Co-occurrence Networks of Soil Microbes in Relation to Soil pH and Organic Matter Fractions

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Abstract
Soil microbial communities exhibit complex interaction patterns that are significantly influenced by environmental factors, particularly soil pH and organic matter composition. This study investigated the co-occurrence networks of soil microbes across different pH gradients and organic matter fractions to understand microbial community assembly and ecological interactions. We analyzed 120 soil samples from agricultural and forest ecosystems using 16S rRNA and ITS gene sequencing, coupled with comprehensive soil chemical analyses. Network analysis revealed distinct microbial co-occurrence patterns across pH ranges, with acidic soils (pH 4.5-5.5) showing higher network complexity and stronger positive correlations compared to alkaline soils (pH 7.5-8.5). Organic matter fractions, particularly dissolved organic carbon (DOC) and particulate organic matter (POM), emerged as key drivers of microbial network topology. Bacterial networks demonstrated greater stability across pH gradients compared to fungal networks, which showed pronounced shifts in community structure. The study identified 23 keystone taxa that maintained network stability across environmental gradients. These findings provide crucial insights into soil microbial ecology and have implications for sustainable soil management practices in agricultural systems.

Keywords: Soil Microbiome, Co-Occurrence Networks, Soil Ph, Organic Matter, Microbial Interactions, Network Topology, Keystone Species, Soil Ecology

Introduction

Soil microbial communities represent one of the most diverse and complex ecosystems on Earth, harboring an estimated 10^9^ bacterial cells per gram of soil [1, 2]. These communities play fundamental roles in biogeochemical cycling, plant health, and ecosystem functioning through intricate networks of ecological interactions [3, 4]. Understanding the patterns and drivers of microbial co-occurrence has become increasingly important for predicting ecosystem responses to environmental changes and developing sustainable land management strategies [5, 6].

Co-occurrence networks provide a powerful framework for analyzing microbial community structure and inferring potential ecological interactions [7, 8]. These networks are constructed based on statistical correlations between microbial taxa across environmental gradients, revealing patterns of species associations that may reflect ecological processes such as competition, cooperation, or shared environmental preferences [9, 10]. Network topology metrics, including modularity, centrality, and clustering coefficients, offer insights into community stability, resilience, and functional organization [11, 12].

Soil pH represents one of the most influential environmental factors shaping microbial community composition and diversity [13, 14]. Previous studies have demonstrated that bacterial diversity typically peaks at neutral pH, while fungal communities often show greater tolerance to acidic conditions [15, 16]. However, the impact of pH on microbial co-occurrence patterns and network structure remains poorly understood, particularly across different ecosystem types and management regimes [17, 18].

Organic matter fractions in soil provide the primary energy and carbon sources for microbial communities, influencing both community composition and metabolic activity [19, 20].

Different organic matter pools, including dissolved organic carbon (DOC), particulate organic matter (POM), and mineral-associated organic matter (MAOM), exhibit distinct chemical properties and bioavailability, potentially leading to specialized microbial associations and niche differentiation [21, 22]. The objectives of this study were to: (1) characterize co-occurrence networks of soil bacteria and fungi across pH gradients in agricultural and forest systems, (2) evaluate the influence of organic matter fractions on network topology and microbial interactions, and (3) identify keystone taxa that maintain network stability across environmental conditions.

Materials and Methods

Study Sites and Sampling Design

Soil samples were collected from 120 locations across three distinct ecosystems in the temperate region: agricultural fields (n=40), deciduous forests (n=40), and mixed grasslands (n=40). Sampling sites were selected to represent a broad pH gradient (4.2-8.7) and varying organic matter content. At each location, five soil cores (0-15 cm depth) were collected within a 10 m² area and composited to form a representative sample. Samples were stored at 4°C during transport and processed within 48 hours of collection.

Soil Chemical Analysis

Soil pH was measured in 1:2.5 soil water suspension using a calibrated pH meter [23]. Total organic carbon (TOC) was determined by dry combustion using a CHN analyzer. Organic matter fractions were separated using established protocols [24]: dissolved organic carbon (DOC) was extracted with 0.5 M K_2SO_4 solution, particulate organic matter (POM) was isolated by density fractionation (>53 µm), and mineral-associated organic matter (MAOM) was calculated as the difference between TOC and POM. Additional soil properties including available nitrogen, phosphorus, and major cations were analyzed using standard methods [25].

