

Phytate and Microbial Suspension Amendments Increased Soybean Growth and Shifted Microbial Community Structure

Bulbul Ahmed

Université de Montréal: Universite de Montreal

Jean-Baptiste Floc'h

Université de Montréal: Universite de Montreal

Zakaria Lahrache

Université de Montréal: Universite de Montreal

Mohamed Hijri (✉ mohamed.hijri@umontreal.ca)

Université de Montréal: Universite de Montreal <https://orcid.org/0000-0001-6112-8372>

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Abstract

Phytate represents an organic pool of phosphorus in soil that require hydrolysis by phytase enzymes produced by microorganisms prior to its bioavailability by plants. We hypothesize that in a greenhouse trial on soybean plants inoculated or non-inoculated with a microbial suspension made from an age-old maple forest's undisturbed soil mineralize phytate. MiSeq Amplicon sequencing targeting bacterial 16S rRNA gene and fungal ITS was performed to assess microbial community changes following treatments. Our results showed that soybean nodulation and shoot dry weight biomass increased when phytate was applied to the nutrient-poor substrate mixture. Bacterial and fungal diversities of the root and rhizosphere biotopes were relatively resilient following inoculation by microbial suspension; however, bacterial community structure was significantly influenced. Interestingly, four arbuscular mycorrhizal fungi (AMF) were identified as indicator species, including *Glomus sp.*, *Claroideoglomus etunicatum*, *Funneliformis mosseae* and an unidentified AMF taxon. We also observed that an ericoid mycorrhizal taxon *Sebacina sp.* and three *Trichoderma* spp. were among indicator species. Non-pathogenic Planctobacteria members highly dominated the bacterial community as core and hub taxa for over 80% of all bacterial datasets in root and rhizosphere biotopes. Overall, our study documented that inoculation with a microbial suspension and phytate amendment improved soybean plant growth.

Introduction

Phosphorus (P) is an essential macronutrient for all living organisms, but due to its low mobility in soil, it is a limiting factor in most agroecosystems. Plants, like other organisms, require adequate P to carry out critical functions for their growth and development [1]. Modern agriculture relies on continuous P fertilizer inputs to maintain high yielding crop production, resulting in global P fertilizer demand increases by 2.4% per year [2]. The majority of P-fertilizers used in agroecosystems are manufactured from phosphate rock (PR) which is a non-renewable resource that is inefficiently used by crop plants [3]. P-fertilizer-released mineral P ions (Pi) react rapidly with binding sites in the soil environment, with plants using only about 20% of the Pi dose applied [1], and residual Pi accumulates in soil, increasing the risk of Pi-related environmental impacts [4]. That said, maintaining soil organic P (Po) paradox is a challenging agriculture.

Soil organic P (Po) is a large reservoir of P. Phytate, non-phytate phosphomonoester and phosphodiester are the three types of phosphatases that mineralize soil Po [5]. In the soil, phytate must be solubilized before phytases can cleave the orthophosphate ions available. Soil microorganisms are the main producers of phytases [6, 7], and phytate hydrolysis can be aided by PGPR-based inoculants [8, 9], and it is degraded in the soil by a variety of yeasts, mycorrhizal fungi, and filamentous fungi [10, 11]. Hence, scientists are interested in improving plant P nutrition productivity by increasing microbial phosphatase activity. The ability of selected strains to evolve and function effectively in various soil environments is critical to the success and effectiveness of plant growth-promoting microbial inoculants for field crops. Plants play an active role in shaping soil microbiota within the soil-plant system, especially in the rhizosphere. *In situ* study of soil microbial interactions has proven challenging [12]. On the other hand, the

ecology of soil microbial communities has been studied using within [13] and interkingdom [14, 15] correlation network analysis based on the cooccurrence of microorganisms. Although network analysis cannot prove the presence of taxonomic interactions, it may help to develop important hypotheses for further research on microbes-mediated soil function. Phytate hydrolysis can be spatially segregated and phosphatase activity in the root and rhizosphere soils varies, but highly efficient Po solubilizing and hydrolyzing microbial strains have been isolated from forest soils [16]. We used phytate as the sole source of P. We applied a microbial suspension from soil obtained in an age-old undisturbed maple forest in Quebec's Gault Nature Reserve, as a source phytate mineralizing microorganisms, where the soil microbial assemblages are unknown. This experiment involved no other lab-grown microorganisms. The bacterial and fungal communities' responses to microbial inoculum and phytate were represented using Illumina MiSeq amplicon sequencing. We hypothesised that the soil of an undisturbed maple forest contains microorganisms that effectively mineralize soil phytate which would be revealed by increased crop P uptake from soil Po via phytate mineralization. Although we found no evidence of significantly increased phytate mineralization in soybean-soil systems treated with the inoculum, we did discover how microbial taxa deal with and react to phytate mineralization to make P-nutrition available to plants in underground environments. We report for the first time, the evidence of Tepidisphaerales abundance in roots of a crop plant like soybean, as well as evidence of frequent tri-party relationships involving plant, bacterium, and fungal.

Results

Soybean biomass, nodulation and P nutrition response to treatments

Comparison of the shoot dry weight using two-way ANOVA indicated significant ($P \leq 0.05$) effect of phytate (M_0P_1) and combined treatment (M_1P_1) compared to control (M_0P_0), whereas inoculum (M_1P_0) was insignificant. The root dry weight was unaffected by inoculum (M_1P_0), phytate (M_0P_1) and their interactions (M_1P_1) (Fig. 1A). Although phytate addition significantly ($P = 0.017$) increased shoot dry weight ($P = 0.037$) and the number of nodules per plant (Table 1), the microbial inoculum had no significant influence on nodulation. Total P levels in inoculated plants (M_1P_1) were higher (22 mg/plant) than in control plants (M_0P_0) (16.78 mg/plant), but the difference was statistically insignificant (Fig. 1B).

