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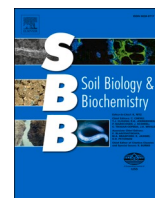
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Bacterial and fungal co-occurrence patterns in agricultural soils amended with compost and bokashi

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ABSTRACT

The living soil harbors a significant number and diversity of bacteria and fungi, which are essential in sustaining soil ecosystem functions. Most studies focus on soil bacteria or fungi, ignoring potential interrelationships between kingdoms that coevolve and synergistically provide ecosystem functions. In a seven-year agricultural field, we explored the effects of organic amendments (OAs; i.e., compost and bokashi) on intra- and inter-kingdom co-occurrence networks of soil bacterial and fungal communities. We observed that OAs changed the co-occurrence patterns of bacteria and fungi. Distinct network modules were observed in the unamended and amended soils, and the physicochemical properties of the soil could partially explain the formation of these modules. We also found that compost and bokashi increased the proportion of positive correlations, and this could reduce competition among microorganisms for soil resources. Our study reveals that soil management with OAs affects relationships between bacterial and fungal subpopulations that physically co-exist, cooperate, and compete in non-random structured networks. It highlights that ecosystem functions may depend on inter-kingdom interactions shaped by different amendments and their applied dose.

1. Introduction

Soil ecosystem functioning is a fundamental component of productive agriculture, largely driven by a myriad of positive and negative, direct and indirect interactions among soil organisms (Wagg et al., 2019). These soil communities generally consist of diverse bacterial and fungal subpopulations that coevolve and synergistically provide ecosystem functions (Fuhrman, 2009; Bezemer et al., 2010; Haq et al., 2014). Intra- and inter-kingdom interactions cannot be understood only based on richness, diversity, and community composition (Wagg et al., 2019), but aggregated organisms that physically co-exist, cooperate and compete in non-random structures that can be conceptually projected and investigated as co-occurrence networks (Barberán et al., 2012; Lu et al., 2013; Ma et al., 2016). While biological activity in agricultural soils facilitates the availability of nutrients for crops from organic amendments (OAs), it remains unclear if common OAs alter the structure and function of soil microbial networks.

The soil microbial biomass is viewed as the “eye of the needle” in the

soil, through which organic matter must pass (Jenkinson, 1977; Sparling, 1985). In this sense, soil microbes are sensitive to the quality and distribution of soil organic pools. Studies have shown that abiotic variables induced by OAs control soil bacterial or fungal community composition (Pérez-Piqueres et al., 2006; Lu et al., 2015; Dumontet et al., 2017), which subsequently shapes the relationships between microbial taxa (Prosser et al., 2007). While several studies have shown that the addition of OAs can affect co-occurrence patterns of soil bacterial or fungal communities, these studies did not interrogate interrelationships between kingdoms (Ling et al., 2016; Schmid et al., 2018; Xue et al., 2018; Yang et al., 2019, 2020; Zhou et al., 2019).

Nonetheless, these studies revealed that the increased complexity of the networks contributed to the stability and resilience of the native soil microbiota (Yang et al., 2019). In addition, modules (a group of nodes more densely connected to each other than to nodes outside the group) in bacterial networks correlated differentially with specific soil variables after applying different OAs (Ling et al., 2016). Here, a node refers to a bacterial or fungal OTU. The connections of nodes in the network are

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called edges, presenting positive or negative correlations between microbes. Exploring whether and how co-occurrences among bacteria and fungi in the soil are shaped by commonly applied OAs should provide a more integrated understanding of the soil microbiome and may provide insights into ecosystem functioning for optimizing agricultural soil management.

In this study, we report a seven-year field experiment in which we investigated how two OAs, compost, and bokashi, applied at high and low doses, affected long-term soil bacterial and fungal intra- and inter-kingdom relationships using co-occurrence network analysis. Compost and bokashi are obtained from aerobic composting and fermentation, respectively. Moreover, bokashi is prepared with the addition of commercial microbial inoculum, which contains five main groups of microorganisms, including lactic acid bacteria, photosynthetic bacteria, yeasts, actinobacteria, and fermenting fungi (Shin et al., 2017). After a seven-year annual application of compost and bokashi, we expect that soil microorganisms co-occur in a non-random way rather than by chance in the compost and bokashi amended soils, with a higher level of non-random networks than the unamended soil. In addition, compost and bokashi are very different regarding the stability of organic substrates and nutrient status. The composting process leads to the removal of labile organic fractions and the concentration of more recalcitrant fractions, stabilizing organic matter with low nutrient content and slow-release fertility (Neher et al., 2015). On the other hand, the C content in bokashi can be more readily metabolized than that in compost due to less efficient mineralization during fermentation than in composting. Therefore, we expect that adding compost and bokashi will shape the physicochemical properties of soil, and this will result in distinct microbial modules. We also expect that adding compost and bokashi will affect the intra- and inter-kingdom associations since compost and bokashi provide organic substrates and nutrients. Antagonistic and mutualistic relationships have been reported between specific bacteria and fungi but depend on the local conditions (Nazir et al., 2012; Fritsche et al., 2014). For instance, fungi can serve as potential providers of nutrients and energy for bacteria in carbon- and nutrient-limited soils (Haq et al., 2014). Therefore, we expect that in soils amended with compost and bokashi, the inter-kingdom associations will be reduced compared with the unamended soil and that a higher OA application dose will lead to a lower percentage of inter-kingdom relations.

