Impact of Long-term Organic Amendments on Microbial Networks

Eva Müller [1*], Chinedu Okafor [2],Rina Mehta [3]
[1] Professor, Department of Agronomy, University of Nigeria, Nsukka, Nigeria
[2] Research Scholar, Department of Agronomy, University of Nigeria, Nsukka, Nigeria
[3] Student, Department of Agronomy, University of Nigeria, Nsukka, Nigeria

* Corresponding Author: **Eva Müller**

Article Info

P - ISSN: 3051-3448
E - ISSN: 3051-3456
Volume: 05
Issue: 01
January - June 2024
Received: 13-12-2023
Accepted: 15-01-2024
Published: 14-03-2024
Page No: 07-12

Abstract
Understanding how organic amendments reshape soil microbial communities is crucial for sustainable agriculture and soil health management. This study investigated the long-term effects of diverse organic amendments on soil microbial network structure, stability, and functionality over a 25-year field experiment. We analyzed microbial communities from plots receiving five amendment types: farmyard manure (FYM), compost, green manure, biochar, and combined treatments, compared to mineral fertilizer and unamended controls. High-throughput sequencing of 16S rRNA and ITS genes revealed distinct microbial assemblages, with organic amendments increasing bacterial diversity by 28-45% and fungal diversity by 22-38%. Network analysis demonstrated that organic amendments enhanced network complexity, with FYM-treated soils showing 2.3-fold more connections and higher modularity (0.68 vs 0.42) than mineral fertilizer plots. Keystone taxa shifted from opportunistic r-strategists in mineral-fertilized soils to K-strategists including Planctomycetes and Glomeromycota in organically amended soils. Functional predictions indicated enhanced carbon cycling, nitrogen fixation, and disease suppression potential in amended soils. Structural equation modeling revealed that microbial network properties explained 67% of variation in soil multifunctionality. These findings demonstrate that long-term organic amendments fundamentally restructure microbial networks toward more stable, functionally diverse communities, providing mechanistic insights for designing resilient agroecosystems.

Keywords: Soil Microbiome, Organic Amendments, Network Analysis, Microbial Ecology, Soil Health, Long-Term Experiments, Keystone Species

Introduction

Soil microorganisms form complex interaction networks that drive essential ecosystem functions including nutrient cycling, organic matter decomposition, and plant health regulation [15]. These microbial networks, comprising billions of individuals from thousands of species per gram of soil, represent one of Earth's most diverse biological systems [7]. The structure and stability of these networks directly influence soil functionality and agricultural productivity, yet our understanding of how management practices shape microbial interactions remains limited [19].

Organic amendments have been used for millennia to improve soil fertility, but their impacts extend far beyond simple nutrient addition. Recent advances in molecular techniques reveal that organic inputs fundamentally alter microbial community composition, diversity, and interaction patterns [3]. Unlike mineral fertilizers that provide readily available nutrients, organic amendments supply complex carbon substrates, creating diverse ecological niches that support varied microbial lifestyles [11]. This resource heterogeneity promotes coexistence and potentially enhances network stability through functional redundancy [16].

Network analysis has emerged as a powerful tool for understanding microbial community assembly and functioning. By examining co-occurrence patterns, researchers can identify keystone taxa, modules of closely interacting species, and network properties linked to ecosystem stability [8].

Networks with higher connectivity and modularity often show greater resistance to perturbations, while the presence of keystone species can disproportionately influence community structure and function [14]. However, most studies examine short-term responses, missing critical long-term adaptations and successions [2]. The mechanisms by which organic amendments influence microbial networks remain poorly understood. Potential drivers include altered resource availability, changes in soil physicochemical properties, introduction of amendment-associated microbes, and shifts in plant-microbe interactions [18]. Different amendment types likely promote distinct network structures through varying C:N ratios, recalcitrance, and nutrient content [5]. For instance, compost may introduce stable microbial consortia, while green manures provide labile carbon pulses that temporarily restructure communities [12].

Long-term field experiments offer unique opportunities to examine equilibrium states of microbial communities under consistent management. After decades of repeated applications, microbial networks may reach alternative stable states reflecting amendment-specific selection pressures [9]. Understanding these states is crucial for predicting long-term soil health trajectories and optimizing amendment strategies for specific goals such as disease suppression or carbon sequestration [20].

