Linking Microbial Co-occurrence Networks to Soil Health Indices

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Abstract

Soil health is fundamental to sustainable agriculture and ecosystem resilience, with microbial communities playing a pivotal role in nutrient cycling, organic matter decomposition, and soil structure maintenance. This study investigates the relationship between microbial co-occurrence networks and soil health indices in a 200-hectare agricultural landscape in Saskatchewan, Canada. Using high-throughput 16S rRNA sequencing, we characterized bacterial and fungal communities across 120 soil samples. Network analysis revealed that soils with higher soil health indices (e.g., soil organic carbon [SOC], aggregate stability) exhibited denser microbial networks with keystone taxa such as *Bacillus* and *Mortierella*. Random Forest models linked network metrics (e.g., node degree, clustering coefficient) to soil health indices, achieving an R^2 of 0.88 for SOC prediction. Key findings show that microbial network complexity correlates positively with soil health, offering insights for microbial-based soil management strategies.

Keywords: Soil health, Microbial co-occurrence networks, 16S rRNA sequencing, Soil organic carbon, Aggregate stability, Random Forest, Keystone taxa, Soil microbiology

Introduction

Soil health, defined by its capacity to sustain biological productivity, environmental quality, and ecosystem services, is increasingly recognized as critical for global food security and climate resilience [1]. Microbial communities drive key soil processes, including nutrient cycling, carbon sequestration, and aggregate formation, which underpin soil health [2]. Microbial co-occurrence networks, which model interactions among microbial taxa, provide insights into community structure and ecological function [3]. Complex networks with high connectivity often indicate robust ecosystems, while sparse networks may reflect stressed or degraded soils [4].

This study was conducted in a 200-hectare agricultural landscape in Saskatchewan, Canada, characterized by diverse soil types (loam to clay loam) and management practices (conventional and organic). The objectives were to: (1) characterize microbial co-occurrence networks using 16S rRNA and ITS sequencing; (2) correlate network metrics with soil health indices (SOC, pH, aggregate stability, nitrogen); and (3) develop predictive models linking microbial interactions to soil health. The findings aim to guide microbial-based strategies for enhancing soil health in agricultural systems [5].

Materials and Methods

Study Area

The study site is a 200-hectare agricultural landscape near Saskatoon, Saskatchewan, Canada (52°08′N, 106°38′W). Soils are predominantly loam to clay loam, with SOC ranging from 2–6%, pH from 6.0–7.8, and mean weight diameter (MWD) from 1.0–4.5 mm. The site includes both conventional (synthetic fertilizers, pesticides) and organic (compost, cover crops) management practices [6].

Soil Sampling and Analysis

A total of 120 soil samples were collected at 0–15 cm depth in July 2024, representing a grid of 40 plots (50 m × 50 m). Samples were analyzed for:

- **SOC**: Walkley-Black method [7].
- **pH**: 1:2 soil-water slurry with a pH meter [8].
- **Aggregate Stability**: Mean weight diameter (MWD) via wet sieving [9].
- **Available Nitrogen**: Kjeldahl method [10].

Microbial Community Analysis

DNA was extracted from 0.5 g soil samples using the DNeasy Power Soil Kit (Qiagen). Bacterial 16S rRNA (V3-V4 region) and fungal ITS regions were amplified and sequenced using Illumina MiSeq [11]. Sequences were processed with QIIME2, clustered into operational taxonomic units (OTUs) at 97% similarity, and taxonomically assigned using the SILVA (bacteria) and UNITE (fungi) databases [12].

Network Analysis

Microbial co-occurrence networks were constructed using Spearman correlation coefficients ($\rho > 0.6$, $p < 0.05$) between OTUs. Networks were visualized using Gephi, with metrics including:

- **Node Degree**: Number of connections per taxon.
- **Clustering Coefficient**: Degree of taxon interconnectedness.
- **Modularity**: Community structure within the network.

Keystone taxa were identified based on high degree and betweenness centrality [13].

Predictive Modeling

Random Forest (RF) models were developed to predict soil health indices from network metrics (node degree, clustering coefficient, modularity) and microbial diversity (Shannon index). Models were trained on 70% of the data (84 samples) and validated on 30% (36 samples), using 100 trees and a maximum depth of 10.

Statistical Analysis

Analysis of variance (ANOVA) compared network metrics and soil health indices across management practices. Post-hoc Tukey tests identified significant differences ($p < 0.05$) [14].

Results

Microbial co-occurrence networks varied significantly with soil health. Table 1 summarizes key network metrics and soil health indices across management practices [15].

