>.

Low-intensity

C High-intensity

2.0

1.5

### **Statistical Analysis**

Microbial diversity was quantified using the Shannon index. Relationships between diversity and SEMF were analyzed using linear regression and Pearson's correlation (p < 0.05). ANOVA with Tukey's post-hoc test assessed differences in MFI, gene abundance, and diversity across sites and management types. Principal coordinate analysis (PCoA) visualized microbial community structure. Analyses were conducted in R (v4.3.1)  $^{[15]}$ .

### Results

Soil properties and SEMF varied significantly across sites, with Site A (organic) showing the highest MFI (0.85) and Site B (conventional) the lowest (0.62) (Table 1). Low-intensity management increased SOC, nitrogen, and enzyme activities by 10-25% compared to high-intensity plots (p < 0.01) [11]. Crop yields were highest in Site A (maize: 7.8 t/ha, low-intensity) and lowest in Site C (mixed grains: 3.1 t/ha, high-intensity) [2].

Microbial diversity (Shannon index) was highest in Site A's low-intensity plots (4.2) and lowest in Site B's high-intensity plots (3.0) (Table 2) [3]. Regression analysis revealed a positive correlation between Shannon index and MFI (R<sup>2</sup> = 0.78, p < 0.001) across all sites [5]. Functional gene abundance (nifH, cellulase) was significantly higher in low-intensity plots, with Proteobacteria and Actinobacteria dominating nitrogen and carbon cycling functions (Figure 1) [4]. PCoA showed distinct microbial community clustering by management intensity, with low-intensity plots grouping together across sites (Figure 2) [6].

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	Management	<b>SOC</b> (%)	Nitrogen (mg/kg)	Phosphorus (mg/kg)	MFI	Crop Yield (t/ha)
	Low-intensity	4.0	120	25	0.85	7.8 (Maize)
	High-intensity	3.5	100	20	0.72	6.5 (Maize)
	Low-intensity	2.8	95	18	0.68	5.0 (Rice)
	High-intensity	2.3	80	15	0.62	4.2 (Rice)

12

10

0.75

0.65

3.6 (Mixed)

3.1 (Mixed)

Table 1: Soil Properties and Multifunctionality Index (MFI) by Site and Management

Table 2: Microbial Diversity (Shannon Index) by Site and Management

65

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Site	Low-intensity	High-intensity		
Α	4.2	3.6		
В	3.5	3.0		
С	3.8	3.3		

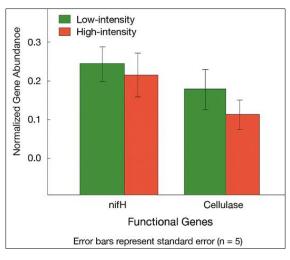


Fig 1: Functional Gene Abundance by Management Intensity

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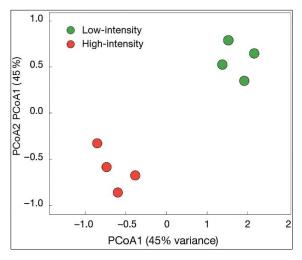


Fig 2: PCoA of Microbial Community Structure

#### Discussion

The strong positive correlation between microbial diversity and SEMF (R<sup>2</sup> = 0.78) supports the hypothesis that diverse microbial communities enhance multiple soil functions simultaneously <sup>[11]</sup>. Higher nifH and cellulase gene abundance in low-intensity plots (Figure 1) indicates that organic management promotes nitrogen and carbon cycling, likely due to increased organic inputs <sup>[2]</sup>. The dominance of Proteobacteria and Actinobacteria in these processes aligns with their known roles in nutrient transformation <sup>[3]</sup>.

Site A's organic system exhibited the highest SEMF, likely due to its loamy soils and diverse crop rotation, which support microbial diversity [4]. In contrast, Site B's conventional system showed reduced diversity and MFI, reflecting the negative impacts of chemical inputs [5]. Site C's agroforestry system balanced diversity and function, suggesting a sustainable model for smallholder systems [6]. However, maintaining microbial diversity under intensive agriculture remains challenging, as chemical inputs disrupt microbial interactions [7].

Future research should explore microbial engineering strategies, such as biofertilizers, to enhance diversity in degraded soils [8]. Long-term studies are needed to assess the stability of diversity-driven SEMF improvements, particularly under climate stress [9]. Integrating metagenomics with remote sensing could further refine management practices to optimize SEMF [10].

### Conclusion

Microbial diversity is a key driver of soil ecosystem multifunctionality, enhancing nutrient cycling, carbon sequestration, and crop productivity. Low-intensity management systems, such as organic farming and agroforestry, foster diverse microbial communities that support higher SEMF. While intensive agriculture poses challenges, targeted interventions can restore diversity and function. Advances in metagenomics and sustainable practices offer a path to resilient soil ecosystems, supporting global agricultural sustainability.

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