2. Materials and methods

2.1. Field experiment description

The field experiment was initiated in autumn 2013 by Agriton (Noordwolde, The Netherlands) at the experimental farm Ebelsheerd of Stichting Proefboerderijen Noordelijke Akkerbouw (SPNA, Nieuw Beerta, The Netherlands). The farm field soil consists of 46% clay, 37% silt, and 12% sand with a pH of 8.2. The soil contains 8.8% organic matter. The area is characterized as strong swelling and shrinking soils with high soil compaction (Bronswijk and Evers-Vermeer, 1990; Roelfsema, 2010). Compost and bokashi were applied yearly before winter wheat (*Triticum aestivum*), with low and high application doses (Table 1). Compost and bokashi were prepared from the same amount of grass that underwent composting and anaerobic fermentation, respectively. The commercial microbial inoculum (EM-1[®], Agriton, The Netherlands) was used to start the fermentation process when preparing Bokashi. All soils received two levels of artificial N fertilization. Table 1 shows the arrangement of the field experiment.

Soil cores (0–15 cm deep, 3 cm diameter) were collected from each plot in October 2020. Soil samples were transported to the laboratory on ice, and each soil sample was divided into three subsamples afterward. The first group of subsamples was stored at 4 °C to measure water content (WC) and organic matter content (OM). The second group of subsamples was dried at 65 °C for three days until a constant weight was

Table 1

Description of the field experiment. Ten sub-treatments were clustered into three treatments: unamended soil (control soil), compost amended soil, and bokashi amended soil. Compost and bokashi had two application doses, and two levels of chemical fertilization were applied as well. Each sub-treatment had three replicates, resulting in 30 plots in total. The plots were randomly distributed. NA: not applicable. F- and F+ represent a low and a high application dose of N fertilizer, respectively.

	Treatment		Organic amendment type	Organic amendment dose (ton/ha)	N Fertilizer dose (kg/ha)
1	Control. F-	Unamended soil (control)	NA	NA	50
2	Control. F+		NA	NA	100
3	CL.F-	Compost amended soil	Compost	3.8	50
4	CL.F+		Compost	3.8	100
5	CH.F-	Compost amended soil	Compost	9.5	50
6	CH.F+		Compost	9.5	100
7	BL.F-	Bokashi amended soil	Bokashi	9.7	50
8	BL.F+		Bokashi	9.7	100
9	BH.F-	Bokashi amended soil	Bokashi	24.25	50
10	BH.F+		Bokashi	24.25	100

reached for measuring pH, electrical conductivity (EC), water extractable nutrients, total nutrients, and dissolved organic carbon (DOC). The third group of subsamples was stored at −20 °C for DNA extraction.

2.2. Physicochemical analysis of soil samples

WC was measured after drying soil samples in a forced-air oven at 105 °C for 8 h. Afterward, samples were burned at 550 °C for 2 h to quantify OM. Soil pH and EC were measured using a Mettler Toledo SevenExcellence™ in a 1:10 soil/MilliQ water suspension (w/v) after 2 h shaking at 25 °C. Water extractable nutrients, including NO₃⁻, NO₂⁻, PO₄³⁻, NH₄⁺, and K⁺, were measured in a 1:10 soil/MilliQ water suspension (w/v). The suspension was centrifuged at 3750 g after 2 h shaking at 25 °C. The supernatant was filtered through a 0.45 μm membrane filter and then analyzed by ion chromatography (Metrohm Compact IC 761). Before analyzing total nutrients, soil samples were ground into fine particles and mixed homogeneously. Total carbon (TC) and total nitrogen (TN) were determined with an elemental analyzer (Interscience FlashSmart CHNSO). Total phosphorus (TP) and total potassium (TK) were analyzed by inductive coupled plasma optical emission spectrometry (Perkin Elmer Optima 5300 DV) after microwave digestion with acids (Milestone Ethos Easy SK-15). DOC was extracted and prepared the same way as water extractable nutrients, then analyzed by a TOC analyzer (Shimadzu TOC-L). The effects of the OAs and chemical fertilization on soil physicochemical properties were tested by ANOVA (alpha = 0.05), followed by pair-wise comparison (TukeyHSD, family-wise error rate 5%). The assumptions of normality and homogeneity of variances were assessed for each ANOVA model.

2.3. Microbial community analysis of soil

DNA was extracted from 0.25 g of soil using the DNeasy Power Soil Kit (Qiagen) following the manufacturer's protocol. DNA concentration was quantified by Quantus (Promega), and purity was checked by a Nanodrop spectrophotometer (Thermo Scientific NanoDrop 1000 spectrophotometer) with OD₂₆₀ and OD₂₈₀. Extracted DNA samples were stored at −20 °C. DNA samples were normalized to 20 ng/μL for library preparation and were sequenced (MrDNA, TX, USA) on a MiSeq (Illumina). Libraries for bacteria were constructed using primers 338F (ACTCCTACGGGAGGCAGCAG) (Fierer et al., 2005) and 806R (GGACTACHVGGGTWTCTAAT) (Caporaso et al., 2011). Libraries for fungi were constructed using primers ITS1F (CTTGGTCATTTA-GAGGAAGTAA) and ITS2R (GCTGCGTTCCTCATCGATGC) (Adams

et al., 2013). The raw sequence data can be accessed at the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB46153 (<https://www.ebi.ac.uk/ena/browser/view/PRJEB46153>). Raw sequence data were analyzed by QIIME2 (version 2019.10) following the methodology in our previous publication (Luo et al., 2022). The downstream analyses of bacterial and fungal communities were performed in RStudio (R version 4.2.1) using the phyloseq package (McMurdie and Holmes, 2013) and the vegan package (Oksanen et al., 2020).

