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Species interactions enhance root allocation, microbial diversity and P acquisition in intercropped wheat and soybean under P deficiency

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Abstract

Belowground interactions in grain legume-cereal intercrops may improve resource acquisition and adaptation to environmental constraints such as phosphorus (P) deficiency. To advance the knowledge of belowground facilitative mechanisms involved in P-deficiency tolerance (root allocation, biochemical and microbial responses), soybean (*Glycine max*) and wheat (*Triticumaestivum*) were grown as mono- and intercrops under P-deficiency and P-sufficiency in soil-filled rhizoboxes. The hypothesis was that intercropping stimulates root microbial diversity, root biomass allocation and P-hydrolyzing acid phosphatases (APase) activity in roots under P deficient conditions. Total root dry weight (RDW), length, and surface area increased in P-deficient intercropped wheat and soybean compared to P-deficient monocrops. Greater root allocation to deeper soil layers was evident for P-deficient intercropped wheat. Shallow roots of intercropped wheat exhibited highly stimulated APase activity under P-deficient conditions while shallow roots of monocropped soybean exhibited higher APase activity in comparison to deeper roots, irrespective of P treatment. Root fungal diversity was significantly higher in intercropped wheat, and was significantly correlated with RDW, root APase activity, shoot P, and soil available P. Root bacterial diversity was higher in both intercrops, and was significantly correlated with RDW and shoot N concentration. The observed shifts in root microbial diversity, root biomass allocation and APase activity provide explanatory mechanisms of relationships between rhizosphere heterogeneity and pathways for increased P acquisition in diversified crops. Advanced belowground metabolomics on root microbial communities are required to reveal the beneficial effect of root microorganisms in associations of different crop species.

Key words: cereals, legumes, phosphatase, microbial diversity, root distribution, T-RFLP54

1. Introduction

Improving growth and yield performance of staple food crops such as legumes and cereals are major concerns for current and future agriculture, especially in stressed environments where increased frequency of abiotic constraints such as drought (Wang et al., 2003; Link et al., 1999), salinity (Munns and Tester, 2008), and low soil fertility (Graham and Vance, 2003) cause drastic yield losses. Low soil nutrient availability, notably phosphorus (P), is among the most important abiotic constraints for plant growth, especially for legumes whose sensitivity to P-deficiency has been attributed to higher P requirements during the symbiotic N₂ fixation process (Vance, 2001; Graham and Vance, 2003; Schulze and Drevon, 2005; Bargaz et al., 2011; Isaac et al., 2012).

While nutrients removed with harvested crops ultimately need to be replaced by recirculation or new inputs to avoid soil nutrient depletion, efficient crop nutrient acquisition is a key function for developing sustainable cropping systems that prevent nutrient losses and are able to maintain high productivity with minimum external inputs. The practice of intercropping legumes with cereals is gaining interest worldwide due to an increasing desire for both reducing and optimizing use of fertilizer (Fujita et al. 1992; Hauggaard-Nielsen and Jensen, 2005; Long et al., 2014; Brooker et al., 2015). Intercropping may mitigate stressful environmental conditions and increase the availability of deficient soil resources (Li et al., 2003; Betencourt et al., 2012; Pang et al., 2013; Isaac et al., 2014; Bargaz et al., 2015). Furthermore, intercropping has enhanced transfer of symbiotically-fixed nitrogen (N) from intercropped legumes to intercropped cereals (Xiao et al. 2004; Isaac et al., 2012), increased soil fertility (Wang et al., 2015), increased yield stability (Nasielskiet al., 2015), and decreased the prevalence of damage by pests, disease, and weeds (Jensen 1996; Trenbath, 1993; De Vallavieille-Pope, 2004; Corre-Hellou and Crozat, 2005; Li et al., 2007; Hauggaard-Nielsen et al., 2009).

Legume-cereal intercrops confer niche differentiation for more efficient exploitation of limiting resources and optimize rhizosphere biochemical heterogeneity (Corak et al., 1987; Li et al., 2001, 2007; Knudsen et al., 2004; Banik et al., 2006; Pang et al., 2013). Of note, the stimulation of legume nodulation and beneficial rooting distribution enhances acquisition of deficient resources, notably P and water, providing an advantage to both species (Li et al., 2007; Betencourt et al., 2012; Pang et al., 2013, Bargaz et al., 2016). Moreover, it has been demonstrated that enhanced nodulation in intercropping also coincided with a higher root biomass thus resulting in a significant correlation between shoot biomass and nodules (Latati et al., 2014, 2016; Bargaz et al., 2016). Faster root growth has been reported in legume-cereal intercrops, associated with higher P uptake, though largely in the cereal rather than the legume (Hauggaard-Nielsen et al., 2001; Li et al., 2006). Furthermore, root length and distribution has been correlated to shoot biomass accumulation in a maize-faba bean or chickpea intercrop (Xia

et al.2013), while Zhang et al. (2001) noted differential contribution from the above- and below-ground parameters to intercropping system performance.

For, specifically, plant P nutrition, rhizospheric interspecific facilitation has been found to increase availability and acquisition of P in several legume-based intercropping systems: wheat-lupine (Horst and Waschkies, 1987), sorghum-pigeon pea (Ae et al., 1990), wheat-chickpea (Li et al., 2003), maize-peanut (El Dessougiet al., 2003), wheat-chickpea (Li et al.,2004; Betencourt et al.,2012), maize-chickpea (Li et al.,2004), maize-faba bean (Li et al.,2003, 2004, 2007), barley-pea (Hauggaard-Nielsen et al., 2009), and maize-cowpea (Latatiet al.,2014). The main physiological mechanisms driving this P use efficiency under P-deficiency (Li et al., 2003; 2004; Veneklaaset al., 2003) are most likely due to the stimulation of rhizodeposits including carboxylates exudation, protons, and the activity of P-hydrolyzing enzymes, such as acid phosphatases (APase). Contribution of APase activity to the facilitation of P uptake in intercropping was reported in studies on maize-chickpea (Li et al., 2004), maize-peanut (Inal et al., 2007), and barley-peanut (Inal et al., 2008) in which the intercropped legume exhibited higher APase activity in roots and rhizosphere soils compared to the intercropped cereal. Induced root and soil APase activity in intercropping (compared to monocropping) has been attributed to an enhanced demand for P to support both intercropped-species (Inal et al., 2007).

Other plant-root exudates, such as organic acids, which are potentially implicated in P solubilization, may also be considered as key drivers in microbial recruitment and proliferation within the different rhizosphere layers e.g. ecto-rhizosphere, rhizoplane, and endo-rhizosphere (Hunter et al., 2014). This also includes root-associated and/or endophytic microbial populations that are responsible for a substantial pool of solubilized inorganic nutrients (e.g. P, Mg, Ca, Fe, etc.) in mixed cropping systems (Loper and Buyer, 1991, Dakora et al., 2003; Hunter et al., 2014). Legume-cereal intercrops show a number of rhizosphere-induced modifications including rhizosphere microbial communities (Morgan et al., 2005; Song et al., 2007; Sun et al., 2009) that coincide with stimulation of plant growth and yield.

Despite clear evidence of improved growth and yield in legume-cereal intercropping systems, further research on the highly intricate plant-soil-microbe continuum is needed, particularly under soil nutrient limitations. We hypothesize that crop diversification by grain legume-cereal intercropping under low P availability will enhance root allocation, root microbial diversity, and APase activity. We also hypothesize that these biophysical traits will be highly stimulated in intercrops resulting in improved P acquisition and growth. The specific objectives of this study were to (1) determine the effects of crop diversity on root allocation and

distribution (in space) as an adaptive mechanism in response to P-deficiency, (2) assess mono- and inter-cropping effects on root microbial diversity and (3) track variations in APase activity under different cropping strategies. To do this, we measured root functional traits, root microbial communities, root APase activity, and aboveground performance (growth) in wheat and soybean grown as mono- and intercrops under contrasting P availability in soil-filled rhizoboxes.

2. Material and methods

2.1. Soil, plant material and experimental design

A greenhouse experiment was conducted at the University of Toronto Scarborough, Canada, during spring and summer 2014 under natural daylight conditions (16 h photoperiod) supplemented with 200 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Day/night temperatures were 25/20 °C, and the relative humidity was kept at approximately 65%. Disease-free seeds of soybean (*Glycine max* L.cv Pioneer P90Y90) and spring wheat (*Triticumaestivum* L. cv Sable) were germinated for ten days in sterilized peat. Seedlings were transplanted into plexiglassrhizoboxes measuring 40 × 30 × 3.5 cm, and constructed with a removable and re-sealable plexiglass front (Bargaz et al., 2015). Rhizoboxes facilitated access to the rhizosphere soil and permitted a non-destructive recovery of roots and nodules. Rhizoboxeswere filled with 6 kg sieved (2-mm) and mixed air-dried soil and sand (30 volume-% soil, 70 volume-% sand). The soil used was collected from the top 20 cm in the agroforestry research station at the University of Guelph (Guelph, Ontario, Canada). Soils at the Guelph site are classified as Gray Brown Luvisols with a sandy-loam soil texture (Order: Alfisols, Group: TypicHapludalf) (Oelbermann and Voroney, 2007). There are characterized as neutral pH, with low total nitrogen (0.07%) and available P (3 mg kg⁻¹) concentration (Isaac et al., 2014). Pooled analysis of soil inorganic N (soil nitrate: 15.76 ± 1.86 mg NO₃⁻ kg⁻¹ and soil ammonium: 9.28 ± 6.10 mg NH₄⁺ kg⁻¹) indicate a moderately N deficient soil.