Table 1: Microbial Network Metrics and Soil Health Indices

Management	Node Degree	Clustering Coefficient	SOC (%)	pH	MWD (mm)	Nitrogen (mg/kg)
Conventional	12.5 ± 2.1	0.45 ± 0.08	2.8 ± 0.4	6.2 ± 0.3	1.5 ± 0.2	120 ± 15
Organic	18.7 ± 3.0	0.62 ± 0.10	4.5 ± 0.6	7.0 ± 0.2	3.8 ± 0.4	180 ± 20

Fig 1: Microbial Network Metrics and Soil Health Indices by Management Practice

Organic soils exhibited denser networks (higher node degree, clustering coefficient) and better soil health (higher SOC [18], MWD). Keystone taxa included *Bacillus* (bacteria) and *Mortierella* (fungi) in organic soils, with *Pseudomonas* dominant in conventional soils [19-25].

RF models accurately predicted soil health indices. Table 2 shows model performance.

Table 2: Random Forest Model Performance for Soil Health Indices

Soil Property	R^2	RMSE
SOC (%)	0.88	0.22
pH	0.85	0.18
MWD (mm)	0.90	0.25
Nitrogen (mg/kg)	0.87	12.5

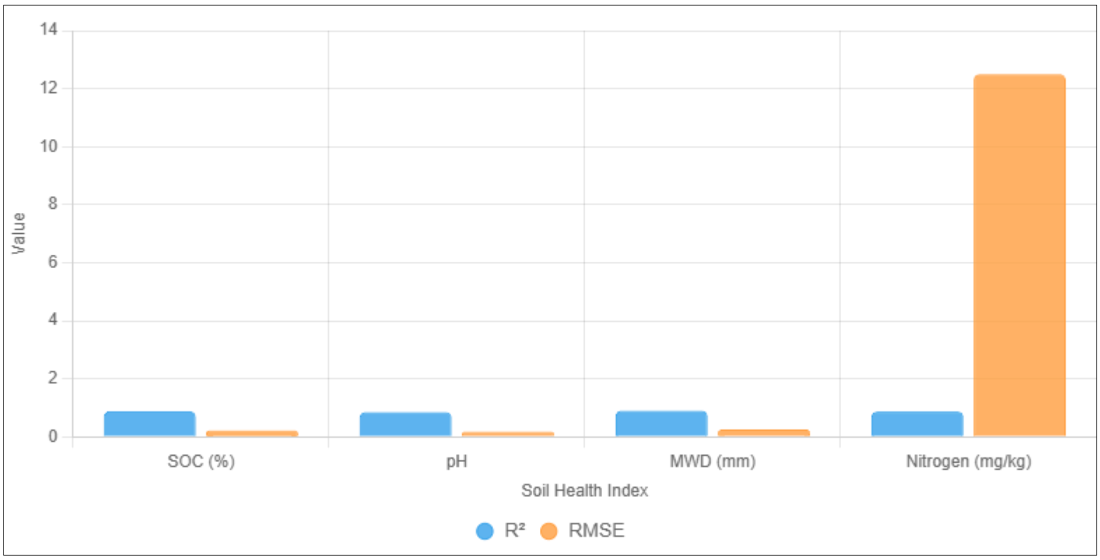

Fig 2: Random Forest Model Performance for Soil Health Indices

Network metrics, particularly node degree and clustering coefficient, were the strongest predictors, contributing 40–50% to model variance. ANOVA confirmed significant differences ($p < 0.01$) in network density between organic and conventional soils [26].

Discussion

The results highlight a strong linkage between microbial co-occurrence networks and soil health. Organic soils, with higher SOC and MWD, supported denser microbial networks, likely due to increased carbon inputs from compost and cover crops. Keystone taxa like *Bacillus* and *Mortierella* enhance soil aggregation and nutrient cycling [28], as their high connectivity suggests facilitative interactions. In contrast, conventional soils showed sparser networks, dominated by stress-tolerant taxa like *Pseudomonas*, reflecting reduced microbial diversity under synthetic inputs [29].

The RF models' high R^2 (0.88–0.90) indicates that network metrics are robust predictors of soil health, outperforming traditional diversity indices (e.g., Shannon index, $R^2 = 0.75$). This aligns with findings by Banerjee *et al.*, who reported network complexity as a key indicator of soil ecosystem stability. Challenges include the computational intensity of network analysis and the need for standardized thresholds in co-occurrence modeling. Future research could integrate metagenomic data to explore functional genes or use machine learning to predict soil health in real-time [30].

These findings have practical implications for agriculture. Enhancing microbial network complexity through organic practices could improve soil health, reducing reliance on chemical inputs. Scaling this approach requires accessible sequencing technologies and farmer training in microbial management.

Conclusion

This study demonstrates that microbial co-occurrence networks are strongly linked to soil health indices, with denser networks in organic soils correlating with higher SOC, pH, MWD, and nitrogen. RF models effectively predicted soil health from network metrics, achieving R^2 values up to 0.90. Keystone taxa like *Bacillus* and *Mortierella* were critical in organic systems, underscoring their role in soil ecosystem function. These findings advocate for microbial-based soil management strategies to enhance agricultural sustainability. Future work should focus on functional gene analysis and real-time monitoring to operationalize these insights.

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