Table 1

Two-way ANOVA from the effects of treatment on fresh and dry weight of shoot and root, nodule formation and total phosphorus.

Source of variation	df	Shoot dry weight		Root dry weight		Nodule		Total P	
		F	Pr(>F)	F	Pr(>F)	F	Pr(>F)	F	Pr(>F)
Inoculum	1	3.060	0.088	3.899	0.058	0.171	0.683	3.391	0.098
Phytate	1	8.018	0.007	0.037	0.847	8.525	0.017	2.901	0.122
Inoculum: Phytate	1	0.983	0.327	0.009	0.921	0.773	0.390	1.465	0.256

Bacterial And Fungal Community Structure In Different Biotopes

Raw data of Illumina MiSeq produced a total of 13,148,932 reads where 7,763,410 reads for the bacterial 16S rRNA and 5,385,522 reads for the fungal ITS region. DADA2's filtering, trimming and quality controlling, resulted on a total of 2,896 335 reads for 16S rRNA (Fig. S2A-C) and 4,234,021 for ITS (Fig. S2D-F). Finally, we assembled forward and reverse filtered reads into 15,613 ASVs for bacteria and 2171 ASVs for fungi. We then analyzed the effects of treatments on the diversity and structure of the bacterial and fungal communities in the roots and rhizosphere biotopes separately.

Alpha diversity indices of bacteria were insignificant for microbial inoculum (Shannon $P=0.1503$ and Simpson $P=0.3776$), phytate (Shannon $P=0.8643$ and Simpson $P=0.908$) and their interactions (Shannon $P=0.7244$ and Simpson $P=0.5756$) (Fig. 1C). Similarly, fungal alpha diversity did not significantly differ for different treatments (Fig. 1D). Bacterial communities clustered by niche along the first axis of the PCoA ordination. Root bacteria formed distinct clusters under M0P0, M1P0, and M0P1, whereas rhizosphere bacteria were much more scattered (Fig. 1E). The clustering pattern of fungal communities showed an opposite pattern to those of bacteria. M₁P₁ caused fungal communities in the rhizosphere to cluster less closely than other treatments (Fig. 1F). According to the PERMANOVA test, microbial inoculum had significant effect on bacteria in the root ($P=0.001$) and rhizosphere ($P=0.004$). Phytate significantly influenced the structure of the bacterial communities in the root biotope ($P=0.024$) (Table 2A). PERMANOVA test was insignificant for the root fungi, but inoculum had a significant ($P=0.007$) impact on the structure of the rhizosphere fungi (Table 2B).

Table 2. Effect of microbial inoculation and phytate on the structure of the bacterial and fungal communities in root and rhizosphere according to PERMANOVA.

A. Bacteria

Variable	Source	DF	SumOfSqs	R ²	F	Pr(>F)
Roots	Inoculation	1	0.2347	0.04366	1.7477	0.001 ***
	Phytate	1	0.1686	0.03136	1.2555	0.024 *
	Inoculation: Phytate	1	0.1384	0.02574	1.0306	0.354
	Residual	36	4.8353	0.89924		
	Total	39	5.3771	1.00000		
Rhizosphere	Inoculation	1	0.2676	0.05702	2.2687	0.004 **
	Phytate	1	0.0878	0.01871	0.7446	0.929
	Inoculation: Phytate	1	0.0908	0.01934	0.7696	0.849
	Residual	36	4.2474	0.90490		
	Total	39	4.6937	1.00000		

B. Fungi

Variable	Source	DF	SumOfSqs	R ²	F	Pr(>F)
Roots	Inoculation	1	0.1592	0.01993	0.7662	0.751
	Phytate	1	0.1368	0.01713	0.6551	0.878
	Inoculation: Phytate	1	0.1730	0.02166	0.8283	0.653
	Residual	36	7.5181	0.94128		
	Total	39	7.9870	1.00000		
Rhizosphere	Inoculation	1	0.1502	0.04113	1.6240	0.007 **
	Phytate	1	0.0789	0.02161	0.8532	0.791
	Inoculation: Phytate	1	0.0928	0.02542	1.0035	0.428
	Residual	36	3.3296	0.91184		
	Total	39	3.6515	1.00000		

Planctobacteria And Ascomycota Dominated Soybean Microbiota

The 15,613 bacterial ASVs were assigned to 39 phyla (Table S1) and 196 orders (Table S2), with Planctobacteria being the most abundant phyla in both root (Fig. 2A) and rhizosphere (Fig. 2C) biotopes. We chose the top 10 orders based on their high relative abundance and eight (Tepidisphaerales, Gemmatales, Isosphaerales, Pirellulales, Planctomycetales, Chthoniobacteriales, Phycisphaerales and Burkholderiales) of the 10 most abundant orders were dominant in both biotopes (Fig. 2B, D). The root biotope was dominated by Tepidisphaerales (Fig. 2B), while the rhizosphere biotope was dominated by Gemmatales (Fig. 2D). Bacterial indicator species analysis revealed 35 ASVs under inoculation treatment, with 19 ASVs enriched in the root (Fig. S3A) and 16 in the rhizosphere (Fig. S3B). Thirteen ASVs were enriched under phytate treatment, with 7 ASVs enriched in root (Fig. S3C) and 6 ASVs in the rhizosphere (Fig. S3D) (Table S3); however, indicator species analysis under combined inoculum and phytate (M₁P₁) treatment significantly ($P \leq 0.05$) revealed BASV738 (*Tepidisphaera mucosa*) and BASV766 (*Candidatus Anammoximicrobium moscowii*) in the root; and BASV1092 (*Pirellula sp.*) in the rhizosphere biotope (Table S4).

