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Management of the mycorrhizal soil infectivity with *Crotalaria ochroleuca*, an indigenous wild legume in the tropics: Impacts on microbial functional diversity involved in phosphorus mobilization processes in a sahelian soil

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ABSTRACT

It is well known that the over exploitation of soil resources decreases the density and diversity of arbuscular mycorrhizal (AM) fungal propagules. It has been postulated that agricultural practices could promote the occurrence and functioning of remaining and resilient AM fungal ecotypes in order to benefit from AM associations. Using *C. ochroleuca*, a highly mycotrophic plant species, the aims of this study were to determine in controlled conditions, the impact of this legume species on: (i) the mycorrhizal soil infectivity (MSI), (ii) the Rock Phosphate (RP) solubilising activity and (iii) the soil microbial functions. The expected benefits of *C. ochroleuca* cultivation have been evaluated by comparing its biological influences with those recorded with a non-mycorrhizal plant species, *Brassica oleracea* in order to attest of the importance of the AM potential in RP weathering and soil functioning. The results show that: *C. ochroleuca* was effective in improving the mycorrhizal soil potential, (ii) functional abilities of soil microflora were influenced by the cultured plant species and the mycorrhizal soil potential and (iii) the efficiency of rock phosphate amendment on plant growth depended on the level of mycorrhizal soil potential and was linkedto the extent of the mycorrhizal network.The *C. ochroleuca* impact was significantly reinforced with KRP amendment (i.e. +29.3% for the Catabolic Eveness, +45.7% for the hyphal length). According to these results, it could be concluded that agricultural strategies based on the management of local biodiversity (i.e. the use of native legume species) could be considered as a sustainable practice to optimally manage AM fungi and to ensure their functionalities in order to maintain a sustainable production of food crops in the tropics.

1. Introduction

Many tropical soils are subjected to many environmental factors that limit food production in annual cropping systems (Wahbi et al., 2016a). The fragility of these soils is mainly due to low nutrient capital, erosion, moisture stress, high P fixation and low soil diversity (Cardoso and Kuyper, 2006). During the last decades, it has been recommended to use pesticides, synthetic fertilizers and high-yielding cultivars to overcome these limitations, to increase crop yield productivity and to improve nutrition (Wahbi et al., 2016b). Unfortunately, a billion people and many rural communities remain affected by a decline in household food production (Wahbi et al., 2016b). Moreover it has been demonstrated that the Green Revolution Technologies largely adopted in the last century could provide

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negative impacts on natural resources and public health (Wahbi et al., 2016b). The major goal for the next 50 years is to double food production without any degradation on the environmental properties. In natural conditions, tropical soils are known to harbour an important biological potential (Verbruggen et al., 2013). Since sustainable food production in the tropics is limited by soil degradation and fertility depletion, a key issue to restore their quality and sustainability is to promote soil biological potential though adequate management strategies in order to improve both plant development and soil quality (Fester and Sawers, 2011). In addition it has been suggested that imitating natural ecosystems rather than planting mono cultures was the best cultural strategy for the tropics by using adequate plant species diversity to maintain soil fertility (Fester and Sawers, 2011).

Ecosystem functioning is subjected to many chemical, physical and biological factors involved in a framework of interactions (Verbruggen et al., 2013). Soil microbiota is of particular importance for the maintenance of soil processes suchas the biogeochemical cycling of nutrients and matter, and to ensure plant health and soil quality (Richardson et al., 2009; Pereira and Castro, 2014). Among soil microorganisms, arbuscular mycorrhizal (AM) fungi are considered essential key components of sustainable soil-plant systems, particularly in semi-arid and arid ecosystems (Fester and Sawers, 2011). This symbiotic process mobilizes and transports nutrients to roots (Smith and Read, 2008), improves soil aggregation in eroded soils and reduces water stress (Ruíz-Lozano et al., 2008; Azcón and Barea, 2010).