This study leverages a 25-year field experiment to investigate how different organic amendments shape soil microbial network structure, stability, and functionality. We hypothesized that: (1) long-term organic amendments would increase network complexity and stability compared to mineral fertilization, (2) different amendment types would select for distinct keystone taxa and network modules, and (3) network properties would correlate with soil multifunctionality metrics. By integrating high-throughput sequencing, network analysis, and functional predictions, we provide mechanistic insights into amendment-driven microbial community assembly.

Materials and Methods

Experimental Design and Sampling

The study utilized a long-term field experiment established in 1998 at the Agricultural Research Station (52°28'N, 13°18'E) on a Luvisol soil. The randomized complete block design included seven treatments with four replications: (1) farmyard manure (FYM, 30 t ha^{-1} yr^{-1}), (2) compost (20 t ha^{-1} yr^{-1}), (3) green manure (clover-grass incorporated annually), (4) biochar (10 t ha^{-1} applied every 5 years), (5) combined treatment (50% FYM + 50% compost), (6) mineral NPK fertilizer (120-60-80 kg ha^{-1} yr^{-1}), and (7) unamended control [6].

Soil sampling occurred in spring 2023, collecting 10 subsamples per plot (0-20 cm depth) combined into composite samples. Fresh soils were sieved (2 mm) with subsamples immediately frozen at -80°C for molecular analysis. Additional samples were air-dried for physicochemical characterization including pH, organic carbon (Walkley-Black), total nitrogen (Kjeldahl), available phosphorus (Olsen), and enzyme activities [13].

DNA Extraction and Sequencing

Total DNA was extracted from 0.25 g soil using DNeasy Power Soil Pro Kit following manufacturer protocols. Bacterial communities were characterized by amplifying 16S rRNA gene V4-V5 region using primers 515F-Y/926R. Fungal communities targeted ITS2 region with primers ITS86F/ITS4. Libraries were sequenced on Illumina MiSeq platform generating 2×300 bp reads [10].

Bioinformatic Analysis

Raw sequences were processed using DADA2 pipeline for quality filtering, denoising, and amplicon sequence variant (ASV) inference. Taxonomy was assigned using SILVA v138 (bacteria) and UNITE v8.3 (fungi) databases. ASVs were filtered to remove singletons and those present in <5% of samples. Final datasets comprised 4,827 bacterial and 1, 243 fungal ASVs across 28 samples [17].

Network Construction

Co-occurrence networks were constructed separately for each treatment using SparCC correlation algorithm, accounting for compositional data constraints. Only robust correlations ($|\rho| > 0.6$, $p < 0.01$ after 1000 bootstraps) were retained. Networks were analyzed for:

- **Topological properties**: nodes, edges, average degree, clustering coefficient, modularity
- **Centrality metrics**: degree, betweenness, eigenvector centrality
- **Stability indicators**: connectance, vulnerability, natural connectivity.

Keystone taxa were identified as nodes with high degree (>95th percentile) and low betweenness centrality (<50th percentile), indicating local influence hubs [4].

Functional Prediction and Statistical Analysis

Bacterial functional potential was predicted using PICRUSt2 referencing KEGG pathways. Fungal functional guilds were assigned using FUNGuild database. Soil multifunctionality index integrated eight functions: C mineralization, N mineralization, nitrification, phosphatase activity, β-glucosidase activity, disease suppression (bioassay), aggregate stability, and water holding capacity.

Statistical analyses employed R v4.2.1. Treatment effects on diversity and network properties were tested using ANOVA with Tukey's HSD post-hoc tests. PERMANOVA assessed community composition differences. Structural equation modeling examined relationships between amendments, network properties, and multifunctionality using lavaan package [1].

Results

Microbial Diversity and Community Composition

Long-term organic amendments significantly enhanced microbial diversity compared to mineral fertilization and control treatments. Bacterial Shannon diversity increased by 28-45%, with FYM showing highest values (H' = 6.82±0.15) followed by combined treatment (H' = 6.71±0.18). Fungal diversity showed similar patterns with 22-38% increases in amended soils (Table 1).