The experimental design was comprised of two factors: (1) cropping treatment (monocropped soybean (S), monocropped wheat (W), and intercropped S and W) and (2) P level (sufficient and deficient). The experiment was performed in a replacement design where the relative plant density remains constant across monocropping and intercropping treatments, a common approach to assess legume-cereal intercropping interactions (Hauggaard-Nielsen et al., 2009). The planting density was four plants per rhizobox, with two plants of each species per rhizobox in the intercropping treatments (4W or 4S for monocropped and 2W + 2S for intercropped) with the abbreviations S for monocropped soybean; W for monocropped wheat; SW-s for intercropped soybean; and SW-w for intercropped wheat. The entire experiment was replicated in three blocks with three rhizoboxes per treatment.

Plants were grown at approximately 80% field capacity using a nutrient solution (Bargaz et al., 2011, 2012) with either 125 or 25 μ mol P per rhizobox per week defined as sufficient and deficient P treatments, respectively. Urea was added at a concentration of 1 mmol (equivalent to 60 mg) per rhizobox in the nutrient solution during the initial 15 days after transplantation (DAT). After this, plants were grown with N-free nutrient solution, *i.e.* totally dependent on soil N and symbiotic N₂ fixation (soybean).

2.2. Measurement of above-and below-ground plant growth performances

At 45 DAT, plants were taken and separated into shoots and roots (nodulated roots for soybean). The rhizosphere soil was obtained by carefully separating roots from the loosely adhering soil, which was then sieved (2 mm) prior to measurements of APase activity and Olsen P concentration. The roots and nodules were carefully washed with distilled water at 4° C. Fresh root fractions were sampled and immediately frozen at -20 °C before they were analyzed for APase activity and microbial community.

Root surface (cm²) and length (cm) were measured using the automated image analysis software WinRhizo 2005b (Regent Instruments. Inc., Canada). These traits were determined in roots of the shallow and deep soil layers by dividing the root system in two: the shallow part (0-15 cm from soil surface) and the deep part consisting of the remaining root system, below 15 cm (Bargaz et al., 2015). Subsequently, dry weight of roots (RDW) shoots (SDW), and nodules (NDW) were determined to evaluate growth parameters (total dry biomass and root to shoot ratio) before they were ground to a fine powder for analyses of N and P concentrations.

2.3. Acid phosphatase activity assay and determination of inorganic P

Roots (100 $\pm$ 5 mg fresh weight (FW)) and 3 mm-diameter nodules (50 $\pm$ 5 mg FW) were ground with an extraction mixture consisting of 500 μ l of 0.1 M sodium acetate buffer (pH 5.6) containing 1 mM dithiothreitol. Homogenates were centrifuged at 13 000 g at 4°C for 30 min and aliquots of 50 μ l of supernatant were used for quantification of APase activity and inorganic phosphorus (Pi) according to Bargaz et al. (2012). Nodule APase and soil APase activities were determined using pNPP as an orthophosphate monoester analogue substrate (Tabataba, 1994). The soil P available for plant uptake (Olsen P) was determined spectrophotometrically at 630 nm using malachite green after extraction in 0.5 M NaHCO₃ at pH 8.5 (Olsen et al., 1954).

2.4. Determination of plant total N and P concentration

Total nitrogen concentration was determined from finely ground shoot subsamples (10 ± 1 mg) using a CHN elemental analyzer (THERMO FISON EA 1108, Waltham, USA). For determination of P concentration, subsamples of shoots were combusted at 550 °C and the obtained ashes were suspended in 25 mL of 1M hydrochloric acid. Suspensions were then filtered and P content was determined using the vanadomolybdate method after reading the absorbance at 430 nm.

2.5. Root microbial analysis

2.5.1. DNA extraction

One composite root sample of 200 ± 20 mg of fresh roots per rhizobox was collected in sterile falcon tubes, surface sterilized with sterile Triton/SDS (1% / 0.5%) for 3 min under moderate agitation, and extensively washed four times with sterile distilled water. Sterilized root samples were used for genomic DNA extractions using FastDNA® SPIN Kit (Q-Biogene, Carlsbad California), following manufacturer's protocol. DNA extracts from all root samples were visualized by agarose gel electrophoresis (0.8%), spectrophotometrically quantified using the NanoDrop™ ND-1000 V3.7.0 (Thermo Fisher Scientific Inc., Wilmington, USA) and diluted down to 15 ng μL^{-1} prior to PCR amplification.

2.5.2. Terminal restriction fragment length polymorphism (T-RFLP) analysis

T-RFLP analysis was conducted using universal primers targeting a conserved region of the 16S rRNA gene for bacteria and the internal transcribed spacer (ITS) region for fungi. The bacterial primers were fluorescently-labeled Eu27f (5'-[6FAM] AGA GTT TGA TCC TGG CTC AG-3') and unlabeled 1492R (5'-GGT TAC CTT GTT ACG ACT T-3') and the fungal primers were fluorescently-labeled ITS1f (5'-[6FAM] CTT GGT CAT TTA GAG GAA GTA A-3') and ITS4r (5'-[HEX] TCC TCC GCT TAT TGA TAT GC -3'). PCR reactions consisted of 10 μL HotStarTaqPlus Master Mix (Qiagen, USA), 1 μL of each primer (10 μM), 7 μL sterile water, and 1 μL of DNA template. PCR amplifications were conducted using a PTC-100 Programmable Thermal Controller (MJ Research, Inc., Canada) with a program of 94°C for 3 min, 36 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min, followed by a final extension at 72°C for 10 min for 16S and a program of 94°C for 3 min, 36 cycles of 94°C for 50 s, 51°C for 1 min, and 72°C for 1 min, ending with final extension at 72°C for 10 min for ITS. In order to suppress plant host plastid and mitochondrial 16S contamination, 2 μL of 10 μM plastid and mitochondrial peptide nucleic acids (pPNA: 5'-GGC TCA ACC CTG GAC AG-3' and mPNA: 5'-GGC AAG TGT TCT TCG GA-3') were added to 16S PCR reactions (Lundberg et al. 2013). Amplified DNA were visualized by agarose gel electrophoresis (2%) containing ethidium bromide for DNA staining and visualization. Amplicons

were then purified using GenElute PCR clean-up kits (Sigma-Aldrich, USA) following the manufacturer's instructions. 16S amplicons were digested with the MspI and ITS amplicons were digested with BfaI and BstNI restriction enzymes (New England BioLabs, USA). Digested fragments were measured relative to commercial standard fragments (ROX 1000) at the University of Guelph Laboratory Services Centre (ON, Canada). After initial data filtering (Abdo et al., 2006), T-RF lengths were rounded to the nearest whole number and proportional abundances of each T-RF were used for further analysis. Samples with two or fewer T-RF peaks were considered outliers and discarded from the dataset.

2.5.3. Pyrosequencing

Pyrosequencing was conducted on pooled root DNA from four treatments: monocropped wheat, intercropped wheat, monocropped soybean, and intercropped soybean. T-RFLP data indicated that P treatment did not affect microbial community composition, so each pooled sample included replicates with both high and low P. The V4 variable region of the 16S rRNA gene was targeted for bacteria, using primers 515f (5'- GTG CCA GCM GCC GCG GTA A-3') and 806r (5'- GGA CTA CHV GGG TWT CTA AT-3') (Caporaso et al. 2011) and ITS primers ITS1f and ITS4r. Sequencing was conducted using a Roche 454 FLX system at MR DNA (www.mrdnalab.com, Shallowater, TX, USA). QIIME (Caporaso et al., 2010) was used to de-noise the raw data and then pick OTUs using uclust, assign taxonomy, and generate an operational taxonomic unit (OTU) table. For 16S data, OTUs were selected with a similarity of 97% and sequences were taxonomically classified using the Greengenes database (13_08). For the ITS data, OTUs were selected with a similarity of 90% and sequences were taxonomically classified using the ITS unite database (12_11). Sequences were deposited at the European Nucleotide Archive (ENA, <http://www.ebi.ac.uk/ena/>) under accession number PRJEB10527.