In the fungal dataset, we identified six phyla: Ascomycota, Basidiomycota, Mucoromycota, Chytridiomycota and Blastocladiomycota with one not assigned (NA) to any phylum (Table S5) and 92 orders (Table S6). Ascomycota was the most abundant phylum in both root and rhizosphere biotopes (Fig. S4A, C). The Sordariales order dominated fungal communities both in the root and rhizosphere biotopes (Fig. S4B, D). Both biotopes shared six (Hypocreales, Sordariales, Pleosporales, Orbiliales, Glomerellales and Pezizales) of the top 10 orders (Fig. S4B, D). Fungal indicator species in the rhizosphere revealed 48 ASVs under inoculation treatment and nine ASVs under phytate addition. Just one fungal ASV, FASV113 (*Arthrotrrys conoides*) was found to be enriched in the root biotope under phytate treatment, while no ASV was found in the root biotope under inoculum treatment (Table S3). In the rhizosphere, four arbuscular mycorrhizal fungi (AMF)- *Glomus sp.*, *Claroideoglomus etunicatum*, *Funneliformis mosseae* and *Glomeromycotina sp.*; one ericoid mycorrhiza *Sebacina sp.* and three *Trichoderma* species - *Trichoderma aeruginum*, *T. Americanum* and *T. simmonsii* were significantly identified as indicator species under inoculum treatment (Fig. S3E); and only *Glomeromycotina sp.* was revealed under phytate treatment (Fig. S3F). Indicator species analysis under M1P1 revealed FASV241 (*Sebacina sp.*) and FASV46 (*Chaetomium grande*) in the root biotope, and twelve fungal ASVs in the rhizosphere, including *Funneliformis mosseae* (Table S7).

Determining Eco- And Core-microbiota

One hundred ASVs were ubiquitous in all roots and 115 ASVs in each rhizosphere and they were attributed as the bacterial eco-microbiota of soybean roots and rhizosphere, respectively (Table S8). The bacterial eco-microbiota in the root belonged to 19 genera, with 91 of them being Planctobacteria (Table S8), whereas the rhizosphere eco-microbiota belongs to 21 genera, with 107 Planctobacteria ASVs (Table S9). *Tepidisphaera mucosa* and *Gemmata sp.* were detected in 32 ASVs in the root and rhizosphere eco-microbiota, respectively (Fig. S5B and Table S9). A Venn diagram identified 63 unique ASVs in the root and 78 distinct ASVs in the rhizosphere, with 37 ASVs shared by the two biotopes (Fig. S5C). Thirty-three

of the 37 shared ASVs belonged to nine bacterial genera (*Tepidisphaera mucosa*, *Gemmata sp.*, *Planctomyces maris*, *Isosphaera sp.*, *Pirellula sp.*, *Planctomicrobium piriforme*, *Lacipirellula parvula*, *Calycomorphotria hydatis*, *Algisphaera agarilytica*), while four were not assigned to any taxon (NA) (Fig. S5D). The shared bacterial taxa were the bacterial core-microbiota in soybean. In the fungal community, only ASV2 (*Humicola fuscoatra*) was discovered as eco-mycobiota in the root (Table S10A). Fifteen ASVs were identified as eco-mycobiota in the rhizosphere and assigned to 14 genera (Fig. S5E, Table S10B). ASV2 (*Humicola fuscoatra*) was found in both biotopes and has been considered as the core-mycobiota (Fig. S5F).

Bacteria Regulates The Connectivity Of Soybean Microbiota

The interkingdom co-occurrence network in the rhizosphere was more complex (452 nodes and 2159 edges) than in the roots (285 nodes and 553 edges) (Fig. 3A, B). Four bacterial ASVs (BASV6, BASV87, BASV16, and BASV58) were classified as hub taxa in the root based on their node degree and betweenness centrality and these hub taxa were Planctobacteria (Fig. S6A and Fig. S7A-H). Based on mutual putative interactions in the subnetwork of the hub taxa in the root, we found: (i) BASV6 (*Tepidisphaera mucosa*) had positive putative interactions with 13 different bacterial ASVs but negative interactions with two different bacterial ASVs (Fig. S7A, E); (ii) BASV16 (*Tepidisphaera mucosa*) interacted negatively with BASV476 but positively with nine different bacterial ASVs and a fungal ASV, FASV2 (Fig. S7B, F); (iii) BASV58 (*Gemmata sp.*) had positive putative interactions with seven different bacterial ASVs but negative interactions with four BASVs (Fig. S7C, G) and (iv) positive putative interactions with seven different bacterial ASVs and negative putative interactions with five bacterial ASVs were found in ASV87 (*Tepidisphaera mucosa*) (Fig. S7D, H). The interkingdom network in the rhizosphere identified four hub taxa- BASV175, BASV148, BASV200 and ASV311 as *Thermostilla marina*, *Chloroflexus aurantiacus*, *Zavarzinella formosa* and *Gemmata sp.*, respectively (Fig. S6B and Fig. S7I-P) and their interaction pattern revealed: (i) BASV175 (*Planctomyces maris*) had positive putative interactions with 10 bacterial ASVs but negative interactions with six bacterial ASVs and three fungal ASVs (Fig. S7I, M); (ii) BASV200 (*Zavarzinella formosa*) had positive interactions with 10 bacterial ASVs and negative interactions with seven bacterial ASVs and a fungal ASV (FASV7) (Fig. S7J, N); (iii) BASV311 (*Gemmata sp.*) had positive interactions with 11 bacterial ASVs and negative interactions with five bacterial and a fungal ASV (FASV74) (Fig. S7K, O); (iv) BASV148 (*Chloroflexus aurantiacus*) had positive interactions with 16 bacterial and a fungal ASV (FASV16), and negative interactions with a bacterial ASV, BASV268 (Fig. S7L, P).