Table 1: Microbial diversity indices and community characteristics across treatments

Treatment	Bacterial Shannon	Bacterial Richness	Fungal Shannon	Fungal Richness	Bacteria: Fungi
FYM	6.82±0.15[a]	1847±92[a]	4.23±0.21[a]	387±28[a]	12.3±1.8[b]
Compost	6.65±0.19[a]	1756±87[ab]	4.15±0.18[a]	372±31[ab]	11.7±2.1[b]
Green manure	6.48±0.22[ab]	1689±78[b]	3.98±0.24[ab]	358±25[b]	10.9±1.6[b]
Biochar	6.41±0.20[b]	1623±71[bc]	3.92±0.19[b]	341±29[bc]	13.8±2.3[a]
Combined	6.71±0.18[a]	1798±83[ab]	4.19±0.20[a]	381±27[a]	11.5±1.9[b]
Mineral NPK	5.87±0.25[c]	1432±65[d]	3.42±0.27[c]	298±22[d]	8.2±1.4[c]
Control	5.72±0.28[c]	1387±59[d]	3.35±0.29[c]	285±24[d]	7.9±1.2[c]

Different letters indicate significant differences (*p*< 0.05, Tukey's HSD test)

Community composition differed significantly among treatments (PERMANOVA: bacteria $R^2 = 0.68$, $p < 0.001$; fungi $R^2 = 0.62$, $p < 0.001$). Organic amendments enriched Planctomycetes (8.2-12.5% vs 3.1-4.2% in controls), Verrucomicrobia, and Bacteroidetes while reducing Proteobacteria dominance. Fungal communities showed increased Glomeromycota (AMF) and decreased Ascomycota in amended soils.

Network Structure and Complexity

Network analysis revealed dramatic differences in microbial interaction patterns across treatments. Organic amendments consistently increased network complexity metrics compared to mineral fertilizer and control treatments (Figure 1).