The improvement of P nutrition of plants has been the most recognized beneficial effects of mycorrhizas (Smith and Read, 2008). As P is the element that usually limits crop production in the tropics, the main strategy to cope with P deficiencies has been the addition of fertilizers in the form of synthetic fertilizers or in the form of Rock Phosphate (RP). It has been showed that plants inoculated with AM fungiutilize more soluble phosphorus from RP than uninoculated plants (Manjunath et al., 1989; Antunes and Cardoso, 1991; Hafidi et al., 2013). The main explanation is that mycorrhiza developed an extramatrical mycelium that increased the root P absorbing sites (Bolan 1991; Ness and Vlek, 2000; Manaut et al., 2015). This mycorrhizal effecthas been frequently observed in RP amended soils and it induced spectacular stimulations of the plant growth and P foliar contents (Guissou et al., 2001).

The overexploitation of soil resources decreases the density and diversity of AM fungal propagules. However mycorrhizal propagules did not completely disappear and it has been postulated that agricultural practices could promote the occurrence and functioning of these remaining and resilient AM fungal ecotypes in order to benefit from AM associations (Fester and Sawers, 2011). For instance crop rotation (i.e. cereal/legume rotations) effects on mycorrhizal functioning have repeatedly been reported (Wahbi et al., 2016b).

Crotalaria species belong to the Fabaceae family, a highly mycotrophic legume species, are worldwide described, mostly from the tropics and at least 500 species have been recorded in Africa. Some of these *Crotalaria* species seem to be good candidates to be used in farming systems. It is also well known that *Crotalaria* species are highly mycorrhiza dependent (Germani and Plenchette, 2004). Unfortunately little is known on these indigenous wild legumes on their impact on soil functions and on RP weathering.

In the present study, one *Crotalaria* species, *C. ochroleuca*, was examined in controlled conditions for its impact on: (i) soil mycorrhizal potential, (ii) RP solubilising activity and (iii) soil microbial functions. The expected benefits of *C. ochroleuca* cultivation have been evaluated by comparing its biological influences with those recorded with a non-mycorrhizal plant species, *Brassica oleracea* in order to attest of the importance of the AM potential in RP weathering and soil functioning.

2. Materials and methods

2.1. Experimental design

Seeds of *C. ochroleuca* and *B. oleracea* were surface sterilized in 95% sulphuric acid for 30 min and in1% NaOCl for 15 min, respectively, rinsed with sterilized distilled water and germinated on 1% water agar at 25 °C in the dark.

A sandy soil was collected in an experimental station localized at Gampella (20 km from Ouagadougou, Burkina Faso), crushed and passed through a 2-mm sieve. Its physico-chemical characteristics were as follows: pH (H_2O) 5.6; clay (%) 4.6; fine silt (%) 0.0; coarse silt (%) 0.8; fine sand (%) 25.5; coarse sand (%) 69.1; carbon (%) 2.04; total nitrogen (%) 0.04; Olsen phosphorus 4.3 mg kg^{-1}; total phosphorus 116 mg kg^{-1}. This sandy soil was mixed with Kodjari Rock Phosphate (KRP) (Burkina Faso) (0.1%, w/v; insolublerock phosphate powder) in one treatment or remained un-amended in another. The KRP was ground with pestle and mortar and passed through a 90 mm sieve. Its chemical characteristics were as follows (%):Ca (%) 32.0; CO_2 (%) 1.0; K (%) 0.119; Na (%) 0.605; Mg (%) 1.06; Fe (%) 0.375; Al (%) 0.488; S (%)0.025; Cl (%) 0.043; F (%) 3.2; total P (%) 13.2 and soluble P(%) 0.032.Each plastic bag (1 dm^3) was filled with the soil mixture and planted with one pre-germinated seed of *C. ochroleuca*, of *B. oleracea* or remained unplanted (control treatment). The pots were arranged in a randomised complete block design with 9 replicates per treatment. They were placed in a glasshouse under natural light (daylight approximately 12 h, mean temperature 25 °C day).