2.5.4. Statistical analyses and calculations

The experiment was performed in a randomized complete block design. All growth and biochemical data were statistically analyzed using Statistica (V.8) software and all T-RFLP and pyrosequencing statistical analyses were performed using R Project for Statistical Computing version 3.1.2 (R Core Team 2014). Two-way analysis of variance (ANOVA) was performed for crop treatment $\times$ P treatment on all analyzed parameters. Comparison of means $\pm$ SE was performed using Fisher's least significant difference test at 5% probability. For T-RFLP data, statistical analysis was conducted in R package {vegan} (Oksanen et al., 2013). One-way permutational analysis of variance (PERMANOVA) with 9999 permutations was conducted on Bray-Curtis dissimilarity matrices to assess the effect of cropping treatment, P treatment, and plant species on bacterial and fungal community

composition. Spearman's rank correlations were conducted between the dissimilarity matrices and Euclidean distances of scaled biochemical variables (Clarke and Ainsworth, 1993) to select the subset of variables that were best correlated with the distribution of T-RF data and Mantel tests (function mantel) with 9999 permutations were used to assess significance. Non-metric multidimensional scaling (NMDS) was conducted on the dissimilarity matrices to visualize treatment effects and correlations. For pyrosequencing data, alpha diversity (Chao 1) rarefaction curves of the bacterial and fungal communities for each sample were created in QIIME. To assess beta diversity, both datasets were rarified to 5000 sequences, after which Bray-Curtis dissimilarity matrices were calculated in QIIME and samples were clustered using PCoA in R package {labdsv} (Roberts, 2013).

3. Results

3.1. Root traits and root distribution

Total wheat RDW was significantly higher ($p<0.001$) in intercropping as compared to monocropping (Table 1). In both cropping treatments, P-deficiency increased ($p<0.001$) total wheat RDW. In soybean, RDW was solely increased ($p<0.05$) in intercropping compared to monocropping in response to P-deficiency. Likewise, under P-deficiency, total root length and surface area of soybean were increased (26 and 28%, respectively) in intercropping compared to monocropping. For root length and surface area of wheat, a highly significant ($p<0.001$) cropping effect was found. Intercropping wheat significantly increased the root length (53%) and surface area (58%) under P-deficiency compared to monocropping. In contrast to the response in intercropping, these root traits had higher values under P-sufficiency than under P-deficiency in monocropped wheat.

Root distribution between shallow and deep soil layers showed clear differences in response to cropping and P treatments (Table 1). In intercropped wheat, RDW nearly doubled both in shallow and deep soil layers compared to monocropped wheat. This increase was clearly seen in the deep soil layer, especially in response to P-deficiency of both intercropped (47% increase) and monocropped wheat (57% increase). For soybean, RDW in the deep soil layer significantly ($p<0.05$) increased (42%) in intercropping under P-deficiency and was 2.14 times higher than that of the same soybean treatment in monocropping. For wheat RDW, root length and surface area markedly increased in response to intercropping in both soil layers. A significantly ($p<0.001$) higher length and surface area were allocated to deep rather than shallow soil layers. Both length and surface area of intercropped wheat increased under P-deficiency, but to a larger extent in the deep soil layer as compared to monocropped wheat.

Soybean root distribution between shallow and deep soil layers showed slight differences, but very similar trends (Table 1). Root length and surface area in the deep soil layer significantly ($p<0.05$) increased in

intercropped soybean regardless of P treatments. These root traits in deep soil layers significantly increased (45 and 65% for length and surface) under P-deficiency in intercropping compared to monocropping.

3.2. Plant growth, N and P concentration

In intercropped wheat (irrespective of P treatment), total plant dry weight (TDW) significantly ($p<0.05$) increased as compared to monocropped wheat (Fig. 1a). Soybean TDW did not vary in response to cropping treatment, whereas a significant ($p<0.05$) decrease (16%) was only noted under P-deficiency in monocropping soybean. The root to shoot dry weight ratio (Fig. 1b) was significantly ($p<0.05$) higher in intercropped wheat (compared to monocropping), in particular under P-deficiency. This ratio did not vary in monocropped soybean irrespective of P treatments, whereas it increased significantly (33%) in response to P deficiency in intercropped soybean.

Shoot N concentration in wheat in both cropping systems did not vary significantly for either P treatments (Fig. 2a). In soybean, shoot N concentration was significantly ($p<0.05$) higher in P-sufficient than P-deficient monocropped soybean. This parameter was also higher in intercropped soybean irrespective of P treatment. Shoot P concentration did not vary significantly in wheat and soybean intercrops regardless of P treatment (Fig. 2b). Nevertheless, both wheat and soybean monocrops had significantly lower shoot P concentration under deficient compared to sufficient P conditions.

3.3. Acid phosphatases activity and P concentration

In both species, root APase activity was significantly influenced by cropping treatment, and wheat root APase activity was highly influenced ($p<0.001$) by P treatment (Table 2). However, APase activity did not vary between the roots in shallow and deep soil layers, apart from the significantly higher ($p<0.05$) root APase activity in deep roots, as compared to shallow roots of the P-deficient monocropped wheat. However, wheat shallow roots exhibited significantly ($p<0.01$) higher APase activity in response to intercropping and P-deficiency. For soybean, significantly ($p<0.05$) higher root APase activity was found in shallow roots under monocropping (regardless of P treatment), whereas a significantly lower root APase activity occurred under intercropping.

Phosphorus deficiency significantly stimulated ($p<0.01$) nodule APase activity in the shallow soil layer under both mono- and intercropping (Table 2). This P-deficiency induced APase activity only occurred in the shallower nodules. In deeper nodules, APase activity was cropping treatment-influenced ($p<0.05$) showing higher APase activity in monocropping. Cropping treatment also significantly ($p<0.05$) affected the rhizosphere soil

APase activity, which was higher in monocropped compared to intercropped soybean under P-sufficiency. However, although P-deficiency slightly increased this enzyme activity in wheat rhizosphere soil (19% in mono- and 25% in intercropping), no significant difference was observed.

Both mono- and intercropped wheat shallow roots had a significantly higher Pi concentration under P-sufficiency (Table 2). Pi significantly ($p<0.05$) decreased under P-deficiency only in intercropped shallow wheat roots, whereas there was no significant difference in deeper root Pi regardless of cropping and P treatments. In both shallower and deeper roots of P-deficient intercropped soybean, a significantly ($p<0.001$) lower Pi concentration was found. Under P-deficiency, a significant ($p<0.05$) decrease was also noticed in monocropped shallower soybean roots, a decrease of 19% that remains smaller compared to 48% reported in shallower roots of intercropped soybean.

Shallower nodules of the P-deficient intercropped soybean also had a significantly ($p<0.05$) lower (40%, as compared to P-sufficiency) Pi concentration (Table 2). A similar observation was found in deeper nodules of P-deficient monocropped soybean, which was as low as that of intercropped deeper nodules under both P treatments. In response to nodule spatial distribution, nodule Pi significantly increased in deeper- as compared to shallower- nodules of P-deficient intercropping (35%) and P-sufficient monocropping (31%) soybean, and that these variations in deeper nodule Pi were found to be highly ($p<0.001$) influenced by cropping treatment.

Intercropped wheat had a significantly ($p<0.05$) higher rhizosphere soil available P concentration as compared to monocropped wheat under P-sufficiency, whereas there was no significant difference between cropping treatments under P-deficiency (Table 2). The rhizosphere soil available P fraction decreased significantly ($p<0.05$) in both P-deficient monocropped- and intercropped-soybean (under both P levels) as compared to P-sufficient monocropped soybean.

3.4. Microbial community composition

Cropping treatment had a significant effect on both the fungal ($p<0.05$) and bacterial ($p<0.01$) community composition in roots, as determined by T-RFLP analysis (Table 3). P treatment had no overall effect, but there was a significant effect ($p<0.05$) of the interaction between cropping and P treatment for the bacterial community (Table 3). Plant species did not affect the fungal community, but did have a strong effect on the bacterial community composition (Table 3). This bacterial community composition was significantly correlated with shoot N concentration and total RDW ($\rho = 0.22$, $p = 0.01$; Fig. 3). Fungal community composition was significantly

correlated with total RDW, root APase activity, soil available P, and shoot P ($p = 0.24$, $p = 0.01$; Fig. 3). However, indicator species analysis revealed that there were no individual OTUs that were significantly associated with one treatment over another.