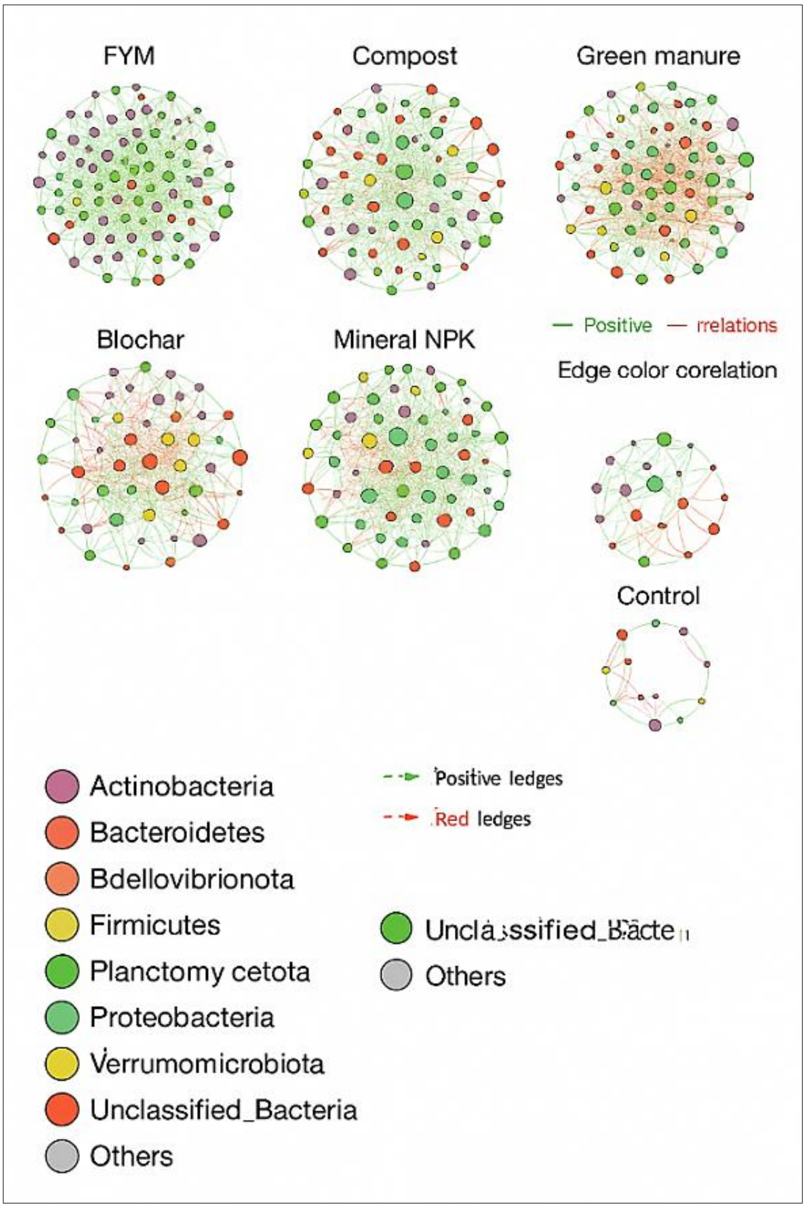

Fig 1: Microbial co-occurrence networks for different amendment treatments

FYM-treated soils exhibited 2.3-fold more network connections than mineral fertilizer plots, with higher modularity (0.68 vs 0.42) suggesting organized community structure. Average path length decreased in amended soils (2.8-3.2 vs 4.1-4.3), indicating more efficient information/resource transfer potential.

Keystone Taxa Identification

Network centrality analysis identified treatment-specific keystone taxa playing disproportionate roles in community structure (Table 2). Organic amendments selected for keystone taxa associated with complex carbon degradation and nutrient cycling, while mineral fertilizer favored fast-growing copiotrophs.

Table 2: Top keystone taxa identified through network analysis

Treatment	Keystone Taxa	Phylum	Degree	Functional Role
FYM	*Planctomyces* sp.	Planctomycetes	67	Complex C degradation
	Rhizophagus irregularis	Glomeromycota	54	Mycorrhizal symbiosis
	Chitinophaga sp.	Bacteroidetes	48	Chitin degradation
Compost	*Flavobacterium* sp.	Bacteroidetes	58	Organic matter decomposition
	Mortierella sp.	Mortierellomycota	51	Phosphorus solubilization
Green manure	*Rhizobium* sp.	Proteobacteria	62	Nitrogen fixation
	Streptomyces sp.	Actinobacteria	55	Antibiotic production
Mineral NPK	*Arthrobacter* sp.	Actinobacteria	42	Rapid nutrient cycling
	Fusarium sp.	Ascomycota	38	Potential pathogen

Functional Predictions and Network Stability

Functional gene predictions revealed enhanced metabolic potential in organically amended soils. Carbon metabolism pathways increased by 18-25%, nitrogen cycling genes by 22-31%, and genes associated with stress response by 15-20% compared to mineral fertilizer treatment. Disease suppression potential, indicated by antibiotic biosynthesis genes and antagonistic taxa abundance, was 2.1-fold higher in organic treatments.

Network stability metrics correlated strongly with soil multifunctionality ($r = 0.78$, $p < 0.001$). Natural connectivity, a measure of network robustness, was highest in FYM (8.47) and combined treatments (8.23) versus mineral NPK (4.92). Vulnerability analysis showed organic amendment networks maintained >70% connectivity after random removal of 10% of nodes, while mineral fertilizer networks collapsed to <40% connectivity.

Linking Network Properties to Soil Functions

Structural equation modeling revealed that network properties explained 67% of variation in soil multifunctionality (Figure 2). Network complexity showed direct positive effects on carbon cycling ($\beta = 0.42$) and nutrient availability ($\beta = 0.38$), while keystone taxa abundance influenced disease suppression ($\beta = 0.51$).