2.4. Co-occurrence network analysis

Bacterial sequence data were first quality trimmed and clustered (*de novo* clustering) into OTUs with a 90% identity threshold using VSEARCH (Rognes et al., 2016) in QIIME2. The 90% identity threshold reduces the size of the OTU table significantly compared with the more standard “species-level” cutoff level of 97% identity, reducing the network complexity and greatly facilitating visualization and analysis of the data (Konstantinidis and Tiedje, 2007; Barberán et al., 2012). Fungal sequence data were quality trimmed and clustered (closed-reference clustering) into OTUs using a 97% identity threshold. After filtering (> 0.01% abundance, > 4 samples prevalence) and clustering, we detected 275 bacterial OTUs (1,636,362 reads) and 270 fungal OTUs (1,591,059 reads) across all soil samples. Bacterial and fungal sequence data were first rarefied at the same depth (29,073) and then concatenated into a single OTU table. Core OTUs for network analysis were defined by retaining OTUs detected in at least five out of six replicate samples of a treatment. Core OTUs comprised > 88.2% of the total relative abundance (Table 2). A total of 181 OTUs were shared across all treatments, and the following OTU counts were retained per treatment: control (n = 244 OTUs), CL (n = 255), CH (n = 260), BL (n = 260), BH (n = 267). Proportions of OTUs shared by and unique to the five treatments are presented as supplementary information (Fig. S1).

To determine if OA treatments differentially affected bacterial and fungal co-occurrences, we used robust correlation analysis that accounts for compositionality and sparsity using SparCC (Sparse Correlations for Compositional data) (Friedman and Alm, 2012), implemented with the SpiecEasi package (Kurtz et al., 2015). The correlation between two OTUs was considered robust if the correlation coefficient > |0.6| and $P < 0.01$ (Barberán et al., 2012). Non-random co-occurrence patterns were evaluated based on checkerboard scores (C-score) using a null model approach that assumes no interactions between microbial taxa (Stone and Roberts, 1990). C-scores were calculated using the vegan package (Oksanen et al., 2020). A higher C-score denotes a higher level of non-randomness or network structure. To assess network topologies, we calculated the average degree, average clustering coefficient,

Table 2

Key properties of networks. Control: unamended soil; CL: soil amended with a low dose of compost (3.8 ton/ha); CH: soil amended with a high dose of compost (9.5 ton/ha); BL: soil amended with a low dose of bokashi (9.7 ton/ha); BH: soil amended with a high dose of bokashi (24.25 ton/ha).

	Control	CL	CH	BL	BH
No. of original nodes	244	255	260	260	267
total relative abundance	93.5%	96.8%	95.4%	95.9%	88.2%
After selection of robust correlation					
No. of nodes	216	242	237	251	249
No. of edges	413	691	693	1104	741
positive edge/negative edge	0.39	0.63	1.24	0.54	0.67
edge density	0.02	0.02	0.02	0.04	0.02
C-score	13.8	29.9	30.0	66.4	31.7
average degree	3.8	5.7	5.8	8.8	6.0
average clustering coefficient	0.3	0.4	0.5	0.4	0.4
average path distance	5.5	5.0	5.9	3.8	4.9
modularity	0.77	0.70	0.72	0.53	0.65
No. of modules	16	11	11	7	11
major modules (degree > 5)	9	9	7	7	9

average path distance, and modularity. All calculations were performed using the igraph packages (Csardi and Nepusz, 2006). Networks were explored and visualized using Gephi (version 0.9.2) (Bastian et al., 2009). Mantel tests were performed to estimate correlations between the major network modules (considering OTUs in each module and their relative abundance) and the physicochemical properties of the soil using the ecodist package (Goslee and Urban, 2007).

3. Results

3.1. Soil physicochemical properties seven years after amendment application

In general, the control soil and the CL soil had more similar physicochemical properties than the other treatments. Specifically, the control soil and the CL soil had lower concentrations of DOC and TC and higher concentrations of TP and TK than the CH, BL, and BH soils (Fig. 1). TN and other water extractable nutrients (i.e., AN, phosphate, and potassium) were generally higher in the amended soils than the control soils. OAs had no significant effects on soil pH, EC, WC, and OM (supplementary information (Table S1; Table S2)). The fertilizer dose had no significant impact on the physicochemical properties, except phosphate (Table S1; Table S2). Therefore, we assessed the impact of OAs and doses on the soil physicochemical variables without distinguishing between the two chemical fertilizer doses (Fig. 1).

3.2. Effects of OAs on co-occurrence networks of soil microorganisms

Chemical fertilization did not influence the richness and composition of the soil microbial community (Fig. S2; Table S3; Table S4). Therefore, we assessed the effects of OAs and doses on soil microbial networks and co-occurrence patterns without distinguishing between the two chemical fertilizer doses.