Meta co-occurrence patterns of hub taxa revealed a network of eight modules (Fig. 3C; Table S11): (i) Module I centered on BASV200 found in the rhizosphere was connected to Module II centered on the hub taxa BASV148 via BASV44 and BASV91; (ii) BASV35 connected Module II to Module III; (iii) Module IV centered on the root hub taxa BASV311 was linked to Module III through BASV308, and BASV577 connected Module IV and Module V; (iv) BASV14 connected Module VI and VII which are respectively centered on the hub taxa BASV87 and BASV16; (v) BASV6, the root interkingdom network's hub taxa is

linked to Module VII via BASV196, and Module VI via BASV57; (vi) BASV30 connected the Module VIII and the Module II; (vii) BASV268 and BASV132, respectively, connected Module V to Module II and the Module VIII (Fig. 3C). BASV91 connecting Module I, II and VIII; and BASV308 connecting Module III, IV and VIII (Fig. 3C). Overall, we identified 11 different bacterial ASVs that established interactions among eight different hub taxa to broaden interactions in soybean microbiota. As a result, these 11 ASVs and eight hub taxa, for a total of 19 ASVs have been designated as global hub taxa (Table S11). These 19 global hub taxa were assigned to seven genera (*Tepidisphaera mucosa*, *Gemmata sp.*, *Chloroflexus aurantiacus*, *Pirellula sp.*, *Ralstonia solanacearum*, *Thermostilla marina* and *Zavarzinella formosa*). Only BASV148 was a Chloroflexi member, while 18 of the 19 ASVs were Planctobacteria (Table S11).

Discussion

We studied the effects of microbial inoculum obtained from an age-old natural maple forest on the response of soybean grown in a greenhouse trial with phytate as phosphorus source. We also demonstrated the effects of this microbial inoculum on the diversity of bacterial and fungal communities in soybean root and rhizosphere biotopes.

Inoculation tends to influence biomass, but sample types sheltered microbial diversity

Our findings showed that microbial inoculum was insignificant to increase the dry weight of shoot dry weight ($P = 0.088$), root ($P = 0.058$), and total P in the shoots ($P = 0.098$), however, phytate significantly increased the dry weight of shoot ($P = 0.007$) and nodulation ($P = 0.017$). This reflected that inoculum tended to increase plant biomass, nodulation and total P accumulation in soybean plants, which may be due to putative phytate hydrolysis since it was the sole source of P in the procedure. Phytate bound to soil constituents can be solubilized by many microorganisms [8, 16–19]; however, in comparison to phytate alone (M_0P_1), the combined application of inoculum and phytate (M_1P_1) had no significant effect on soybean P-nutrition in our study. Many soil microbial species have been shown to be able to solubilize P [5, 20] and inoculum may not have increased P dissolution in the growth substrate beyond that of non-inoculated soil. Inoculation did not increase phytase activity or have an indirect effect on soybean and associated soil microbial communities in our systems if phytate solubilization was the rate limiting stage in phytate mineralization, as it is often the case [5]. Previous research has focused on the use of microbial inoculation for plant biomass production, regardless of whether the effect on plant biomass is statistically significant [21–24].

A statistically insignificant effect of microbial inoculation on phytate hydrolysis can hardly be attributed to inoculation failure. Microbial inoculum and phytate had no statistically significant impact on microbial communities; however, according to beta study, inoculum significantly affected the structure of the fungal microbiota in the rhizosphere. This meant that microbial inoculum displayed a tendency for phytate hydrolysis for P-nutrition and impacted microbial community pattern. Nonetheless, the efficacy of diversity indices and species richness was tracked to see if the results of various treatments were investigated. This may be explained by the fact that multiple treatments resulted in increased microbial

diversity in the rhizosphere, as well as the ability to recruit certain microbes that could be beneficial to plant [25, 26]. Differences in the niche used for the analysis had an important effect on the differences observed in beta-diversity in the bacterial and fungal communities.

Planctobacteria And Ascomycota Predominate In The Soybean Microbiota

Planctobacteria orders, Tepidispheerales and Gemmatales were the most abundant bacterial taxa which reported for over 80% of bacteria. Tepidispheeraceae has recently been found abundant in soil with recurrent soybean straw returns [27] which would be coherent with a particular association between this bacterial taxa and soybean. In the rhizosphere bacterial communities associated with wild beet, Planctobacteria came second to Proteobacteria [28]. In most cases, Planctobacteria relative abundance ranges from 1–18% in soil [29], < 1–4% in root and 5–18% in rhizosphere [30], and 40% more abundant in greenhouse pots than in natural soil (Kurtz, Müller [31]. The co-culture of *Isophaera pallida*, a Planctobacteria was found to be obligate for *Heliuthrix. uregonensis* [32] suggesting that at least some Planctobacteria may be symbiotic. We assume this is the first evidence of Planctobacteria absolute dominance in the root and rhizosphere of a healthy soybean grown in a greenhouse. The order Gemmatales was recently established by parsing genomic data from a few culturable taxa, including *Gemmata sp.* [33]. Gemmatales genera are gram negative aerobic chemotrophs and most of them are unculturable [34], meaning that they depend on biotrophic associations. As a result, Planctobacteria are largely undescribed, which explains their low taxonomic resolution and functional studies in crop plants. Planctobacteria may use organic materials in soil but not fresh plant residues, as shown by a ¹³C-labelled experiment [35], implying that they depend on associated organisms to meet their carbon requirements. Organic matter concentrations were relatively higher in the underlying soil layers in the Gault Nature Reserve, where we collected soil as a source of microbial inoculum [36]. It should be noted that the microbial association discovered in this study is most likely due to the source's microbe availability. In this study, Ascomycota was found to be the most abundant phylum. Previous studies have shown that Ascomycota is more abundant in fertilisation interactions such as carbon, nitrogen and phosphorus [37, 38]. In the rhizosphere biotope, four AMFs, an ericoid mycorrhiza and three Trichoderma were reported as fungal indicator species under inoculum treatment, and an AMF *Glomeromycotina sp.* was listed as indicator species in the root biotopes. Furthermore, an ericoid mycorrhizal fungus, *Sebacina sp.* and an AMF *Funneliformis mosseae* were reported as indicator species in roots and rhizosphere biotopes, respectively under the treatment of M₁P₁. More than 80% of terrestrial plants roots have symbiotic relationships with AMF [39]. Despite the fact that AMF hyphae have phosphatase activity [40], only a few studies have looked into whether AMF can hydrolyze phytate and thus increase plant P uptake. Phytate was used by *Funneliformis mosseae* hyphae to transport released P to maize roots [10]. Feng, Song [41] showed that hyphae in the root-free compartment acquired P from sodium phytate and transferred it to the red clover plant, but they did not observe hyphal growth. Plants have been shown to recruit microbes