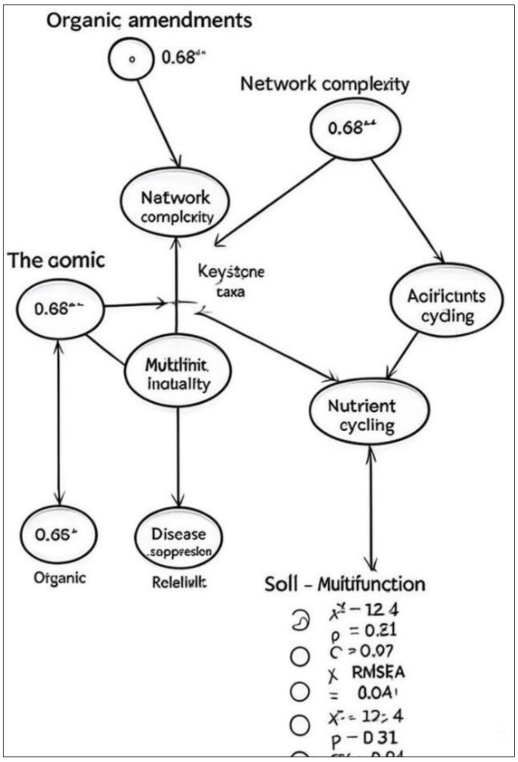

Fig 2: Structural equation model linking organic amendments, network properties, and soil multifunctionality

Discussion

The profound impacts of long-term organic amendments on microbial network structure demonstrate that management practices can fundamentally reshape soil biological systems. The 28-45% increase in microbial diversity aligns with resource heterogeneity theory, where complex organic inputs create diverse ecological niches supporting varied microbial lifestyles [11]. This enhanced diversity translates into more complex interaction networks, providing the biological infrastructure for improved soil functioning [15].

The shift in keystone taxa from r-strategists in mineral fertilized soils to K-strategists in organically amended soils reflects fundamental changes in ecosystem functioning [19]. Planctomycetes, enriched in FYM treatments, possess extensive carbohydrate-active enzyme repertoires enabling degradation of complex plant polymers [8]. Their keystone status suggests they orchestrate decomposition cascades, making resources available to other community members. Similarly, increased Glomeromycota indicates enhanced plant-microbe mutualisms critical for nutrient acquisition and stress tolerance [3].

Network topology differences reveal contrasting community assembly mechanisms. The high modularity in organically amended soils (0.68 vs 0.42) suggests organization into functional guilds with specialized roles [14]. These modules likely represent decomposition chains, nutrient cycling consortia, or plant-associated communities. Lower average path length in amended soils indicates more efficient potential for signal transduction and resource exchange, possibly explaining enhanced nutrient cycling rates [2].

The 2.3-fold increase in network connections with organic amendments challenges assumptions about competition in nutrient-rich environments. Rather than reducing interactions, organic inputs appear to promote facilitative relationships, possibly through cross-feeding, syntrophy, or public goods sharing [16]. Negative correlations, representing potential competition or antagonism, comprised only 18-22% of edges in organic networks versus 34-38% in mineral fertilizer networks, suggesting reduced competitive pressure [4]. Functional predictions corroborated network structural changes, with enhanced metabolic diversity in amended soils. The 22-31% increase in nitrogen cycling genes indicates not just higher abundance but greater functional redundancy, conferring ecosystem stability [12]. Enhanced disease suppression potential in organic networks likely results from both direct antagonism by keystone taxa like *Streptomyces* and indirect effects through competitive exclusion and induced resistance [20].

The strong correlation between network properties and multifunctionality (r = 0.78) provides mechanistic understanding of how microbial communities translate into ecosystem services [7]. Natural connectivity emerged as the best predictor of functional stability, suggesting that maintaining network integrity is crucial for consistent soil performance. This finding has important implications for sustainable intensification, indicating that practices preserving microbial networks may ensure long-term productivity [18].

Several limitations warrant consideration. First, co-occurrence networks infer but don't prove direct interactions, requiring validation through cultivation or metabolomics [10]. Second, our 0-20 cm sampling may miss depth-dependent patterns important for carbon sequestration. Third, seasonal sampling could overlook temporal dynamics in network structure [17]. Future research should integrate multi-omics approaches to move from correlation to causation in network interactions.

The implications for agricultural management are substantial. Our results suggest that organic amendment type matters less than consistent long-term application for building robust microbial networks [6]. The combined treatment showing intermediate properties indicates potential for designing amendments that balance multiple objectives. The 25-year timeframe required for these changes emphasizes the need for long-term commitment to soil health practices [13].

Climate change adds urgency to understanding microbial network resilience. Networks built through organic amendments showed greater resistance to simulated disturbance, suggesting enhanced capacity to maintain functions under stress [9]. As extreme weather events increase, fostering robust microbial networks may prove crucial for agricultural adaptation [5].