Overall, fungal diversity (Chao 1) was higher in the intercropped-wheat roots than in any other treatment, whereas bacterial diversity was higher for roots from intercropped treatments than when compared to monocropped treatments, regardless of plant species (Fig. 4). Sequencing analysis indicated that the two soybean treatments had relatively similar microbial communities, especially for fungi, whereas there were large differences in the composition of the bacterial and fungal communities in the two wheat treatments. The wheat treatments were similar in their most abundant bacterial families, but the monocropped samples had more *Rhizobiaceae* (including *Agrobacterium* spp.), *Hyphomicrobiaceae*, *Pseudomonadaceae* and *Xanthomonadaceae*, whereas the intercropped samples had more *Bradyrhizobiaceae*, *Comamonadaceae*, and *Devosia* spp. (Table 4). The fungal community in the monocropped wheat treatment was dominated by members of the *Chaetomiaceae* and *Basidiomycota*, which only made up a small percentage of the fungal community in the other three treatments. Similarly, the intercropped wheat and monocropped soybean treatments were dominated by *Paraphomaspp.* Intercropped-soybean roots were also dominated by *Cercophora* spp. and *Alternaria* spp. compared to the other three treatments. Also, there was also a strong effect of plant species on the bacterial communities, mainly due to a higher proportion of *Rhizobiaceae*, *Bradyrhizobiaceae* and *Comamonadaceae* families in roots of soybean (Table 4).

4. Discussion

The present study contributes knowledge on the plant-soil-microbe continuum, specifically root phenotypic response and microbial diversity in intercropping systems. We show a significant effect of intercropping on root parameters and associated microbes that can improve the functioning of intercropping systems under soil limitation such as P-deficiency.

4.1. Root distribution and consequences for plant growth

This study highlights the importance of root distribution under intercropping (compared to monocropping) and suggests this distribution between shallow and deeper soil layers is a key mechanism that increases belowground niche complementarity, especially under P-deficiency. We found two main root-distribution patterns across the intercropped species under P-deficiency (as compared to monocropping): 1) soybean exhibited higher rooting

(DW, surface area, and length) in the deeper soil layer while 2) wheat increased both deeper and shallower roots. The development of deeper roots was clearly seen at the bottom of the rhizoboxes, where roots of intercropped wheat massively spread deep underneath the soybean roots and occupied a greater soil volume (given that wheat root surface was almost twice that of soybean root surface in intercropping under P-deficiency) indicating an important intercropping effect in the deeper soil layer. Similar root distribution has also been shown for the intercrops (regardless of P treatment) of pea-barley (Hauggaard-Nielsen et al., 2001), faba bean-maize, wheat-maize (Li et al., 2006), and cassava-maize (Lose et al., 2003). In intercropping, the barley root system was shown to grow deeper and faster than pea roots (Hauggaard-Nielsen et al., 2001). Such a rapid barley root growth made P uptake happen nearly 10 days ahead than that of intercropped pea (Hauggaard-Nielsen et al., 2001). Along with this root stimulation, acceleration of the onset of soil N uptake and alleviation of drought and limited nutrient constraints were also reported in barley rather than pea as intercrops (Jensen, 1996; Hauggaard-Nielsen et al., 2001).

Analysis of the spatial distribution of root biomass between shallow and deeper soil layers has revealed a noticeable intercropping advantage for both crops. This was especially pronounced in P-deficient wheat; compared to monocropping, shallow roots of intercropped plants had higher biomass (43%) and APase activity (19%), which coincided with improved P concentration (15%) and dry weight (27%) of shoots. This finding indicates that roots in the shallow soil layer might be important for the P acquisition in wheat, supporting productive shoot growth under P-deficiency. This increased allocation of roots in the shallow soil layer would also facilitate the coexistence of wheat with soybean, by increased belowground niche complementarity. Hauggaard-Nielsen et al. (2001) emphasized a similar example of complementary resource use in intercropping of pea and barley where shallow-rooted pea contributed to almost 90% of the P uptake in the shallow soil layer (0-12.5 cm). However, this shallow-rooted pea decreased use of deeper soil nutrients (especially water) owing to the extensive intercropped-barley root deployment at the deeper soil layer. This was a situation where reduced resource availability may have compromised pea growth in intercropping as compared to monocropping (Ofori and Stern, 1987; Hauggaard-Nielsen et al., 2001). It has also been reported that shallow-rooted common bean genotypes were associated with higher P acquisition and plant growth under P-deficiency whereas deep-rooted genotypes grew better under water deficiency (Liao et al., 2004; Ho et al., 2005).

Xia et al., (2013) demonstrated that belowground spatial distribution and length of shallow roots in maize differentially varied with the intercropped legume species. These belowground root parameters were significantly

correlated to the aboveground acquisition of P and shoot biomass of maize intercropped with faba bean and chickpea, while no effect was observed with soybean (Xia et al., 2013). In the present study, it is possible that the absence of a beneficial intercropping effect on soybean aboveground biomass could be attributed to increased competition and soil occupancy by wheat roots, especially under P-deficiency. Increased root biomass allocation is important for plant responses to nutrient availability as the root mass fraction contributes to an increase in total root length, especially the fine roots, which are responsible for resource acquisition (Ostonenet al., 2007; Poorterand Ryser, 2015).

4.2. Associated belowground biochemical changes

These root growth patterns may explain why wheat root Pi concentrations did not change under either cropping system, but Pi in intercropped soybean roots markedly decreased (58%) under P deficiency. This decrease may be a result of a lower root APase activity (Table 2) and high shoot P demand under P-deficiency, but to a larger extent in intercropping than monocropping (Fig. 2). However, it remains unclear why there was no variability in terms of root Pi and soil APase activity in parallel to the important deeper rooting for the intercropped wheat. An alternative explanation for such a response may be attributed to the weak involvement of deeper roots in P uptake as compared to shallow roots, given that P availability in a natural soil is greater in shallow as compared to deeper soil layers. In mixed crops, P uptake and growth of *Trifoliumsubterraneum* was significantly enhanced when intercropped with the shallow-rooted annual legume “*Cullen pallidum*” than with the deeper-rooted “*Medicago sativa*” (Pang et al., 2013). The degree of similarity in growth as well as the degree of root interactions is presumably a key factor for a compatible intercrop in order to avoid the asymmetric interspecific belowground interactions, a phenomenon described in detail by Li et al. (2001, 2006). In this study, the greater competition between wheat over soybean in intercropping may be a consequence of asymmetric interspecific belowground interactions. Conversely, symmetric interspecific facilitations were found in the faba bean-maize intercropping where both species over-yield owing to a compatibility of their spatial and temporal root distribution (Li et al., 2006).

Furthermore, it is also possible that roots of intercropped wheat (given its higher soil occupancy) benefited from both the available P fraction and the rhizosphere APase activity of intercropped soybean (15% (soil P) and 32% (soil APase activity) increase in the soybean rhizosphere compared to the wheat rhizosphere, regardless of P treatment). This is consistent with the findings by Li et al. (2004) that intercropped-chickpea aboveground biomass did not vary between cropping (mono- and inter-cropping with maize) and P treatments (inorganic and organic

P). However, an important belowground facilitation occurred under P-deficiency through an increased APase activity in intercropped chickpea (root and rhizosphere soil) that was associated with higher root length in intercropped maize; two main factors that enhanced the P use efficiency and facilitated the intercropped-maize growth (Li et al., 2004). Similarly, wheat growth was also facilitated when intercropped with chickpea under an organic P source (Li et al., 2003).

4.3. Associated shifts in microbial communities

This study has also shown clear variations in the root microbial community structure between both intercrops; these variations appear to be species- rather than P-driven. This result, besides the induced root growth, provides evidence of a strong belowground rhizosphere heterogeneity that may rely on the interaction between crops and is particularly apparent in the intercropped wheat roots which harbor more diverse bacterial and fungal communities, compared to monocropped wheat. On the other hand, the large difference in terms of bacterial community found in intercropped soybean appears to be significantly ($p<0.05$) influenced by the interaction “cropping $\times$ P”. This may suggest that root microbial diversity depends on plant and/or root P status rather than soil P level alone, as the latter did not affect either bacterial or fungal community (Table 3). An alternative mechanism is that because intercropping led to increased root growth and nutrients uptake, enough P was available (for both plant and root-associated microbes) that the root microbial community did not need to respond to soil P level. This theory may also be supported by the positive relationship between the microbial composition and soil biochemical parameters, especially root APase activity, soil available P, and shoot contents in P and N (Fig. 3). However, advanced studies on how root-associated microbial communities would influence (or be influenced by) intracellular nutrients (especially P) are needed in mixed plant culture. For example, it would be worth investigating whether increased microbial diversity has also contributed to the higher root APase activity quantified in intercropped wheat that made P available (within the rhizoplane and root parenchyma) for uptake. This is in accordance with a study by Maougalet al. (2014) that suggested P hydrolysis in root parenchyma of common bean would be performed by endophytic phosphatase producing bacteria such as *Bacillus*.