from the soil that may be beneficial to them [37]. This consistency may have happened in our study since soybean plants benefitted from the mycorrhizal symbiosis.

Microbial Amendment Influences The Community Composition

Ten bacterial and one fungal taxa were identified as core microbiota (Fig. 5D, F). We found two bacteria, *Tepidisphaera mucosa* and *Gemmata sp.*, as hub taxa in the root biotope and three bacteria, *Thermostilla marina*, *Gemmata sp.*, and *Zavarzinella formosa*, as hub taxa in the rhizosphere, all of which are Planctobacteria. Their wide distribution [42] and ability to degrade plant-derived polymers and exopolysaccharides formed by other bacteria has recently been documented [43]. Six Planctobacteria and a Chloroflexi were identified as global hub taxa in the co-occurrence network (Fig. 3C), but no fungal taxon was included in the hub microbiota list. This indicated that bacteria had a greater impact than fungi on community assemblies and Planctobacteria may have a significant impact on multifunctionality in soybean. Planctobacteria [44] and Chloroflexi [45] were abundant in response to nitrogen and phosphorus nutrition in tomato. Overall, further research using culture-dependent approaches to isolate and characterize members of these core and global hub microbiota that may play a key role in hydrolysing phytate for P-nutrient for soybean may complement our findings. Given the adaptability of global hub microbiota, we speculate that they may recruit other microbes to establish interactions and occupy multiple niches, as well as serve as a central route for outlining other microbes. The absence of fungi as hub taxa in our network study could indicate that these bacteria are highly conserved. It is also possible that abundance and distribution of bacteria in the source microbial suspension is fully dominated in their native habitat. In our study, there is not significant influence of microbial inoculum recorded in P uptake in soybean shoots. There might be several potential justifications. One of the possible justifications is that Planctobacteria diversity in soil is related to soil history, including abundance and diversity, which appeared to occur highly significant in the soil uninhabited for > 45 years [46].

We prepared inoculum from an undisturbed old-growth maple forest, it was therefore rich in Planctobacteria. The second possibility is that microbial community composition might be masked by the impact of non-specific changes in soil composition and growth practises in the greenhouse. The third possibility, a complementary opinion, is that Planctobacteria abundant could be a competitor for P-nutrition and may be able to store P and therefore did not respond favourably to its supply for soybean. Although the third possibility is not proved experimentally, previous reports anticipated similar circumstances for Planctobacteria-phosphorus relationships in aquatic ecosystems [47]. These possible variabilities may implicate the soil microbial composition in relation to the abundance to its origin. To the best of our knowledge, several studies had focused on the pattern of trees and microenvironmental effects on the distribution and abundance on the mountain trees of the Gault Nature Reserve from where we collected soil as the source of microbial amendments [48–50], but no study had been reported until recently on soil microbial diversity. Hence, we are unable to refer that such microbiological diversity and

abundance might deliberate reasonable interaction benefits in soybean. Overall, our study offers a baseline understanding of microbial attributes of microbial amendments from an undisturbed age-old maple forest in a greenhouse grown crop plant.

We speculated that the composition of microbial communities observed in this study should be taken into consideration when studying microbial abundance as well as their role in nutritional acquisition for the benefit of plants like soybean. This study suggests that discovering phytate hydrolysing microbes will help us better understand how microbial amendments from an age-old maple forest respond to phytate, a phosphorus source, as well as uncover the roles of microbial taxa that have been understudied; however, further research into Planctobacteria-phytate relationships is required to enhance our understanding of how to use unknown soil microbial inoculum for crop production.

Experimental Procedures

Experimental design, treatments and sampling

The study was performed in a greenhouse trial from May 20th to August 24th, 2019, in a randomized complete block design with 10 blocks including four treatments with two replicates of each treatment. The average temperature during the experiment ranged from 22 to 27°C with a photoperiod of 16/8 h. Detail experimental procedure are available in the supporting information (Methods S1). Briefly, four L plastic pots (7.5"x7.25") were filled with substrate made from a mixture of sand, turface and sandy loam soil (1:1:1). Seeds of Viking 2518N non-GMO soybean (*Glycine max* L.) were purchased from William Dam Seeds Ltd (ON, Canada). Phytic acid sodium salt (Sigma-Aldrich, ON, Canada) was used as a source of phytate and the dose of phytate (3.3 mg/pot) was adjusted according to previous studies [51, 52]. Microbial suspension was prepared from soil collected from an age-old maple forest in the Gault Nature Reserve at Mont Saint-Hilaire, in Quebec (Fig. S1). Soybean shoots, roots and rhizosphere soil were collected per treatment and immediately transported to the laboratory and measured different parameters. Detail information is available in the supporting information (Methods S2).