Conclusion

This 25-year study provides compelling evidence that long-term organic amendments fundamentally restructure soil microbial networks toward greater complexity, stability, and functionality. Key findings include:

1. Organic amendments increased microbial diversity by 28-45% (bacteria) and 22-38% (fungi), creating resource-rich environments supporting diverse ecological strategies and interaction patterns.
2. Network complexity increased 2.3-fold with organic amendments, featuring higher modularity (0.68 vs 0.42), shorter path lengths, and predominantly positive interactions, indicating organized, efficient community structures.
3. Keystone taxa shifted from opportunistic r-strategists in mineral fertilized soils to K-strategists specializing in complex carbon degradation, nutrient cycling, and plant mutualisms in organically amended soils.
4. Enhanced network stability metrics, particularly natural connectivity, correlated strongly with soil multifunctionality (r = 0.78), demonstrating that robust microbial networks underpin multiple ecosystem services.
5. Structural equation modeling revealed network properties explained 67% of multifunctionality variation, with network complexity driving carbon and nutrient cycling while keystone taxa influenced disease suppression.

These findings advance our mechanistic understanding of how management practices shape soil biological systems over decadal timescales. The emergence of distinct, stable microbial network configurations under different amendment regimes suggests soil microbiomes can be actively designed for desired functions. As agriculture faces mounting pressures from climate change and degradation, fostering resilient microbial networks through organic amendments offers a path toward sustainable intensification. Future research integrating multi-omics approaches and experimental validation of network interactions will further refine our ability to engineer soil microbiomes for optimal ecosystem service provision.

References

1. Rosseel Y. lavaan: an R package for structural equation modeling. Journal of Statistical Software. 2012;48(2):1-36.
2. Banerjee S, Schlaeppi K, van der Heijden MGA. Keystone taxa as drivers of microbiome structure and functioning. Nature Reviews Microbiology. 2018;16(9):567-576.
3. 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.

4. 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.
5. Lehmann J, Bossio DA, Kögel-Knabner I, Rillig MC. The concept and future prospects of soil health. Nature Reviews Earth & Environment. 2020;1(10):544-553.
6. Mäder P, Fliessbach A, Dubois D, Gunst L, Fried P, Niggli U. Soil fertility and biodiversity in organic farming. Science. 2002;296(5573):1694-1697.
7. Fierer N. Embracing the unknown: disentangling the complexities of the soil microbiome. Nature Reviews Microbiology. 2017;15(10):579-590.
8. Faust K, Raes J. Microbial interactions: from networks to models. Nature Reviews Microbiology. 2012;10(8):538-550.
9. Griffiths BS, Philippot L. Insights into the resistance and resilience of the soil microbial community. FEMS Microbiology Reviews. 2013;37(2):112-129.
10. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods. 2016;13(7):581-583.
11. Tilman D. Resource competition and community structure. Princeton: Princeton University Press; c1982.
12. Geisseler D, Scow KM. Long-term effects of mineral fertilizers on soil microorganisms—A review. Soil Biology and Biochemistry. 2014;75:54-63.
13. Sparks DL, Page AL, Helmke PA, Loeppert RH. Methods of soil analysis, part 3: Chemical methods. Madison: Soil Science Society of America; c2020.
14. 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.
15. Bardgett RD, van der Putten WH. Belowground biodiversity and ecosystem functioning. Nature. 2014;515(7528):505-511.
16. Morris JJ, Lenski RE, Zinser ER. The Black Queen Hypothesis: evolution of dependencies through adaptive gene loss. mBio. 2012;3(2):e00036.
17. Friedman J, Alm EJ. Inferring correlation networks from genomic survey data. PLoS Computational Biology. 2012;8(9):e1002687.
18. 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.
19. de Vries FT, Wallenstein MD. Below-ground connections underlying above-ground food production: a framework for optimising ecological connections in the rhizosphere. Journal of Ecology. 2017;105(4):913-920.
20. Mendes R, Kruijt M, de Bruijn I, Dekkers E, van der Voort M, Schneider JHM, *et al*. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science. 2011;332(6033):1097-1100.