Robust correlation analysis revealed that OA amended soils retained more nodes (OTUs) and edges than control soil (Fig. 2 and Table 2). Higher C-scores were observed in the amended soils, indicating that the addition of either compost or bokashi increased the network structure of the soil microbial community. The control soil community network had a C-score of 13.8 ($p < 0.001$), which also indicated a non-random structure. The addition of a low dose of bokashi (BL) resulted in the highest C-score when all treatments were compared. The microbial taxa (nodes) in the amended soils were densely connected into seemingly more tightly knit groups than the nodes in the control soil, as indicated by higher average degrees and higher average clustering coefficients.

In all treatments, soil microbial networks were structured by modules (modularity index > 0.5; Table 2; Fig. 3). The modularity was highest in control soil, which also had more modules than any of the treatments (Table 2). The number of major modules (degree > 5) across all treatments was similar; however, the size (number of nodes) of major modules in the amended soils was generally larger than in the control. This was also reflected by the average degree and average clustering coefficient.

In addition, the formation of major modules in treatment-specific networks was correlated differentially with the soil physicochemical parameters (Fig. 4A). EC, DOC, TC, TN, AN, and phosphate were the main soil physicochemical measures that significantly correlated to treatment-specific modules, but few significant correlations ($P < 0.05$) were observed. The addition of compost and bokashi increased the number of bacterial-fungal correlations compared with the unamended soil, particularly in the BL treatment (Fig. 4B). This suggests that more bacterial and fungal taxa in the amended soils co-occurred and potentially interacted more frequently than in control soil. The addition of compost and bokashi increased the proportion of positive correlations while decreased the proportion of negative correlations among taxa (Table 2 and Fig. 4B). Notably, the addition of compost and bokashi decreased the proportions of negative correlations within kingdoms (i.

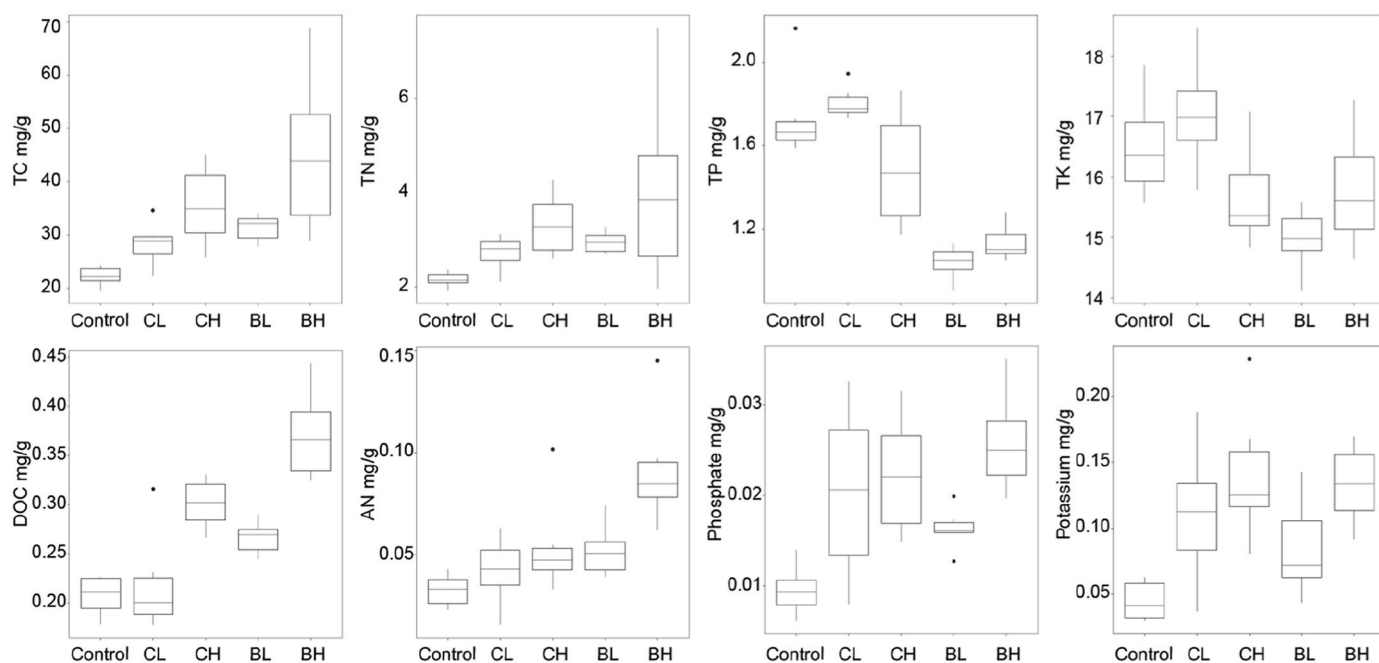


Fig. 1. Effects of compost and bokashi on the physicochemical properties of soil seven years after application. Box plots are shown representing the median and quartiles of the data. Experimental replication $n = 6$ (without distinguishing the two levels of chemical fertilization). DOC: dissolved organic carbon; TC: total carbon; TN: total nitrogen; TP: total phosphorus; TK: total potassium; AN: water extractable nitrogen, the sum of NO_3^- , NO_2^- , and NH_4^+ . Control: unamended soil; CL: soil amended with a low dose of compost (3.8 ton/ha); CH: soil amended with a high dose of compost (9.5 ton/ha); BL: soil amended with a low dose of bokashi (9.7 ton/ha); BH: soil amended with a high dose of bokashi (24.25 ton/ha).