DNA Extraction and Sequencing

Total genomic DNA was extracted from 0.5 g soil using the Power Soil DNA Isolation Kit (Qiagen) following manufacturer's protocols. Bacterial 16S rRNA genes were amplified using primers 515F/806R targeting the V4 region, while fungal ITS1 region was amplified using primers ITS1F/ITS2 [26, 27]. PCR products were purified, indexed, and pooled for paired-end sequencing (2×250 bp) on an Illumina MiSeq platform.

Bioinformatics and Statistical Analysis

Raw sequences were processed using QIIME2 pipeline [28]. Quality filtering, denoising, and chimera removal were performed using DADA2. Taxonomic assignment was conducted against SILVA database (v138) for bacteria and UNITE database (v8.3) for fungi. Operational taxonomic units (OTUs) with <0.01% relative abundance across all samples were removed to reduce noise.

Co-occurrence networks were constructed separately for bacterial and fungal communities using SparCC algorithm to account for compositional data characteristics [29]. Networks were built for different pH categories: acidic (pH <6.0), neutral (pH 6.0-7.5), and alkaline (pH >7.5). Only correlations with |r| >0.6 and P <0.01 (FDR-corrected) were retained. Network visualization and topology analysis were performed using Gephi and I graph packages.

Results

Soil Characteristics and Microbial Diversity

Soil samples exhibited substantial heterogeneity in chemical properties across the study sites (Table 1). pH values ranged from 4.2 to 8.7, with agricultural soils showing the broadest pH range (4.8-8.3). Total organic carbon content varied from 12.3 to 67.8 g kg⁻¹, with forest soils generally containing higher organic matter levels.

Table 1: Soil characteristics across ecosystem types

Parameter	Agricultural (n=40)	Forest (n=40)	Grassland (n=40)	Overall Range
pH	6.8±1.2	5.4±0.9	6.2±1.1	4.2-8.7
TOC (g kg^{-1})	28.4±12.6	45.7±15.3	33.2±11.8	12.3-67.8
DOC (mg kg^{-1})	67.3±28.4	98.6±35.7	74.2±26.9	23.5-156.7
POM (g kg^{-1})	8.2±4.1	15.6±6.8	11.3±4.9	2.1-28.4
MAOM (g kg^{-1})	20.2±9.7	30.1±12.4	21.9±8.6	8.7-52.3

DOC concentrations ranged from 23.5 to 156.7 mg kg^{-1}, representing 2.1-4.8% of total organic carbon.

A total of 3,847 bacterial OTUs and 1,923 fungal OTUs were identified across all samples. Bacterial communities were dominated by Acidobacteria (18.3%), Proteobacteria (16.7%), and Actinobacteria (14.2%), while fungal communities were primarily composed of Ascomycota (52.4%) and Basidiomycota (31.8%). Alpha diversity showed significant variation across pH gradients, with bacterial richness peaking at neutral pH (Shannon diversity: 7.2±0.4) and declining in both acidic (6.1±0.6) and alkaline conditions (5.8±0.5).

Network Topology Across pH Gradients

Co-occurrence networks exhibited distinct topological properties across pH categories (Table 2). Acidic soils displayed the most complex networks with higher node connectivity and modularity compared to neutral and alkaline conditions. Bacterial networks consistently showed greater stability across pH gradients than fungal networks.

Table 2: Network topology metrics across pH categories

Network Property	Acidic pH	Neutral pH	Alkaline pH
Bacterial Networks			
Nodes	487	523	391
Edges	1,243	1,089	678
Average degree	5.1	4.2	3.5
Modularity	0.67	0.58	0.52
Clustering coefficient	0.43	0.38	0.31
Fungal Networks			
Nodes	298	267	201
Edges	562	423	287
Average degree	3.8	3.2	2.9
Modularity	0.71	0.63	0.59
Clustering coefficient	0.39	0.34	0.28

Influence of Organic Matter Fractions

Organic matter fractions showed differential effects on network structure and microbial associations. DOC concentration emerged as the strongest predictor of network complexity ($r = 0.72$, $P < 0.001$), followed by POM content ($r = 0.58$, $P < 0.01$). Networks in soils with high DOC content exhibited increased modularity and stronger positive correlations between taxa (Figure 2).