While P status had no effect on the dominant bacterial families, bacterial families were different under intercrops compared to monocrops, but this effect was especially apparent in wheat roots. The key changes were a decline in members of the *Pseudomonadaceae* and *Xanthomonadaceae* families, along with an increase in members of the *Hyphomicrobiaceae* (*Devosia* spp.), *Bradyrhizobiaceae*, *Comamonadaceae*, and *Rhodospirillaceae* which all may contribute to N nutrition of the host plant in the intercropped treatments. We are

not aware of any other study describing an increase in the genus *Devosia* in intercropped wheat. Currently, eight *Devosia* species are known; *D. neptuniae*, first described by Rivas et al. (2002), nodulates and fixes N in the aquatic legume species “*Neptunianatans*”. In other studies, *Devosia*spp were among some *Alphaproteobacteria* assimilating root exudates, and were described to promote plant growth by improving inorganic nutrients availability (Valverde et al., 2007; Haicharet et al., 2012). Moreover, the presence of *Rhodospirillaceae* family (four times more abundant in intercropped wheat and soybean than in monocrops) may contribute to the host-plant N economy. For example, some *Azospirillum* (members of *Rhodospirillaceae* family) can substantially ameliorate plant N by releasing NH_4^+ intracellularly in paranodes (Christansen-Weniger and Vanderleyden 1994), symbiotic performances, and hair root production in common bean (Burdman et al., 1997). This is also consistent with a study by Riggs et al. (2001) reporting a positive relationship between the enhanced maize performance (higher nutrient acquisition, plant growth and yield) and inoculation with the diazotrophic endophytes *Herbaspirillum seropedicae* Z152 and *Klebsiella pneumonia* 342. It is worth mentioning *H. seropedicae* and *K. pneumonia* to be N_2 fixing endophytes belonging to *Beta*- (high relative percentage in intercropped wheat) and *Gamma-proteobacteria* (high relative percentage in intercropped soybean) classes, respectively.

In this study, the higher occurrence of the *Bradyrhizobiaceae* family in intercropped wheat roots agrees with several studies reporting rhizobia to occur commonly as natural endophytes that act either as N_2 -fixing or plant growth-promoting rhizo-bacteria for many non-legume crops such as wheat (Biederbeck et al., 2000), rice (Yanniet al., 1997), canola (Lupwayi et al., 2000), maize and cotton (McInroy and Kloepper, 1995; Martinez-Romero et al. 2000). Furthermore, rhizobia can act as biocontrol agents in natural and agricultural ecosystems. For example, *Bradyrhizobium japonicum* that was reported to significantly decrease sporulation of many bacterial and fungal pathogens (Tu, 1979; Dakora et al., 1993; Dakora, 2003). In this context, the higher *Bradyrhizobiaceae* occurrence in the intercropped wheat roots may influence the observed decline in endophytes members of the *Pseudomonadaceae*, but contrast with the appearance of few pathogenic fungi of the genera *Alternaria*, *Fusarium*, and *Cercospora*, which perhaps indicate high susceptibility of intercropped soybean to pathogenic fungi.

4.4. Interspecific belowground interactions: microbial, root biomass and biochemical changes

In intercropping, the significant correlation between root microbial community and RDW may indicate a microbial role in plant nutrient acquisition and root biomass allocation. This relationship coherently varied with root biochemical analyses, particularly the stimulated APase activity along with a decreased Pi in the P-deficient intercropped-wheat roots (Table 1), which may mean that both fungi and bacteria probably compete with plants

for the root-associated P pool. This effect was also seen in P-deficient soybean, although to a lower extent than in intercropped wheat. On the other hand, shoot P concentration in P-deficient soybean increased significantly (45%) in response to intercropping, which indicates that both increased root growth and relocation of P from roots to shoots might be part of an adaptation to P deficiency in intercropped soybean. However, even though this study mechanistically advanced knowledge on belowground information in wheat-soybean intercropping, it remains unclear whether rhizosphere biochemical properties in intercrops (especially wheat intercrop in this study) rely on a direct (root-induced changes) or indirect (microbial activities) rhizosphere effect in order to buffer the effect of low P availability. Overall, the higher root biomass allocation, the increased root microbial diversity, and the significant correlation between root microbial community and both APase activities and dry weight of roots (Fig. 3) may plausibly support an efficient intra-tissue recycling of P from the intercropped-wheat roots (lower P_i than monocrop) to shoot (higher P_i and biomass than monocrop). The higher APase activity and lower P_i in shallow roots of P-deficient intercropped wheat may also indicate a possible root P turnover as to recycle senescent-root P (from shallow roots), given that nutrient recycling is one of multiple strategies that enable diversified crops to over-yield as compared to monocrops (Zhang et al., 2010; Li et al., 2014). Nevertheless, it remains to be clarified to what extent mixed crops (such as intercropping) may maximize remobilization of both above- and below-ground senescent-tissue P pools under P-deficiency.

The significant correlation between root microbial community, shoot nutrients (P and N) and RDW presumably links to higher biomass in intercropped species (especially wheat). Moreover, differences in root microbial community between plant species driven by variations in the above- and below-ground biomass, especially carbon allocation (quantitatively and qualitatively) to roots, has been considered one of the key factors that shape the microbial community within the roots and rhizosphere (Hernández et al., 2015). This may explain the higher microbial community diversity in intercrops as compared to monocrops, especially for wheat intercrop whose higher belowground C allocation would result from an optimal photosynthetic activity and growth. It is estimated that up to 60% of the photosynthesis-fixed C in wheat, pea, maize, and tomato, is belowground-translocated where root-associated microorganisms can metabolize it or use it for the benefit of plant growth and the rhizosphere microbiome (Hernández et al., 2015; Morgan et al., 2005). This is in agreement with several studies that intercropping legume-cereal stimulates plant growth and yield in parallel to numerous rhizosphere-induced changes including soil respiration (Ibrahim et al., 2013), rhizosphere microbial communities (Morgan et al., 2005; Song et al., 2007; Sun et al., 2009), rhizobial symbiosis efficiency (Latatiet et al., 2014, 2016), rhizosphere acidification and P availability (Betencourt et al., 2012; Latatiet et al., 2016).

5. Conclusions

Under P limited conditions, this study provides evidence of significant differences in root microbial community structure and root biomass allocation patterns between inter- and mono-cropped wheat and soybean. At the rhizosphere level, the findings contribute to a mechanistic understanding of belowground interactions in a wheat-soybean intercropping, arguably necessary to understand the widely described enhanced crop performance under suboptimal conditions in intercropping systems. The enhanced microbial diversity in concert with root-induced morphological and biochemical changes provide evidence of a positive belowground rhizosphere heterogeneity that contributes to improve aboveground biomass and nutrient (P and N) acquisition. On the other hand, the observed prevailing wheat growth over soybean in intercropping may reveal an asymmetric interaction both temporally and spatially. However, stimulation of root / rhizosphere activities and interaction mechanisms in intercropping (root-soil-microbial interactions, soil exploration, nutrient mobilization and acquisition) that result from a higher crop functional diversity remain complex. Improving the understanding of multi-functional belowground interactions in diversified cropping systems will rely on further research on the coordination between specific rhizosphere metrics, notably soil and root microbial communities and their physiological function, for instance, root nutrient absorptive capacity and whole plant production.

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References

- Ae, N., Arihara, J., Okada, K., Yoshihara, T., Johansen, C.,1990.Phosphorus uptake by pigeon pea and its role in cropping systems of the Indian subcontinent. *Science*.248, 477–480.
- Abdo, Z.,Schüette, U.M.E., Bent, S.J., Williams, C.J., Forney, L.J., Joyce, P.,2006. Statistical methods for characterizing diversity of microbial communities by analysis of terminal restriction fragment length polymorphisms of 16S rRNA genes. *Environ.Microbiol*.8, 929-938.

507 Banik, P.A., Midya, B.K., Sarkar, S., Ghose, S., 2006. Wheat and chickpea intercropping systems in an additive
508 series experiment: Advantages and weed smothering. *Eur. J. Agron.* 24, 325–332.

509 Bargaz, A., Ghoulam, C., Faghire, M., Aslan, Attar, H., Drevon, J-J., 2011. The nodule conductance to the O₂
510 diffusion increases with high phosphorus content in the *Phaseolus vulgaris*-rhizobia symbiosis. *Symbiosis*. 53,
511 157–164.

512 Bargaz, A., Ghoulam, C., Amenc, L., Lazali, M., Faghire, M., Abadie, J., Drevon, J.J., 2012. A phosphoenol
513 pyruvate phosphatase gene transcript is induced in the root nodule cortex of *Phaseolus vulgaris* under P
514 deficiency. *J. Exp. Bot.* 63, 4723–4730.

515 Bargaz, A., Isaac, M.E., Jensen, E.S., Carlsson, G., 2015. Intercropping of faba bean and wheat under low water
516 availability promotes faba bean nodulation and root growth in the deeper soil layers. *Proc. Environ. Sci.* 29,
517 111–112.

518 Bargaz, A., Isaac, M.E., Jensen, E.S., Carlsson, G., 2016. Nodulation and root growth increase in lower soil layers
519 of water-limited faba bean intercropped with wheat. *J. Plant Nutr. Soil Sci.* 179, 537–546.

520 Betencourt, E., Duputel, M., Colomb, B., Desclaux, D., Hinsinger, P., 2012. Intercropping promotes the ability of
521 *durum wheat* and chickpea to increase rhizosphere phosphorus availability in a low P soil. *Soil.*
522 *Biol. Biochem.* 46, 21–33.