e., bacteria-bacteria, fungi-fungi) compared to the unamended soil (Fig. 4B). However, increased negative correlations across kingdoms (i. e., bacteria-fungi) were observed in the amended soils, especially in the BL treatment. Common taxa of bacteria and fungi were observed in major modules (Fig. 4C). Taxa within Ascomycota fungi were highly prevalent in the networks across all treatments. Other fungal taxa (including Basidiomycota, Mortierellomycota, Glomeromycota, and Chytridiomycota) were also observed in major modules. Diverse bacterial phyla were also observed in major modules: Chloroflexi, Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes, Verrucomicrobia, Acidobacteria, and Planctomycetes were dominant across all treatments.

4. Discussion

4.1. Non-random co-occurrence patterns induced by OAs

Non-random community assembly may be a general characteristic across all life domains (Horner-Devine et al., 2007; Barberán et al., 2012). Our study, which shows significant non-random co-occurrence patterns in the soil with and without the application of OAs, supports this general notion. Our observation that the OA treatment increases the complexity of networks in native soil is in line with previous studies (Ling et al., 2016; Yang et al., 2019; Zhou et al., 2019). Our data support that the microbial community in the amended soils was more organized and potentially had a larger operational community that was functionally interrelated than that in control because of a higher C-score, more edges, and more densely correlated nodes in the modules.

Interestingly, in our study, a decrease in the proportion of negative correlations was found in the amended soils when compared to the control soil, suggesting that amelioration of soil with compost and bokashi may decrease competition among microorganisms for resources (Blagodatskaya and Kuzyakov, 2008; Mau et al., 2015). Other studies also reported that adding OAs or nutrients reduced the number and strength of negative correlations between microorganisms (Banerjee et al., 2016; Yang et al., 2019, 2020). The addition of OAs introduced

extra C and nutrients to the soil. This may reduce competition and increase the number of multiple trophic levels or resource cascades in the food web, which could improve energy use efficiency (Coyte et al., 2015; Banerjee et al., 2016). However, theoretical studies predict that compartmentalization and the presence of negative interactions will increase the stability of networks under disturbances, as negative links stabilize co-oscillation in communities (Coyte et al., 2015; Kuiper et al., 2015). In addition, a recent experimental study demonstrated that negative interactions are more common than reciprocal positive interactions in soil bacterial communities. Negative interactions can be desirable by aiding in resistance against invasive species and maintaining the stability of the community (Palmer and Foster, 2022). Even though the proportion of negative correlations was reduced in the OA amended soils, the absolute number of negative correlations was higher than in the control soils. Therefore, the addition of compost or bokashi may contribute to the stability of the microbial community by enhancing resistance or resilience to disturbance.

4.2. Distinct modules observed in the amended soils

In all treatments, we observed high modularity indices, indicating that the microbial community was structured into modules consisting of groups of bacteria and fungi that formed strong associations. A modularity index > 0.4 suggests that the network can be well-divided into modules or clusters with a strong modular structure (Newman, 2006). These findings support our hypothesis that the addition of different doses of compost and bokashi will result in distinctive microbial modules in the co-occurrence networks. In addition, the size (number of nodes) of major modules was larger in the amended soils than in the control, particularly in the BL soil. This also suggests that the addition of OAs may have changed the function of the soil microbial community with larger operational communities that could be functionally correlated.

Interestingly, only a few major modules significantly correlated with soil variables (i.e., EC, DOC, TC, TN, AN, and phosphate). Our results