Measurement of total phosphorus in plant shoot

Total P concentration in a shoot was measured following the dry ashing protocol [53]. In brief, the top 10 cm shoots of soybean plants were taken and grinded. Grinded samples of 0.5 g were taken in a porcelain crucible, placed in a furnace muffle and ashed for 4h at 500°C. Once cooled, dry ashed samples were placed in a 100 mL Erlenmeyer borosilicate flask and wet with 6 mL of concentrated H₂SO₄ and 1 mL of HNO₃. The solution was evaporated on a hot plate at 200°C until the solution was clear. The solution was filtered and transferred to a volume flask and filled up with 100 mL of distilled water. The solution was stirred and immediately used to determine the total P using the molybdene blue colorimetric method [54].

DNA extraction and amplicon sequencing

100 mg roots were taken for DNA extraction using DNeasy Plant mini kit (Qiagen, Toronto, ON, Canada) according to the manufacturer's recommendations. Soil DNA was extracted from 250 mg rhizosphere soil samples using DNeasy PowerSoil Pro kit (Qiagen, Toronto, ON, Canada), following the manufacturer's suggestions. DNA was eluted in 30 µL elution buffer and stored at -20°C. Extracted DNAs were quantified using NanoDrop™ 2000/2000c Spectrophotometer (ThermoFisher Scientific, Canada) and further visualized by gel electrophoresis on 1% agarose gel and GelDoc System (BioRad, Montreal, QC, Canada). PCR amplification was performed targeting bacterial 16s rRNA and fungal ITS region and sequenced using an Illumina MiSeq at the Genome Quebec Innovation Centre (Montreal, QC, Canada). Detailed information are in the supporting information (Methods S3).

Bioinformatics pipeline and processing of data

We performed all bioinformatics, data processing and graphical analyses using R4.0.2 software [R Core 55]. Detailed information on bioinformatics pipeline and data processing are available in the Supporting Information (Methods S4). Briefly, DADA2 was used to obtain Amplicon Sequence Variants (ASV) table and taxonomy was assigned to ASV using the reference dataset SILVA [56] for 16S rRNA, and the UNITE database [57] for ITS. The relative abundance of taxa were analysed using dplyr v2.0.0 [58] in R. The vegan package v 2.5.6 [59] was used for the alpha (Shannon and Simpson) and beta diversity indices (PCoA) and performed PERmutational Multivariate ANalysis Of VAriance (PERMANOVA) [60]. Tukey's *post-hoc* tests was performed in comparing treatments and sample types using agricolae v1.3-3 [61]. We visualized taxa abundance at order level with metacoder v 0.3.4 [62]. We performed indicator species analysis using the package indicspecies v 1.7.9 [63] in R4.0.2. using Šidák correction for multiple comparison in the R package 'RVAideMemoire' v 0.9–78 [64]. A co-occurrence network analysis was performed using the algorithm glasso of the SPIEC-EASI v 1.0.6 [13] and were imported in Cytoscape v 3.8.0 for plotting [65].

Declarations

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Conflicts of interest

There is no conflict of interests among the authors.

Data availability

All sequences are accessible in NCBI SRA database under the accession number PRJNA720672.

Code availability

All the R code used for the analysis is available on github.

(https://github.com/bulbul2020/soya_microbiome).

Ethics approval

This is an original scientific work that has not been previously published or is not being considered for publication elsewhere.

Consent to participate

All authors have agreed on the authorship, and all participants in this study have given their informed consent.

Consent for publication

All study participants provided approval for data publishing. To protect the privacy of the study participants, all data has been de-identified.

Author Contributions

BA: Designed the experiment, sampling, data analysis and wrote the manuscript; ZL: experimental design and sampling; JF: Data analysis; MH: Conceptualization, supervision and contribution to the manuscript writing.