After 4-month culture, plants were uprooted and the root systems gently washed. The oven-dried (1 week at 65 °C) of the shoots was measured. After drying, 1 g of leaf tissue from each plant was ground, ashed (500 °C), digested in 2 ml HCl 6 N and 10 ml HNO_3N andthen analysed by colorimetry for P (John 1970). The roots were cleared and stained according to the method of Phillips and Hayman (1970). They were placed on a slide for microscopic observation at ×250 magnification (Brundrett et al., 1985). About one hundred 1-cm root pieces were observed per plant. The extent of mycorrhizal colonization was expressed as the number of mycorrhizal root pieces/number of non-mycorrhizal root pieces × 100. Root nodules along *C. ochroleuca* root systems and induced by indigenous rhizobia were numbered and weighed. Then the dry weight of roots was measured (65 °C, 1 week). Soils collected from each pot were kept at 4 °C for further analysis.

2.2. Catabolic diversity of microbial communities in soil treatments

Microbial functional diversity in soil treatments was assessed by measurement of the patterns of *in situ* catabolic potential (ISCP) of microbial communities (Degens and Harris, 1997). Twenty six substrates, comprising a range of amino acids (L-asparagine, L-glutamic acid, L-serine, L-phenylalanine), carbohydrates (D-Glucose, D-Mannose, Sucrose), organic acids (ascorbic acid, citric acid, α-ketoglutaric acid, malonic acid, succinic acid, quinic acid, tartaric acid, uric acid, oxalic acid, formic acid, gallic acid, malic acid, DL-α-Hydroxybutyric acid, gluconic acid, α-ketobutyric acid), amines (L-glutamine, D-glucosamine) and one amide (succinamide), were screened for differences in SIR responsiveness between soil treatments. One g equivalent dry weight soil was mixed to each substrate suspended in 2 ml sterile distilled water in 10 ml bottles(West and Sparling, 1986). The amines, amides and amino acids were added at 15 mM, car-

bohydrates at 75 mM and carboxylic acids at 100 mM (Degens and Vojvodic-Vukovic, 1999). CO_2 production from basal respiratory activity in the soil samples was also determined by adding 2 ml sterile distilled water to 1 g equivalent dry weight of soil. After the addition of the substrate solutions to soil samples, bottles were immediately closed and kept at 28 °C for 4 h. CO_2 fluxes from the soils were measured using an infrared gas analyser (IRGA) (Polytron IR CO_2, Dräger) in combination with a thermal flow meter (Heinemeyer et al., 1989). Results were expressed as $\mu g\, CO_2\, g^{-1}$ soil h^{-1}. Catabolic evenness (E) that indicates the catabolism variability of a substrate among the range of tested substrates, was calculated using the Simpson-Yule index, $E = 1/p^2_i$ with p_i = [respiration response to individual substrates]/[total respiration activity induced by all substrates for a soil treatment] (Magurran, 1988). Microbial biomass C (MBC) was calculated with thesubstrate-induced respiration (SIR) method as describedby Sparling (1995). Oven-dried weight of soil (1 g) was suspended in 2 ml of 75 mM glucose solution in 10 ml bottles, sealed with a Vacutainer stopper and incubated at 25 °C for 4 h. After correction for CO_2 produced in bottles with only deionized water added, MBC was calculated asMBC ($\mu g\, C\, g^{-1}$ soil) = 50.4 × respiration rate ($\mu g\, C\, g^{-1}$ soil h^{-1}). The metabolic quotient (qCO_2) was calculated dividing the CO_2 basal respiration by the MBC content.