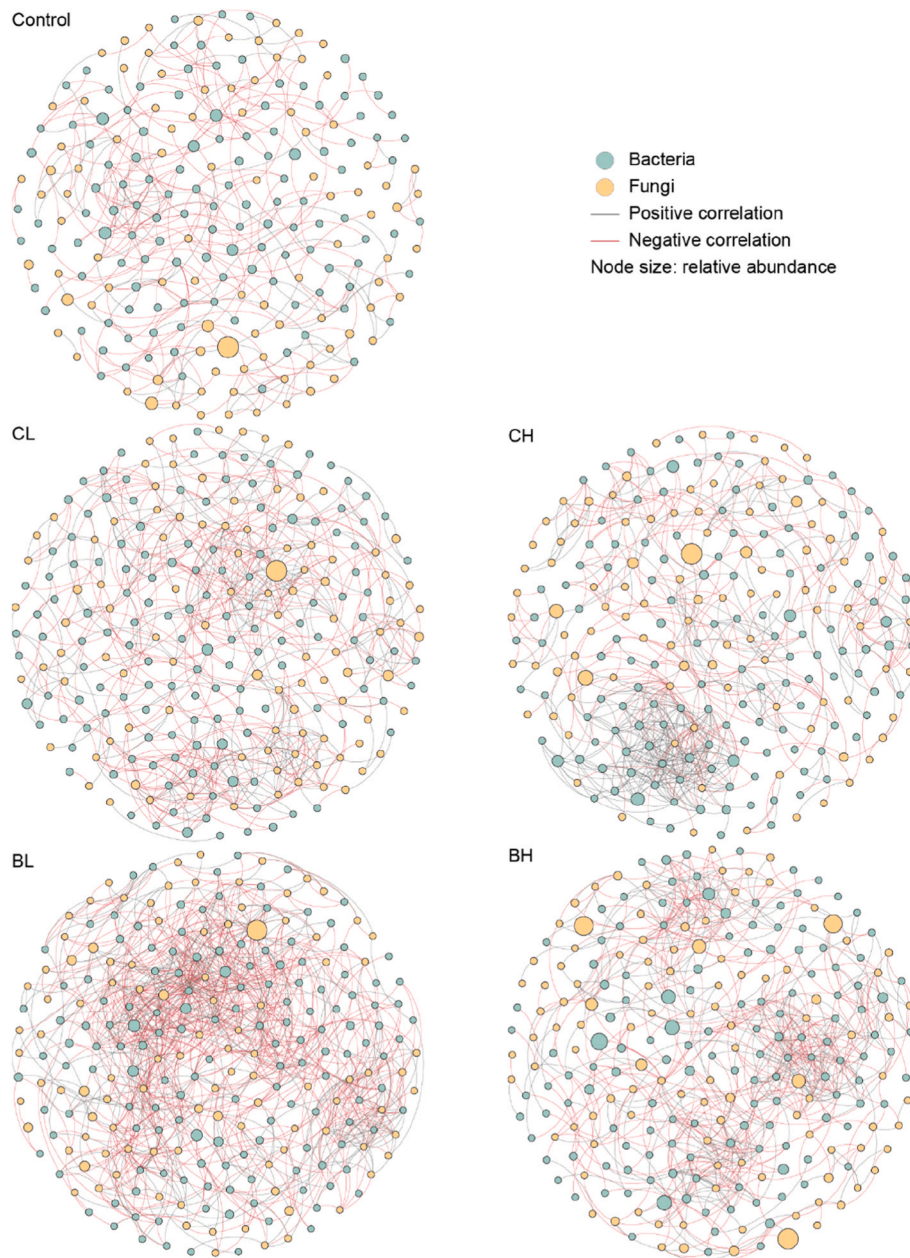


Fig. 2. Network visualization of co-occurring bacteria and fungi per treatment. Control: unamended soil; CL: soil amended with a low dose of compost (3.8 ton/ha); CH: soil amended with a high dose of compost (9.5 ton/ha); BL: soil amended with a low dose of bokashi (9.7 ton/ha); BH: soil amended with a high dose of bokashi (24.25 ton/ha).

suggest that the physicochemical properties of the soil might not be the primary factors driving the formation of the network modules. We speculate that the heavy clay soil in this study field, characterized by strong compaction (Roelfsema, 2010), may weaken the influence of the physicochemical properties of the soil on the microbial network. Alternatively, there may be unknown factors (e.g., specific and multiple interactions with protists, Archaea, and other soil organisms) that are more important than the physicochemical properties of the soil in determining the soil microbial structure (Fuhrman, 2009; Deveau et al., 2018). Further studies are suggested to examine the influence of OAs on microbial networks in different soil types (e.g., sand and clay) and how the underground food web shapes microbial networks. Nevertheless, the microbial network might be susceptible to changes in EC, DOC, TC, TN, AN, and phosphate, which provide necessary nutrients for the growth, activity, and metabolism of soil microorganisms.

4.3. Organic amendments changed intra- and inter-kingdom correlations

Bacteria and fungi operate in the same environment, where complex interactions occur between these kingdoms, including predation, parasitism, competition, commensalism, and mutualism. Bacteria associated with soil fungi, including saprotrophic and mycorrhizal ones, can benefit from nutritional interactions (Haq et al., 2014). Nutritional interactions imply that bacteria feed on fungi or on their released products (De Boer et al., 2005; Leveau and Preston, 2008) or vice versa (Hildebrandt et al., 2002, 2006). Such nutritional interactions often imply commensalism or true mutualism (Nazir et al., 2012). We expected that adding OAs would weaken nutritional interactions between bacteria and fungi due to the provisioning of additional nutrients; thus, fewer bacterial-fungal correlations would be detected in the amended soils than in the unamended soil. However, this was not in line with our observation. We observed that the addition of compost and bokashi increased bacterial-fungal