523 Biederbeck, V.O., Lupwayi, N.Z., Hanson, K.G., Rice, W.A., Zentner, R.P., 2000. Effect of long-term rotation
524 with lentils on rhizosphere ecology and on endophytic rhizobia in wheat. In: Book of Abstracts, 17th North
525 American Conference on Symbiotic Nitrogen Fixation, 23–28 July 2000, Quebec, Canada p80.

526 Brooker, R.W., Bennett, A.E., Cong, W.F., et al., 2015. Improving intercropping: a synthesis of research in
527 agronomy, plant physiology and ecology. *New Phytol.* 206, 107–117.

528 Burdman, S., Kigel, J., Okon, Y., 1997. Effects of *Azospirillum brasilense* on nodulation and growth of common
529 bean (*Phaseolus vulgaris* L.). *Soil. Biol. Biochem.* 29, 923–929.

530 Christensen-Weniger C., Vanderleyden, J., 1994. Ammonium-excreting *Azospirillum* sp. become intracellularly
531 established in maize (*Zea mays*) para-nodules. *Biol. Fert. Soils*. 17, 1–8.

532 Corak, S.J., Blevins, D.G., Pallardy, S.G., 1987. Water transfer in an alfalfa/maize association. *Plant. Physiol.* 84,
533 582–586.

534 Corre-Hellou, G., Crozat, Y., 2005. N₂ fixation and N supply in organic pea (*Pisum sativum* L.) cropping systems
535 as affected by weeds and pea weevil (*Sitona lineatus* L.). *Eur. J. Agron.* 22, 449–458.

536 Dakora, F.D., Joseph, C.M., Phillips, D.A., 1993. Alfalfa (*Medicago sativa* L.) root exudates contain isoflavonoids
537 in the presence of *Rhizobium meliloti*. *Plant. Physiol.* 101, 819–824.

538 Dakora, F.D., 2003. Defining new roles for plant and rhizobial molecules in sole and mixed plant cultures
539 involving symbiotic legumes. *New Phytol.* 158, 39–49.

540 De Vallavieille-Pope, C., 2004. Management of disease resistance diversity of cultivars of a species in single
541 fields: controlling epidemics. *CR. Biologies.* 327, 611–620.

542 El Dessougi, H., Dreele, A.Z., Claassen, N., 2003. Growth and phosphorus uptake of maize cultivated alone, in
543 mixed culture with other crops or after incorporation of their residues. *J. Plant. Nutr. Soil. Sci.* 166, 254–261.

544 Fujita, K., Ofori-Budu, K.G., Ogata, S., 1992. Biological nitrogen fixation in mixed legume-cereal cropping
545 systems. *Plant. Soil.* 141, 155–175.

546 Graham, P.H., Vance, C.P., 2003. Legumes: importance and constraints to greater use. *Plant Physiol.* 31, 872–877.

547 Haichar, F.Z., Roncato, M.A., Achouak, W., 2012. Stable isotope probing of bacterial community structure and
548 gene expression in the rhizosphere of *Arabidopsis thaliana*. *FEMS Microbiol. Ecol.* 81, 291–302.

549 Hauggaard-Nielsen, H., Ambus, P., Jensen, E.S., 2001. Interspecific competition, N use and interference with
550 weeds in pea–barley intercropping. *Field Crops Res.* 70, 101–109.

551 Hauggaard-Nielsen, H., Jensen, E.S., 2005. Facilitative root interactions in intercrops. *Plant Soil* 274, 237–250.

552 Hauggaard-Nielsen, H., Gooding, M., Ambus, P., et al., 2009. Pea-barley intercropping for efficient symbiotic
553 N₂-fixation, soil N acquisition and use of other nutrients in European organic cropping systems. *Field Crops*
554 *Res.* 113, 64–71.

555 Hernández, M., Dumont, M.G., Yuan, Q., Conrad, R., 2015. Different Bacterial Populations Associated with the
556 Roots and Rhizosphere of Rice Incorporate Plant-Derived Carbon. Appl Environ
557 Microbiol doi:10.1128/AEM.03209-14.

558 Ho, M.D., Rosasdahl, L., Brown, K.M., Lynch, J.P., 2005. Root architectural tradeoffs for water and phosphorus
559 acquisition. J.Theor. Biol. 226, 331–340.

560 Horst, W.J., Waschkies, C., 1987. Phosphorus-nutrition of spring wheat (*Triticumaestivum* L) in mixed culture
561 with white lupin (*Lupinus albus* L). J. Plant Nutr. Soil. Sci.-Zeitschrift für Pflanzenernährung und
562 Bodenkunde 150, 1–8.

563 Hunter, P.J., Teakle, G.R., Bending, G.D., 2014. Root traits and microbial community interactions in relation to
564 phosphorus availability and acquisition, with particular reference to Brassica. Front. Plant Sci. 5, 1–18.

565 Ibrahim, H., Hatira, A., Pansu, M., 2013. Modelling the functional role of microorganisms in the daily exchanges
566 of carbon between atmosphere, plants and soil. Proc. Environ.Sci. 19, 96-105.

567 Inal, A., Gunes, F., Zhang, I., Cakmak, C., 2007. Peanut/maize intercropping induced changes in rhizosphere and
568 nutrient concentrations in shoots. Plant Physiol. Biochem. 45, 350-356.

569 Inal, A., Gunes, A., 2008. Interspecific root interactions and rhizosphere effects on salt ions and nutrient uptake
570 between mixed grown peanut/maize and peanut/barley in original saline-sodic-boron toxic soil. J. Plant
571 Physiol. 165, 490-503.

572 Isaac, M.E., Hinsinger, P., Harmand, J.M., 2012. Nitrogen and phosphorus economy of a legume tree-cereal
573 intercropping system under controlled conditions. Sci. Total Environ. 434, 71-78.

574 Isaac, M.E., Carlsson, G., Ghoulam, C., Makhani, M., Thevathasan, N.V., Gordon, A., 2014. Legume performance
575 and nitrogen acquisition strategies in a tree-based agroecosystem. Agroecol. Sustain. Food Syst. 38, 686-703.

576 Jensen, E.S., 1996. Grain yield, symbiotic N₂ fixation and interspecific competition for inorganic N in pea-barley
577 intercrops. Plant Soil 182, 25-38.

578 Knudsen, M.T., Hauggaard-Nielsen H, Jensen, ES., 2004. Pea, faba bean and lupin intercropped with spring
579 barley-performance and N₂ fixation at two temperate locations. J. Agr. Sci. 142, 617-627.

580 Latati, M., Blavet, D., Alkama, N., Laoufi, H., Drevon, J.J., Gérard, F., Pansu, M., Ounane, S.M., 2014. The
 581 intercropping cowpea-maize improves soil phosphorus availability and maize yields in an alkaline soil. *Plant*
 582 *Soil* 385, 181-191.

583 Latati, M., Bargaz, A., Belarbi, B., Lazali, M., Benlahrech, S., Tellah, S., Ghiles, K., Drevon, J.J., Ounane, S.M.,
 584 2016. The intercropping common bean with maize improves the rhizobial efficiency, resource use and grain
 585 yield under low phosphorus availability. *Eur. J. Agron.* 72, 80-90.

586 Li, L., Sun, J.H., Zhang, F.S., Li, X.L., Yang, S.C., Rengel, Z., 2001. Wheat/maize or wheat/soybean strip
 587 intercropping. I. Yield advantage and interspecific interactions on nutrients. *Field Crops Res.* 71, 123-137.

588 Li, L., Tang, C., Rengle, Z., Zhang, F.S., 2003. Chickpea facilitates phosphorus uptake by intercropping wheat
 589 from an organic phosphorus source. *Plant Soil* 248, 305-312.

590 Li, L., Sun, J., Zhang, F., Guo, T., Bao, X., Smith, F.A., Smith, S.E., 2006. Root distribution and interactions
 591 between intercropped species. *Oecologia* 147, 280-290.

592 Li, L., Li, S.M., Sun, J.H., Zhou, L.L., Bao, X.G., Zhang, H.G., Zhang, F.S., 2007. Diversity enhances agricultural
 593 productivity via rhizosphere phosphorus facilitation on phosphorus-deficient soils. *Proceedings of the National*
 594 *Academy of Sciences, USA* 104, 11192–11196.

595 Li, S.M., Li, L., Zhang, F.S., Tang, C., 2004. Acid phosphatase role in chickpea/maize intercropping. *Ann. Bot.*
 596 94, 297–303.

597 Liao, H., Yan, X.L., Rubio, G., Beebe, S.E., Blair, M.W., Lynch, J.P., 2004. Genetic mapping of basal root
 598 gravitropism and phosphorus acquisition efficiency in common bean. *Funct. Plant Biol.* 31, 959–970.