2.3. Assessment of the mycorrhizal soil potential

AM hyphal length was measured on membrane filters according to Jakobsen and Rosendahl (1990). The Mycorrhizal Soil Infectivity (MSI) was determined for each soil treatment. It was calculated with a bioassay based on a dose (quantity of a non disinfected soil) – response (mycorrhizal status of test plants) according to the biological assay principle (Plenchette et al., 1989). The method involved cultivation of a population of mycotrophic plantlets on a range of concentrations of natural soil diluted with the same disinfected soil. Six dilutions of each soil samples were carried out by mixing the original soil in various quantities (100, 48, 24, 12, 6 and 3%, w:w) with the same autoclaved soil (140 °C, 40 min) to give a range of concentrations. There were 5 replicates per dilution. Seeds of millet (*Pennisetum typhoides* L.) were pre-germinated for two days in Petri dishes on humid filter paper. Ten germinated seeds were transplanted into plastic pots (5.5 cm diameter; 6 cm high) filled with 100 g of each dilution. Pots were placed in a glasshouse (30 °C day, 20 °C night, 10-h photoperiod) and watered daily with tap water. After 2 weeks culture, the entire root system of each seedling was collected, gently washed under tap water, clarified in 10% KOH for 30 min at 90 °C and stained for 15 min with acid fuchsin (0.05% in lactoglycerol). Each entire root system was mounted on a microscope slide and checked at a 250× magnification for the presence of endomycorrhizal structures. A single arbuscular mycorrhizal hyphal entry was considered as a record of mycorrhizal infection to give an all or nothing quantitative response. The infected plants were counted and the results were expressed as percentages of mycorrhizal plants per pot.

For each soil treatment, the percentage of mycorrhizal plants was plotted against the logarithm of undisinfected soil concentration. Regression curves (model $Y = BX + A$) were calculated for each soil treatment and variance analysis was performed to test the non-equality of their slope. The Mycorrhizal Soil Infectivity (MSI) unit was calculated using a regression line equation (Duvert et al., 1990) and defined as the minimum dry weight (g) of soil required to infect 50% (MSI_{50}) of a plant population under the bioassay conditions and calculated for Y = 50%.

Table 1
Growth, leaf mineral content, mycorrhizal colonization and nodule formation of *C.ochroleuca* and *B. oleracea* in the soils amended or not with Kodjari Rock Phosphoate (KRP) after 4 months of cultivation in glasshouse conditions.

	C. ochroleuca		*B. oleracea*	
	– KRP	+ KRP	– KRP	+ KRP
Shoot biomass (mg dry weight)	163.5 a [a]	582.2 b	412.4 a	706.7 b
Root biomass (mg dry weight)	11.6 a	26.8 b	23.4 a	39.4 b
Total biomass (mg dry weight)	175.1 a	609.1 b	435.8 a	746.1 b
Leaf P content ($\mu g\, g^{-1}$)	0.043 a	0.487 b	0.035 a	0.154 b
AM colonization (%)	16.7 a	53.3 b	0	0
Number of nodules per plant	3.7 a	4.6 a	0	0
KRP amendment efficiency (%)[b]	–	71.2 b	–	41.6 a

[a] For each plant species, data in the same line followed by the same letter are not significantlydifferent according to the Newman &Keuls test ($p < 0.05$).
[b] Growth efficiency of KRP amendment was determined as follow: [(Total biomass of plants growing in KRP amended soil) – (Total biomass of plants growing in non amended soil)/(Total biomass of plants growing in KRP amended soil)] × 100.

2.4. Statistical analysis

Data were treated with one-way analysis of variance.Means were compared using the Newman-Keul's test ($p < 0.05$). The patterns of ISCP of microbial communities from soilsamples submitted to the six treatments (control with or without KRP amendment, *C. ochroleuca* with or without KRP amendment, *B. oleracea* with or without KRP amendment) were analyzed using the between-group analysis (BGA, Dolédec and Chessel, 1989; Culhane et al., 2002). BGA is an ordination method considered as a robust alternative to the discriminant analysis (Huberty, 1994). A permutation test (Monte-Carlo method)was used to check the statistical significance of the between group differences. BGA computations were performed with the free ADE 4 software (Thioulouse and Dray, 2007). The percentages of the mycorrhizal colonization were transformed by arcsin (sqrt) prior statistical analysis. For the assessment of the mycorrhizal soil infectivity, relationships between the extent of AM colonization and soil dilutions were fitted by covariance analysis models. Covariance analysis allowed comparing theslopes of the regression model for each treatment (Control, *C. ochroleuca* and *B. oleracea,* with or without KRP amendment).The *p*-values for model fits were calculated byANOVA (Venables and Ripley, 2002). Computations were performed with the R software (R Development Core Team, 2010).