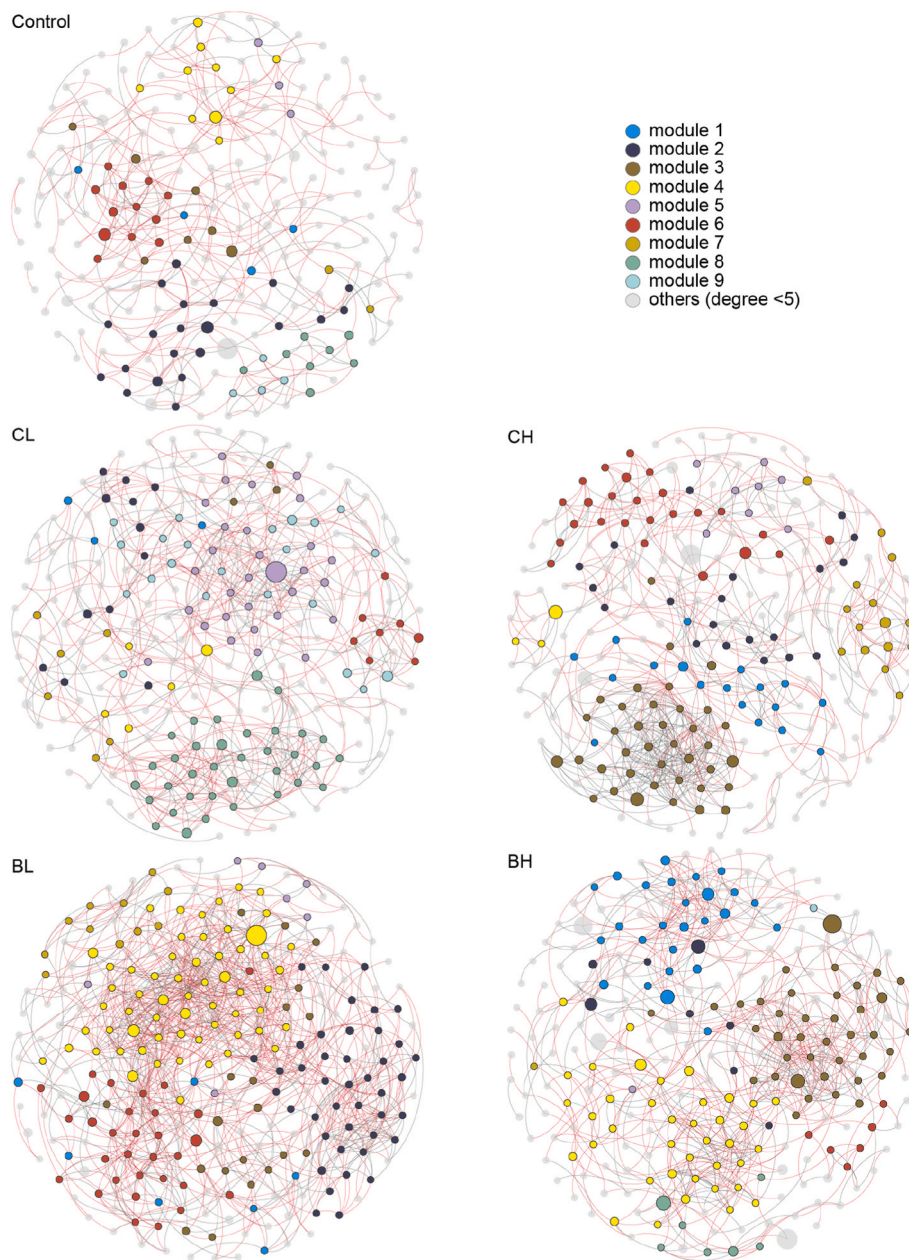


Fig. 3. Major modules (degree > 5) highlighted in networks per treatment. Control: unamended soil; CL: soil amended with a low dose of compost (3.8 ton/ha); CH: soil amended with a high dose of compost (9.5 ton/ha); BL: soil amended with a low dose of bokashi (9.7 ton/ha); BH: soil amended with a high dose of bokashi (24.25 ton/ha).

correlations, mainly increasing their negative correlations compared to control, while positive correlations of bacteria-bacteria and fungi-fungi were increased after applying OAs. We hypothesize that the provisioning of nutrients (TC and TN) and the availability of nutrients (DOC, AN, and phosphate) by OAs may alleviate resource competition within kingdoms but stimulate competition across kingdoms. It is unclear what drives this. Even though efforts have been taken to uncover mechanisms driving bacterial-fungal interactions; challenges remain since the outcome of these interactions are the combined results of the physical associations (biofilm, free cell), molecular communication between (micro)organisms, and the local soil environment (Deveau et al., 2018). Further research should identify essential parameters in the soil system that can drive bacterial-fungal interactions.

We emphasize that the intra and inter-kingdom co-occurrence patterns are correlations between bacterial and fungal taxa, which indicates potential positive, negative, or neutral interactions but cannot uncover

causal mechanisms structuring the communities (Barberán et al., 2012; Banerjee et al., 2016). Moreover, correlation analyses inherently imply technical challenges when analyzing microbial associations in soil, such as how to pre-process compositional abundance data (Faust, 2021). In addition, the performance of the statistical methods (including simple, direct correlation measures like Pearson and Spearman correlations) for inferring microbial association does not account for data properties that may yield spurious associations (Weiss et al., 2016; Hirano and Takemoto, 2019; Faust, 2021). Spearman correlation is still commonly applied to infer microbial networks from non-independent relative abundance data, but negative correlations are often overrepresented regardless of the true correlations underlying absolute abundances (Friedman and Alm, 2012). Methodological choices for network analyses need careful consideration of the data characteristics. Particularly, correlation techniques should be considered that account for sparsity and compositionality when necessary, such as SparCC (Friedman and