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Figures

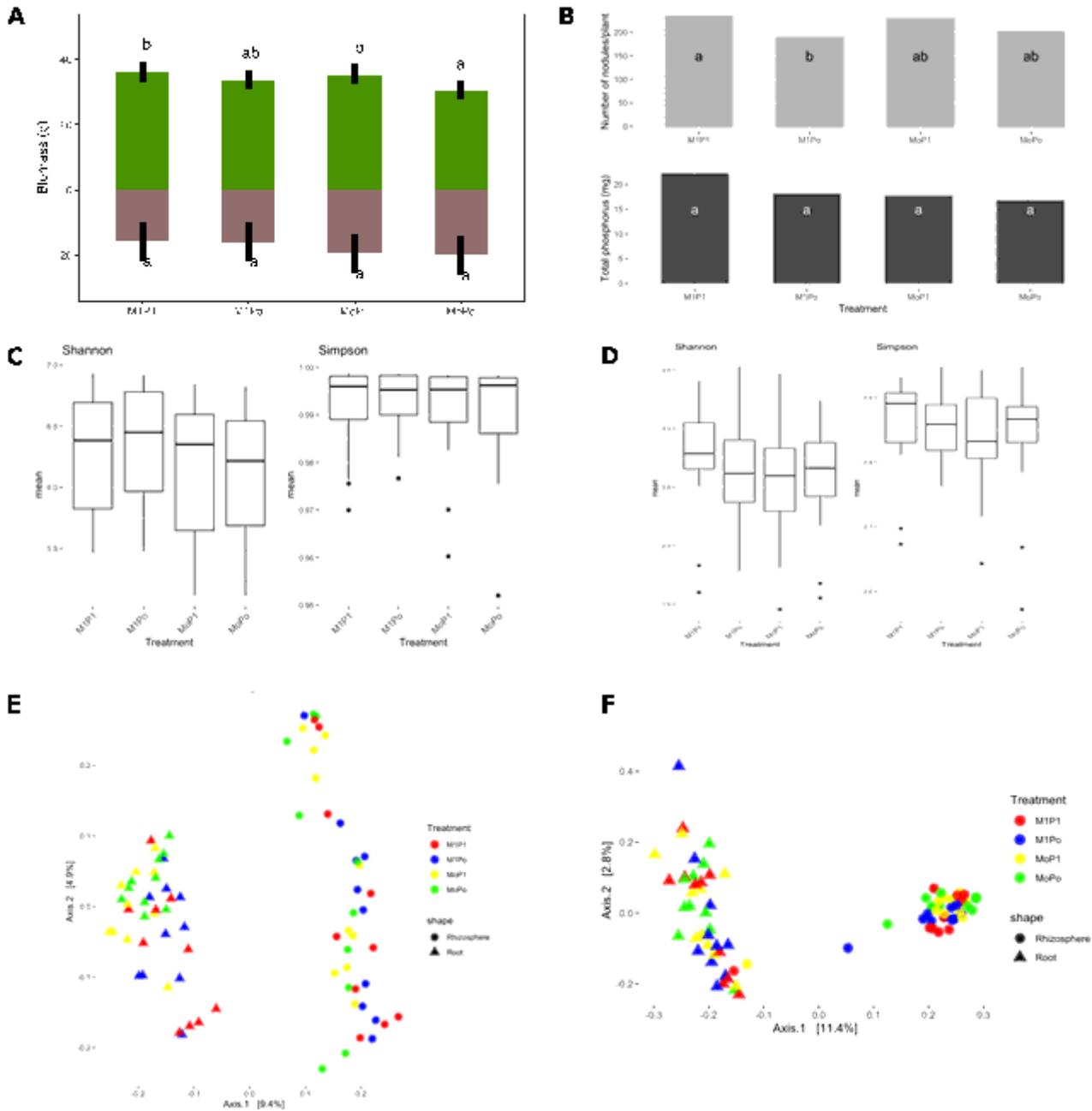


Figure 1

Measures of plant biomass, nodulation and total phosphorus, and microbial community structure. (A) Dry weight (g) of shoot and root. Green boxplots represent shoot dry weight, and gray boxplots represent root dry weight.; (B) number of nodules per plant and total phosphorus measured in shoots. With each treatment group, means with the same letter are not significantly different by a Tukey's range test. M1P1 = presence of both microbial inoculum and phytate; M1P0 = only microbial inoculum; M0P1 = only phytate, and M0P0 = absence of both microbial inoculum and phytate. (C) Shannon and Simpson diversity for bacterial microbiota; (D) Shannon and Simpson diversity for fungal microbiota. M1P1 = presence of both microbial inoculum and phytate; M1P0 = only microbial inoculum; M0P1 = only phytate, and M0P0 = absence of both microbial inoculum and phytate. Principal coordinates analysis (PCoA)

showing the community compositions assignments of (E) bacterial 16S r RNA genes and (F) fungal ITS genes data. The variation shown in axes 1 and 2 of the ordinations is indicated in parenthesis. Circular and triangle shape represents samples from rhizosphere soil and root, respectively. Each colour represents a sample. M1P1 = microbial inocula and phytate; M1P0 = only microbial inocula; M0P1 = phytate only and M0P0 = absence of both microbial inocula and phytate.