3. Results

After 4-months culture and for both plant species, the shoot and root biomasses, the leaf P content were significantly higher in the KRP treatments than in the non-amended soil with the Rock phosphate (Table 1). The efficiency of KRP amendment was significantly higher with the legume species than with *B. oleracea* (Table 1). Root mycorrhizal colonization was only found with *C. ochroleuca* as well as root nodules (Table 1).

Soil microbial biomass increased after KRP amendment into the soil for all the treatments (Table 2). Without KRP amendment, soil microbial biomasses ranged among the treatments as follows: *C. ochroleuca* > *B. oleracea* > control (Table 2). The specific respiration rate of the soil microbial biomass (qCO_2) was significantly higher in the soil collected under *C. ochroleuca* and KRP amended (Table 2). The hyphal network was more important in the soil samples from the *C. ochroleuca* treatments than in the other treatments and the highest length was recorded when KRP was added to the soil (Table 2). The MSI_{50} was significantly lower in the *C. ochroleuca* treatments than those measured in the control and in the *B. oleracea* treatments (with or without KRP amendment) (Table 3).The positive effect of *C. ochroleuca* on the mycorrhizal propagule abun-

Table 2
Microbial biomass, hyphalmycorrhizal length and microbial functionalities (Catabolic evenness and average SIR responses) of the soils cultivated with *C. ochreuleuca*, *B. oleracea*or non-planted and amended or not with Kodjari Rock Phosphoate (KRP) after 4 months of cultivation in glasshouse conditions.

	Control (non planted soil)		*C. ochroleuca*		*B. oleracea*	
	− KRP	+ KRP	− KRP	+ KRP	− KRP	+ KRP
Microbial biomass (μg C g^{-1} soil) (MBC)	314.6 a [a]	822.8 b	956.1 b	2280.1 c	426.3 a	1007.6 b
Hyphal length (m g^{-1} soil)	1.1 a	1.2a	1.9 b	3.5 c	1.3 a	1.1 a
qCO_2 (μg-C-CO_2 g^{-1} MBC h^{-1})	0.12 a	0.12 a	0.15 a	0.20 b	0.11 a	0.12 a
Catabolic eveness	12.8 a	13.1 a	16.2 b	22.9 c	14.8 ab	13.9 ab
Carboxylic acids	26.57 a	24.21 a	34.33 a	54.07 b	25.83 a	48.73 b
Amino-acids	8.5 a	8.9 a	18.5 b	41.7 c	9.7 a	36.6 bc
Amines	8.5 a	8.9 a	18.5 b	41.7 c	9.7 a	10.6 a
Amides	9.8 a	13.1 b	38.1 d	49.9 d	22.9 c	27.4 c
Carbohydrates	46.1 a	53.6 b	91.1 c	108.6 d	39.7 a	49.2 ab

[a] Data in the same line followed by the same letter are not significantlydifferent according to the Newman & Keuls test ($p < 0.05$).

Table 3
Substrate-Induced Respiration (SIR) responses (μg CO_2 g^{-1} soil h^{-1}) with carboxylic acids known to be involved in the process of phosphate solubilization in the soils cultivated with *C. ochreuleuca* or *B. oleracea* and amended or not with Kodjari Rock Phosphoate (KRP) after 4 months of cultivation in glasshouse conditions.

	Control (non planted soil)		*C. ochroleuca*		*B. oleracea*	
	− KRP	+ KRP	− KRP	+ KRP	− KRP	+ KRP
Citric acid	6.2 a[a]	3.5 a	3.5 a	55.3 c	20.7 b	28.4 b
Malonic acid	6.0 ab	1.7 a	10.3 b	12.9 c	13.3 c	15.8 c
α-Hydroxybutyric acid	13.9 a	11.5 a	14.5 a	29.5 b	40.8 c	55.6 c
Malic acid	5.9 a	7.6 a	17.4 b	31.6 c	4.8 a	20.9 b
Oxalic acid	20.4 a	33.6 b	44.1 b	86.5 c	17.5 a	39.6 b
Tartaric acid	5.9 a	2.6 a	6.4 a	20.8 b	5.5 a	19.7 b
Gluconic acid	5.7 a	2.5 a	2.9 a	18.8 b	2.9 a	13.3 b
α-Ketobutyric acid	37.7 b	23.8 a	72.1 c	55.2 c	32.6 ab	68.9 c

[a] For each plant species, data in the same line followed by the same letter are not significantlydifferent according to the Newman &Keuls test ($p < 0.05$).