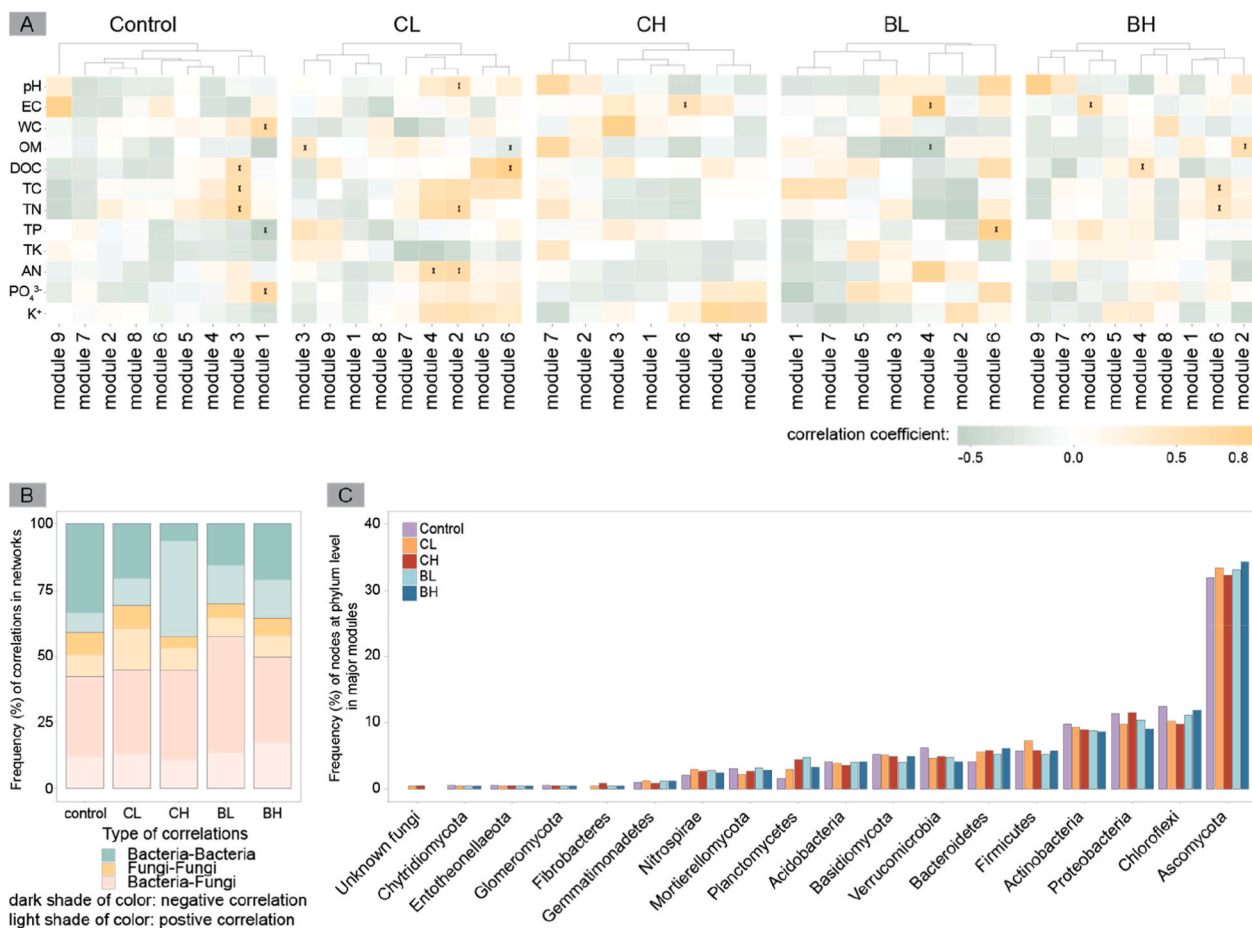


Fig. 4. (A) Correlation between major modules and the physicochemical properties of the soil based on the Mantel test. Significant correlations ($P < 0.05$) were marked with “X”; (B) Frequency of intra- and inter-kingdom correlations in the networks. The proportion of negative and positive correlation within each type of correlation is indicated by different color intensities: the dark shade of the color represents negative correlations, and the light shade represents positive correlations; (C) Frequency of nodes (bacteria and fungi) at phylum level in major modules detected in the networks. Control: unamended soil; CL: soil amended with a low dose of compost (3.8 ton/ha); CH: soil amended with a high dose of compost (9.5 ton/ha); BL: soil amended with a low dose of bokashi (9.7 ton/ha); BH: soil amended with a high dose of bokashi (24.25 ton/ha). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Alm, 2012). Nonetheless, our results highlight the importance of considering prokaryotic and eukaryotic components of the soil microbial community, which is often neglected when microbial communities are investigated as compartmentalized groups (De Menezes et al., 2015).

5. Conclusion

Compost and bokashi changed the intra- and inter-kingdom co-occurrence patterns of microorganisms in soils. Soil microbial communities appeared to have a higher level of non-randomness in the networks after applying compost and bokashi. This was particularly so when bokashi was applied at a low dose. OAs drove distinct network modules in terms of module compositions and the size of major modules. Our results suggest that network modules correlated with soil recourses (i.e., EC, DOC, TC, TN, AN, and phosphate). We also observed that OAs decreased the proportion of negative correlations, reducing potential competition among microorganisms for resources and increasing energy use efficiency. The addition of OAs increased bacterial-fungal correlations, particularly their negative correlations. Further studies should be conducted to identify key factors driving the changes in bacterial-fungal interactions after OAs application. In addition, it is essential to have experimental evidence validating the hypotheses generated by microbial networks (e.g., how abiotic properties of soil shape biotic correlations) since network analyses do not uncover causal relationships. The links between networks and soil ecological functions (e.g., plant growth,

C sequestration, and soil aggregation) also merit further research.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2022.108831>.

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