599 Link, W., Abdelmula, A.A., Von Kittlitz, E., Bruns, S., Riemer, H., Stelling, D., 1999. Genotypic variation for
 600 drought tolerance in *Vicia faba*. *Plant Breeding* 118, 477–483.

601 Long, Li., Tilman, D., Lambers, H., ZanghF-S., 2014. Plant diversity and overyielding: insights from belowground
 602 facilitation of intercropping in agriculture. *New Phytol.* 203, 63–69.

603 Loper, J.E., Buyer, J.S., 1991. Siderophores in microbial interactions on plant surfaces. *Mol. Plant Microbe* 4, 5–
 604 13.

605 Lose, S.J., Hilger, T.H., Leihner, D.E., Kroschel, J., 2003. Cassava, maize and tree root development as affected
 606 by various agroforestry and cropping systems in Bénin, West Africa. *Agr.Ecosyst. Environ.* 100, 137–151.

607 Lupwayi, N.Z., Rice, W.A., Clayton, G.W., 2000. Endophytic rhizobia in barley and canola in rotation with field
 608 peas. In: Book of Abstracts, 17th North American Conference on Symbiotic Nitrogen Fixation 23–28 July
 609 2000 Quebec Canada. 80. University of Laval, p 51.

610 Maougal, R.T., Bargaz, A., Sahel, C., Amenc, L., Djekoun, A., Plassard, C., Drevon, J.J., 2014. Localization of
 611 the *Bacillus subtilis* beta-propeller phytase transcripts in nodulated roots of *Phaseolus vulgaris* supplied with
 612 phytate. *Planta* 239, 901-908.

613 Martinez-Romero, E., Gutierrez-Zamora, M.L., Estrada, P., Caballero-Mellado, J., Hernandez-Lucas, I.,
 614 2000. Natural endophytic association between. *Rhizobium Etli* and maize. In: Book of Abstracts, 17th
 615 North American Conference on Symbiotic Nitrogen Fixation, 23–28 July, 2000. Quebec, Canada. University
 616 of Laval, p 51.

617 McInroy, J.A., Kloepper, J.W., 1995. Survey of indigenous endophytes from cotton and sweet corn. *Plant Soil* 173,
 618 337–342.

619 Morgan, J.A.W., Bending, G.D., White, P.J., 2005. Biological costs and benefits to plant–microbe interactions in
 620 the rhizosphere. *J. Exp. Bot.* 56, 1729–1739.

621 Munns, R., Tester, M., 2008. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* 59, 651–681.

622 Nasielski, J., Furze, J., Tan, J., Bargaz, A., Thevathasan, N.V., Isaac, M.E., 2015. Soybean morphological and
 623 physiological responses to in-situ water deficit in a tree-based intercropping system. *Agron. Sustain.*
 624 *Dev.* 35, 1541-1549.

625 Oelbermann, M., Voroney, R.P., 2007. Carbon and nitrogen in a temperate agroforestry system: Using stable
 626 isotopes as a tool to understand soil dynamics. *Ecol. Eng.* 29, 4342-349.

627 Ofori, F., Stern, W.R., 1987. Cereal-legume intercropping systems. *Adv. Agron.* 41, 41–90.

628 Pang, J., Wang, Y., Lambers, H., Tibbetd, M., Siddique, K.H.M., Ryan, M.H., 2013. Commensalism in an
 629 agroecosystem: hydraulic redistribution by deep-rooted legumes improves survival of a droughted shallow-
 630 rooted legume companion. *Physiol. Plant* 149, 79-90.

631 Poorter, H., Ryser, P., 2015. The limits to leaf and root plasticity: what is so special about specific root length?
632 New Phytol. 206, 1188-1190.

633 Riggs, P.J., Chelius MK, Iniguez AL, Kaeppler SM, Triplett EW., 2001. Enhanced maize productivity by
634 inoculation with diazotrophic bacteria. Aust. J. Plant Physiol. 28, 829–836.

635 Rivas, R., Velázquez, E., Willems, A., et al., 2002. A new species of *Devosia* that forms a unique nitrogen-fixing
636 root nodule symbiosis with the aquatic legume *Neptunia natans* (L.f.) Druce. Appl. Environ. Microbiol. 68,
637 5217–5222.

638 Schulze, J., Drevon, J.J., 2005. P-deficiency increases the O₂ uptake per N₂ reduced in alfalfa. J. Exp. Bot. 56,
639 1779-1784.

640 Song, Y.N., Marschner, P., Li, L., Bao, X.G., Sun, J.H., Zhang, F.S., 2007. Community composition of ammonia-
641 oxidizing bacteria in the rhizosphere of intercropped wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), and
642 faba bean (*Vicia faba* L.). Biol. Fert. Soils 44, 307-314.

643 Sun, Y.M., Zhang, N.N., Wang, E.T., Yuan, H.L., Yang, J.S., Chen, W.X., 2009. Influence of intercropping and
644 intercropping plus rhizobial inoculation on microbial activity and community composition in rhizosphere of
645 alfalfa (*Medicago sativa* L.) and Siberian wild rye (*Elymus sibiricus* L.). FEMS Microbiol. Ecol. 70, 218–226.

646 Trenbath, B.R., 1993. Intercropping for the management of pests and diseases. Field Crops Res. 34, 381-405.

647 Tu, J.C., 1979. Evidence of differential tolerance among some root rot fungi to rhizobial parasitism *in vitro*.
648 Physiol. Plant Pathol. 14, 171–177.

649 Valverde, A., Igual, J.M., Cervantes, E., 2007. Polyphasic characterization of phosphate-solubilizing bacteria
650 isolated from rhizospheric soil of the north-eastern region of Portugal. E. Velázquez and C. Rodríguez-Barrueco
651 (eds.). First International Meeting on Microbial Phosphate Solubilization 102, 273–276.

652 Vance, C.P., 2001. Symbiotic nitrogen fixation and phosphorus acquisition. Plant nutrition in a world of declining
653 renewable resources. Plant Physiol. 127, 390-397.

654 Veneklaas, E.J., Stevens, J., Cawthray, G., R Turner, S., Grigg, A.M., Lambers, H., 2003. Chickpea and white lupin
655 rhizosphere carboxylates vary with soil properties and enhance phosphorus uptake. Plant Soil 248, 187-197.

656 Wang, W.X., Vinocur, B., Altman, A., 2003. Plant responses to drought, salinity and extreme temperatures:
657 towards genetic engineering for stress tolerance. *Planta* 218, 1-14.

658 Wang, Z.G., Bao, X.G., Li, X.F., Jin, X., Zhao, J.H., Sun, J.H., Christie, P., Li, L., 2015. Intercropping maintains
659 soil fertility in terms of chemical properties and enzyme activities on a timescale of one decade. *Plant Soil*
660 391, 265-282.

661 Xia, H-Y., Zhao, J-H., Sun, J-H., Bao, X-G., Christie, P., Zhang, F-S., Li, L., 2013. Dynamics of root length and
662 distribution and shoot biomass of maize as affected by intercropping with different companion crops and
663 phosphorus application rates. *Field Crops Res.* 150, 52–62.

664 Xiao, Y.B., Li, L., Zhang, F.S., 2004. Effect of root contact on interspecific competition and N transfer between
665 wheat and faba bean using direct and indirect ¹⁵N techniques. *Plant Soil* 262, 45–54.

666 Yanni, Y.G., Rizk, R.Y., Corich, V., et al., 1997. Natural endophytic association between *Rhizobium*
667 *leguminosarum* bv. *trifolii* and rice roots and assessment of its potential to promote rice growth. *Plant Soil* 194,
668 99–114.

669 Zhang, F., Shen, J., Zhang, J., Zuo, Y., Li, L., Chen, X., 2010. Rhizosphere processes and management for
670 improving nutrient use efficiency and crop productivity: implications for China. *AdvAgron* 107, 1–32.

Tables

Table 1 Dry weight (g), length (cm) and surface area (cm²) of roots of soybean bean (S) and wheat (W) grown in monocropping (S, W) or intercropping (SW-s, SW-w) under phosphorus sufficiency (high P) *versus* phosphorus deficiency (Low P) conditions. Data are means and SE of six replicates harvested at forty-fifth days after transplantation. Mean values labeled with the same letter were not significantly different at P<0.05. Means within a column (all traits between shallow and deep soil layers) followed by letter (x) are significantly different at P<0.05. CT, crop treatment; PT, P treatment; and their interaction CT ×PT.