Specific microbial taxa showed preferential associations with different organic matter pools. Copiotrophic bacteria, including members of *Betaproteobacteria* and *Gammaproteobacteria*, were strongly correlated with DOC availability. Conversely, oligotrophic taxa such as Acidobacteria showed stronger associations with MAOM fractions, suggesting specialized metabolic strategies for accessing recalcitrant organic compounds.

Keystone Taxa Identification

Network analysis identified 23 keystone taxa that maintained high centrality values across different pH conditions and organic matter gradients (Table 3). These keystone species included representatives from major bacterial phyla (Proteobacteria, Acidobacteria, Actinobacteria) and fungal groups (Ascomycota, Basidiomycota). Notably, several keystone bacteria belonged to genera known for their versatile metabolic capabilities, including *Pseudomonas*, *Streptomyces*, and Rhizobium.

Table 3: Selected keystone taxa and their network properties

Taxon	Phylum	Betweenness Centrality	Degree	pH Range
Pseudomonas sp.	Proteobacteria	0.087	23	4.5-8.2
Streptomyces sp.	Actinobacteria	0.076	19	5.1-8.0
Acidobacteria GP1	Acidobacteria	0.069	17	4.2-7.8
Mortierella sp.	Mortierellomycota	0.063	15	4.8-7.5
Rhizobium sp.	Proteobacteria	0.058	14	5.5-8.3

Discussion

This study provides comprehensive insights into the complex relationships between soil pH, organic matter fractions, and microbial co-occurrence networks. The observed increase in network complexity under acidic conditions contrasts with traditional views of pH effects on microbial diversity, suggesting that while species richness may decline in acidic soils, the remaining taxa form more intricate interaction networks.

The differential response of bacterial and fungal networks to pH gradients reflects fundamental differences in their ecological strategies and stress tolerance mechanisms. Fungi generally exhibit greater tolerance to acidic conditions due to their ability to actively regulate intracellular pH and secrete organic acids. This physiological advantage may explain the maintained network stability observed in fungal communities across pH gradients. The strong correlation between DOC concentration and network complexity highlights the importance of readily available carbon sources in supporting diverse microbial interactions. High DOC environments may promote cooperative relationships among microorganisms through cross-feeding and metabolic complementarity. The preferential association of copiotrophic bacteria with DOC pools aligns with their fast-growth strategies and high resource requirements.

The identification of keystone taxa provides valuable insights for ecosystem management and restoration efforts. These taxa likely play critical roles in maintaining community stability and ecosystem functioning through their central positions in interaction networks. The prevalence of metabolically versatile genera among keystone species suggests that functional diversity, rather than taxonomic diversity alone, may be key to network stability.

Conclusion

This study demonstrates that co-occurrence networks of soil microbes are significantly influenced by both soil pH and organic matter fractions, with distinct patterns emerging across environmental gradients. Acidic soils support more complex microbial networks despite lower overall diversity, while organic matter quality, particularly DOC availability, serves as a major driver of network topology. The identification of keystone taxa provides a foundation for developing targeted management strategies to maintain soil microbial diversity and ecosystem functionality. Future research should investigate the temporal dynamics of these networks and their responses to anthropogenic disturbances to better understand soil ecosystem resilience and stability.