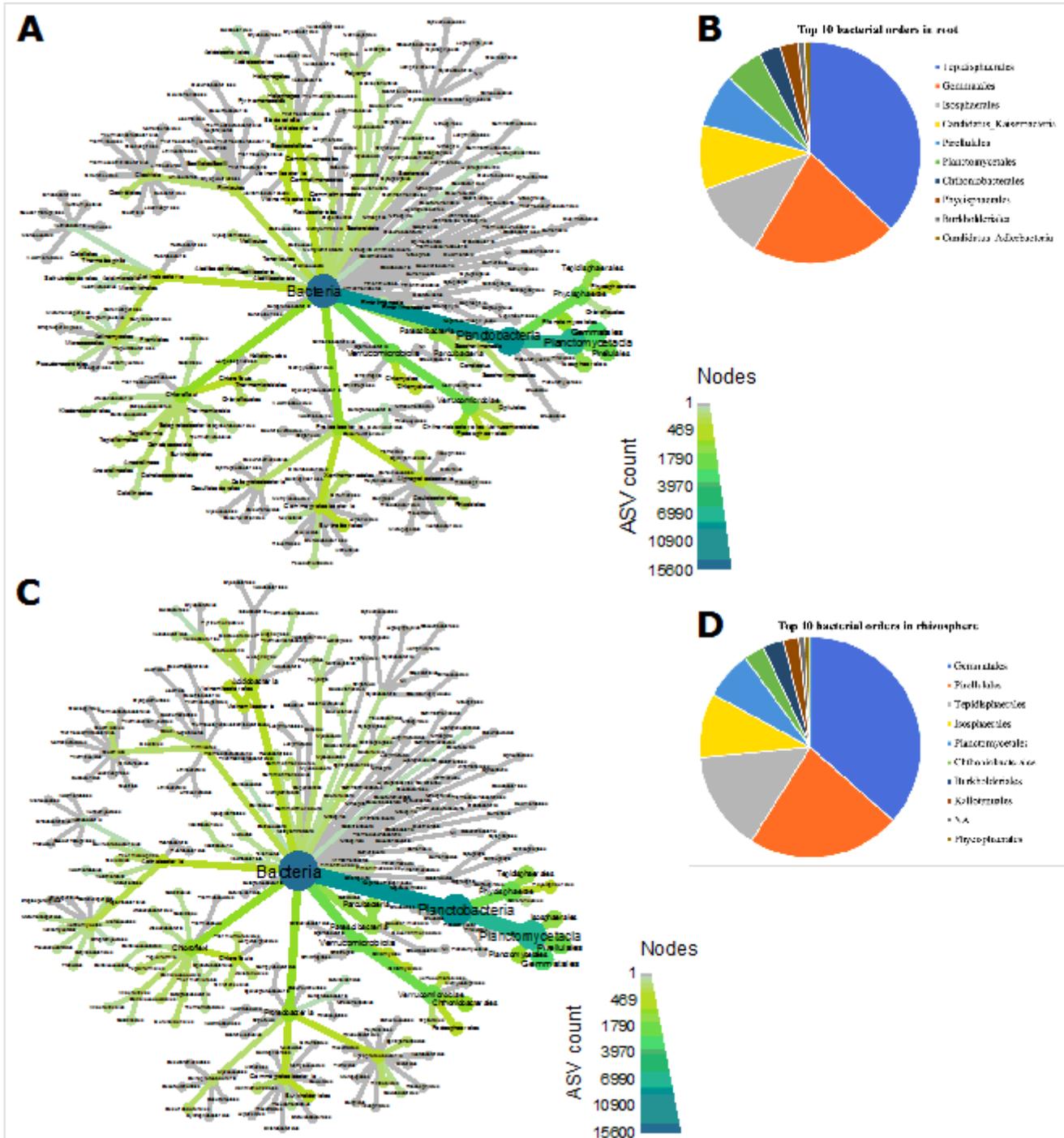


Figure 2

Taxonomic hierarchy and associated observations of ASVs (taxmap) for bacterial communities. Taxmap at order level in root (A) and relative abundance of top 10 orders in root (B). Taxmap at order level in rhizosphere (C) and relative abundance of top 10 orders in rhizosphere biotope (D).

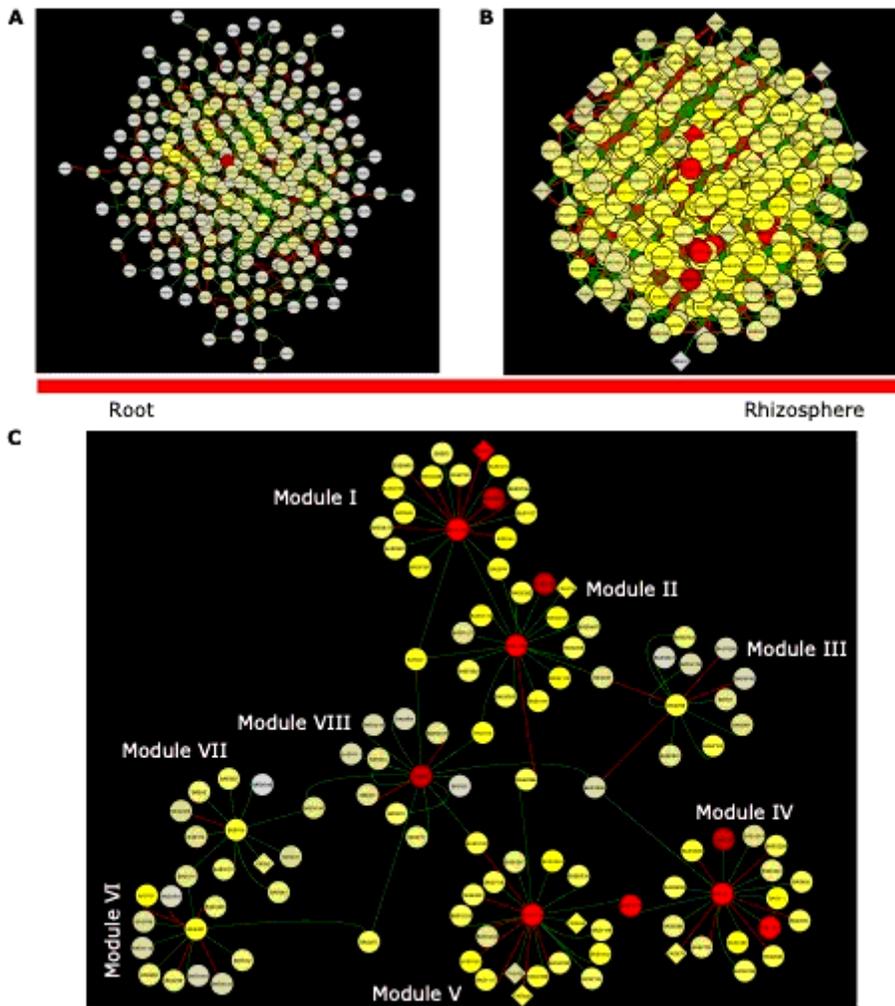


Figure 3

Network analysis in soybean microbiome. Inter-kingdom network in the root (A) and rhizosphere (B) biotopes. The node shapes represent bacterial (circular) and fungal (rhombus) communities. Nodes are coloured according to the relative abundance of the corresponding ASVs. The ribbon shows the relative complexity of the inter-kingdom network in root and rhizosphere biotopes. (C) A network built from ASVs of hub taxa and their inter-connection clustered into eight different modules. Each node represents a ASV from the microbiome. Green link represents a positive interaction and red link represents a negative interaction. Nodes are coloured according to the relative abundance of the corresponding ASVs, and node shapes denote bacterial (circular) and fungal (rhombus) ASVs. Details of the ASVs corresponding global hub taxa are in the Table S11.

Supplementary Files

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