Crop treatment		S		SW-s		W		SW-w		Treatments effect		
P treatment	Location	High P	Low P	High P	Low P	High P	Low P	High P	Low P	CT	PT	CT ×PT
Root traits										S/W	S/W	S/W
Dry weight	Total	0.54a	0.37b	0.44ab	0.56a	0.17d	0.25c	0.34b	0.44a	ns/***	ns/***	**/ns
	Shallow	0.30a	0.22a	0.22a	0.25a	0.1b	0.11b	0.16a	0.19a	ns/***	ns/*	ns/ns
	Deep	0.25ab	0.14d (x)	0.21bc	0.30a	0.07d (x)	0.11c	0.17b	0.25a (x)	*/ ***	ns/***	***/ns
Length	Total	986a	706b	1002a	956a	1071c	880d	1463b	1669a	ns/***	ns/ns	ns/***
	Shallow	394a	382a	349a	349a	372b	304c	637a	584a	ns/***	ns/*	ns/ns
	Deep	588a (x)	393b	632a (x)	607a (x)	699b (x)	575c (x)	826b (x)	1085a (x)	ns/***	ns/ns	ns/***
Surface	Total	57a	39b	52ab	54a	55b	38c	86a	91a	ns/***	ns/ns	*/**
	Shallow	24a	19a	20a	21a	20c	16d	36a	31b	ns/***	ns/**	ns/ns
	Deep	33a (x)	20b	32a (x)	33a (x)	34b (x)	23c (x)	50a (x)	59a (x)	ns/***	ns/ns	*/**

Significance levels: n.s.: P > 0.05; *P < 0.05; **P < 0.01; ***P < 0.001.

Table 2 Acid phosphatases activity and inorganic P concentration in roots, nodules, and rhizosphere soil of soybean bean (S) and wheat (W) grown in monocropping (S, W) or intercropping (SW-s, SW-w) under phosphorus sufficiency (high P) *versus* phosphorus deficiency (Low P) conditions. Data are means and SE of six replicates harvested at 45 days after transplantation. Mean values labeled with the same letter were not significantly different at P<0.05. Means within a column (all traits between shallow and deep soil layers) followed by letter (x) are significantly different at P<0.05. CT, crop treatment; PT, P treatment; and their interaction CT × PT.

Crop treatment		S		SW-s		W		SW-w		Treatments effect		
P treatment	Location	High P	Low P	High P	Low P	High P	Low P	High P	Low P	CT	PT	CT ×PT
<u>Acid phosphatase nmol g⁻¹ fresh weight min⁻¹</u>										S / W	S / W	S / W
Roots	Shallow	71a	79a	48b	51b	41bc	44b	38c	54a	*** / *	ns / ***	ns / **
	Deep	65ab	70a	56b	56b	49ab	62a (x)	38b	49ab	*** / ns	ns / ns	ns / ns
Nodules	Shallow	232c	369ab	257bc	382a					ns / -	** / -	ns / -
	Deep	384a (x)	347a	251b	312ab					* / -	ns / -	ns / -
Rhizosphere soil (10 ⁻³)		121a	129a	84b	106ab	100ab	124a	68b	91ab	* / *	ns / ns	ns / ns
<u>Inorganic P mg g⁻¹ FW</u>												
Roots	Shallow	0.57a	0.46b	0.56ab	0.29c	0.30ab	0.24bc	0.34a	0.18c	* / ns	*** / **	* / ns
	Deep	0.65a	0.55a	0.65a	0.27b	0.24a	0.21a (x)	0.22a (x)	0.20a	** / ns	*** / ns	** / ns
Nodules	Shallow	1.18a	1.23a	1.21a	0.73b					* / -	* / -	* / -
	Deep	1.70a (x)	1.26b	1.06b	1.12b (x)					*** / -	* / -	** / -
Rhizosphere soil (10 ⁻³)		2.34a	1.1b	1.66b	1.33b	1.07b	1.14ab	1.34a	1.2ab	ns / ns	** / ns	* / ns

Significance levels: n.s.: P > 0.05; *P < 0.05; **P < 0.01; ***P < 0.001.

Table 3Permutational multivariate analysis of variance (PERMANOVA) testing the effects of cropping treatment (CT, monocropped*vs.* intercropped), phosphorus treatment (PT, high P *vs.* low P), plant species (wheat (W)*vs.* soybean (S)), and the interaction of cropping × P treatments on the root microbial community composition as assessed using T-RFLP of 16S and ITS genes. Abbreviations: df, degrees of freedom; SS, sum of squares; MS – mean square

ITS- & 16S-T-RFLP	Df	SS	MS	F-value	R ²	<i>p</i> -value
ITS T-RFLP						
CT	1	0.58	0.58	1.86	0.08	0.0189
PT (high/low)	1	0.35	0.35	1.13	0.05	0.3036
Species (W/S)	1	0.48	0.48	1.54	0.06	0.0741
CT× PT	1	0.21	0.21	0.69	0.03	0.8282
Residuals	19	5.91	0.31		0.78	
16S T-RFLP						
CT	1	0.65	0.65	3.96	0.15	0.0030
PT (high/low)	1	0.26	0.26	1.61	0.06	0.1347
Species (W/S)	1	0.86	0.86	5.24	0.19	0.0004
CT× PT	1	0.37	0.37	2.27	0.08	0.0458
Residuals	14	2.29	0.16		0.52	

Table 4Phylogenetic distribution of 16S and ITS sequences belonging to most-abundant bacterial and fungal families of wheat and soybean bean as monocrop (W, S) or intercrop (SW-w, SW-s). Bacteria: Percentage of 16S sequences belonging to families that compose at least 5 % of the total community in each sample. Listed genera are the five most-abundant bacterial genera. Fungi: Percentage of ITS sequences belonging to the eight most-abundant fungal OTUs (identified to genus-level, when possible).

Family	Wheat		Soybean	
	W	SW-w	S	SW-s
Bacteria				
<i>Rhizobiaceae</i>	22.3	20.1	27.7	16.1
Unknown <i>Rhizobiaceae</i>	9.5	7.0	12.2	6.4
<i>Agrobacterium</i> spp.	7.7	6.5	8.5	5.1
<i>Hyphomicrobiaceae</i>	22.2	17.5	14.2	15.5
<i>Devosias</i> spp.	7.4	13.5	4.1	2.8
<i>Bradyrhizobiaceae</i>	7.5	10.0	15.3	17.5
Unknown <i>Bradyrhizobiacea</i>	3.3	5.0	8.4	8.1
<i>Comamonadaceae</i>	2.7	8.2	15.2	18.7
<i>Rubrivivax</i> spp.	0.3	1.8	4.6	6.8
<i>Pseudomonodaceae</i>	5.9	1.1	0.3	0.7
<i>Rhodospirillaceae</i>	0.2	0.8	0.3	1.1
Fungi				
<i>Chaetomium</i> spp.	52.3	0.5	6.4	4.0
<i>Basidiomycota</i> endophyte	25.4	0	0.2	3.1
<i>Alternaria</i> spp.	1.2	2.8	0.9	23.5
<i>Paraphoma</i> spp.	5.9	12.9	20.2	11.1
Unknown <i>Sordariales</i>	2.0	0.1	0.1	9.8
<i>Fusarium</i> spp.	0.6	0.7	1.7	8.9
Unknown endophyte	0	0	0	7.7
<i>Cercophora</i> spp.	1.5	0.2	0.3	10.9

Figure legends

Fig. 1 Total dry weight (g plant^{-1}) and the root dry weight and shoot dry weight (RDW : SDW) ratio of soybean bean (S) and wheat (W) grown in monocropping (S, W) or intercropping (SW-s, SW-w) under phosphorus sufficiency (open bars) *versus* phosphorus deficiency (dark bars) conditions. Data are means and SE of three pooled replicates (corresponding to three rhizoboxes) harvested at forty-fifth days after transplantation. For each species, mean values labeled with the same letter were not significantly different at $P < 0.05$.

Fig. 2 Concentrations of total N and P (mg g^{-1}) in shoots of wheat (W) and soybean (S) grown in monocropping (W, S) or intercropping (SW-w, SW-s) under phosphorus sufficiency (open bars) *versus* phosphorus deficiency (dark bars) conditions. Data are means and SE of three replicates harvested at forty-fifth days after transplantation. For each species, mean values labeled with the same letter were not significantly different at $P < 0.05$.

Fig. 3 Non metric multidimensional (NMDS) ordination of Bray-Curtis dissimilarity matrices of T-RF proportional abundance in roots of wheat (W: monocropping; SW-w: intercropping) and soybean (S: monocropping; SW-s: intercropping). Colors indicate plant species and shapes indicate cropping treatments. Phosphorus conditions (deficient and sufficient) are combined. Vectors indicate the subset of measured morphological and biochemical variables that had significant Spearman's correlations ($P < 0.05$) with taxa distribution according to Mantel tests. RDW: total root dry weight; R.Apase: root acid phosphatase activity; Soil P: soil available P; Shoot P: shoot P concentration; Shoot N: shoot N concentration.

Fig. 4 Chao1 diversity estimates for fungi and bacteria in in roots of wheat (W: monocropping; SW-w: intercropping) and soybean (S: monocropping; SW-s: intercropping). Phosphorus conditions (sufficient and deficient) are combined.

Fig.1

Fig.2

Fig. 3

Fig. 4