References

1. Whitman WB, Coleman DC, Wiebe WJ. Prokaryotes: the unseen majority. Proceedings of the National Academy of Sciences of the United States of America. 1998;95(12):6578-6583.
2. Roesch LFW, Fulthorpe RR, Riva A, Casella G, Hadwin AKM, Kent AD, *et al*. Pyrosequencing enumerates and contrasts soil microbial diversity. The ISME Journal. 2007;1(4):283-290.
3. Falkowski PG, Fenchel T, Delong EF. The microbial engines that drive Earth's biogeochemical cycles. Science. 2008;320(5879):1034-1039.
4. van der Heijden MGA, Bardgett RD, van Straalen NM. The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters. 2008;11(3):296-310.
5. Shade A, Peter H, Allison SD, Baho DL, Berga M, Bürgmann H, *et al*. Fundamentals of microbial community resistance and resilience. Frontiers in Microbiology. 2012;3:417.
6. Wagg C, Bender SF, Widmer F, van der Heijden MGA. Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proceedings of the National Academy of Sciences of the United States of America. 2014;111(14):5266-5270.
7. Faust K, Raes J. Microbial interactions: from networks to models. Nature Reviews Microbiology. 2012;10(8):538-550.
8. Berry D, Widder S. Deciphering microbial interactions and detecting keystone species with co-occurrence networks. Frontiers in Microbiology. 2014;5:219.
9. Barberán A, Bates ST, Casamayor EO, Fierer N. Using network analysis to explore co-occurrence patterns in soil microbial communities. The ISME Journal. 2012;6(2):343-351.
10. Zhou J, Deng Y, Luo F, He Z, Tu Q, Zhi X. Functional molecular ecological networks. mBio. 2010;1(4):e00169-10.
11. Newman MEJ. Modularity and community structure in networks. Proceedings of the National Academy of Sciences of the United States of America. 2006;103(23):8577-8582.
12. Olesen JM, Bascompte J, Dupont YL, Jordano P. The modularity of pollination networks. Proceedings of the National Academy of Sciences of the United States of America. 2007;104(50):19891-19896.
13. Fierer N, Jackson RB. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences of the United States of America. 2006;103(3):626-631.
14. Lauber CL, Hamady M, Knight R, Fierer N. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Applied and Environmental Microbiology. 2009;75(15):5111-5120.
15. Rousk J, Bååth E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG, *et al*. Soil bacterial and fungal communities across a pH gradient in an arable soil. The ISME Journal. 2010;4(10):1340-1351.
16. Tedersoo L, Bahram M, Põlme S, Kõljalg U, Yorou NS, Wijesundera R, *et al*. Global diversity and geography of soil fungi. Science. 2014;346(6213):1256688.
17. Zhalnina K, Dias R, de Quadros PD, Davis-Richardson A, Camargo FAO, Clark IM, *et al*. Soil pH determines microbial diversity and composition in the park grass experiment. Microbial Ecology. 2015;69(2):395-406.
18. Delgado-Baquerizo M, Oliverio AM, Brewer TE, Benavent-González A, Eldridge DJ, Bardgett RD, *et al*. A global atlas of the dominant bacteria found in soil. Science. 2018;359(6373):320-325.
19. Cotrufo MF, Wallenstein MD, Boot CM, Denef K, Paul E. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization. Global Change Biology. 2013;19(4):988-995.
20. Kallenbach CM, Frey SD, Grandy AS. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. Nature Communications. 2016;7:13630.
21. Lavallee JM, Soong JL, Cotrufo MF. Conceptualizing soil organic matter into particulate and mineral-associated forms to address global change in the 21st century. Global Change Biology. 2020;26(1):261-273.
22. Lehmann J, Kleber M. The contentious nature of soil organic matter. Nature. 2015;528(7580):60-68.
23. Thomas GW. Soil pH and soil acidity. In: Sparks DL, editor. Methods of soil analysis. Part 3. Chemical methods. Madison: Soil Science Society of America Book Series; c1996. p. 475-490.
24. Cambardella CA, Elliott ET. Particulate soil organic-matter changes across a grassland cultivation sequence. Soil Science Society of America Journal. 1992;56(3):777-783.
25. Robertson GP, Coleman DC, Bledsoe CS, Sollins P. Standard soil methods for long-term ecological research. New York: Oxford University Press; c1999.
26. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, *et al*. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences of the United States of America. 2011;108(Suppl 1):4516-4522.
27. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. New York: Academic Press; c1990. p. 315-322.
28. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, *et al*. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology. 2019;37(8):852-857.
29. Friedman J, Alm EJ. Inferring correlation networks from genomic survey data. PLoS Computational Biology. 2012;8(9):e1002687.