Table 4
Relationships between soil dilutions and percentage of mycorrhizal plantlets of *Pennisetumtyphoides* and values of the MSI_{50} units.

Treatments	*Y*-intercept	Regression coefficient	MSI_{50} units (g per 100 g)
Control (non planted soil) − KRP	0.137 a[a]	0.61	>100d
Control (non planted soil) + KRP	0.207 b	0.54	73.3d
C. ochroleuca − KRP	0.326 c	0.72	32.2b
C. ochroleuca + KRP	0.409 c	0.74	14.2 a
B. oleracea − KRP	0.252 b	0.67	60.5 cd
B. oleracea + KRP	0.281 b	0.62	59.2 c

[a] Data in the same columnfollowed by the same letter are not significantlydifferent ($p < 0.05$).

dance was significantly reinforced with KRP amendment (Table 3). This result suggests that *C. ochroleuca* cultivation has promoted the multiplication of infective mycorrhizalpropagules into the soil (Table 4).

In the un-amended soils, plant cultivation has significantly enhanced the soil catabolic evenesses (Table 2). Compared to the control (non-planted soil), average SIR responses with amino-acids, amines, amides and carbohydrates were significantly higher in the *C. ochroleuca* treatment whereas these significant effects were only recorded with amides and carbohydrates with the *B. oleracea* treatment (Table 2). In the KRP amended soils, the catabolic evenness was significantly higher in the *C. ochroleuca* soil than in the other treatments (Table 2). In the control treatment, KRP amendment had significantly enhanced the average SIR responses with amides and carbohydrates (Table 2). After *C. ochroleuca* cultivation, KRP amendment has significantly promoted average SIR responses with carboxylic acids, amino-acids, amines and carbohydrates, whereas with *B. oleracea*, this positive impact was only recorded with carboxylic acids and amines (Table 2).

The permutation test of BGA showed that the six treatments gave very different SIR profiles ($P < 0.001$). The six treatments were very well separated, with the soils cultured with *C. ochroleuca* or *B. oleracea* without KRP amendment on the left and on the right, the soils influenced by both species but not amended with the Rock phosphate (Fig. 1b). For the planted treatments, the KRP amendment has induced higher respirations with all the tested substrates whereas this effect was not recorded with the control treatment (non-planted soil) (Fig. 1a). Most of the substrates were preferentially used in samples collected from the planted treatments amended with KRP. The substrates preferentially used in the *Crotalaria* treatments and in the non-planted soil amended with KRP were mannose, sucrose, aspartic acid and glucose whereas they were malonic acid, citric acid and α-OHbutyric acid in the *Brassica* treatments and in the non-planted soil without KRP (Fig. 1b).

In the un-amended soil treatment, the highest SIR responses with carboxylic acids were recorded with the malic and α-ketobutyric acids for *C. ochroleuca*, and with the citric, malonic and α-OH-butyric acids for *B. oleracea* (Table 3).In the control treatment, the KRP amendment significantly enhanced SIR responses with oxalic acid and decreased that with α-OH butyric acid. With *C. ochroleuca*, mixing the rock phosphate with the soil led to an improvement of all SIR responses except with α-OH butyric acid whereas, in the *B. oleracea* treatment, KRP amendment improved SIR responses with malic acid, oxalic acid, tartaric acid, gluconic acid and α-OH butyric acid (Table 3).

4. Discussion

From the results of this study, the following three main points deserve discussion:

(i) Whether *C. ochroleuca* was effective in improving the mycorrhizal soil potential,
(ii) Whether functional abilities of soil microflora was influenced by the cultured plant species and the mycorrhizal soil potential and

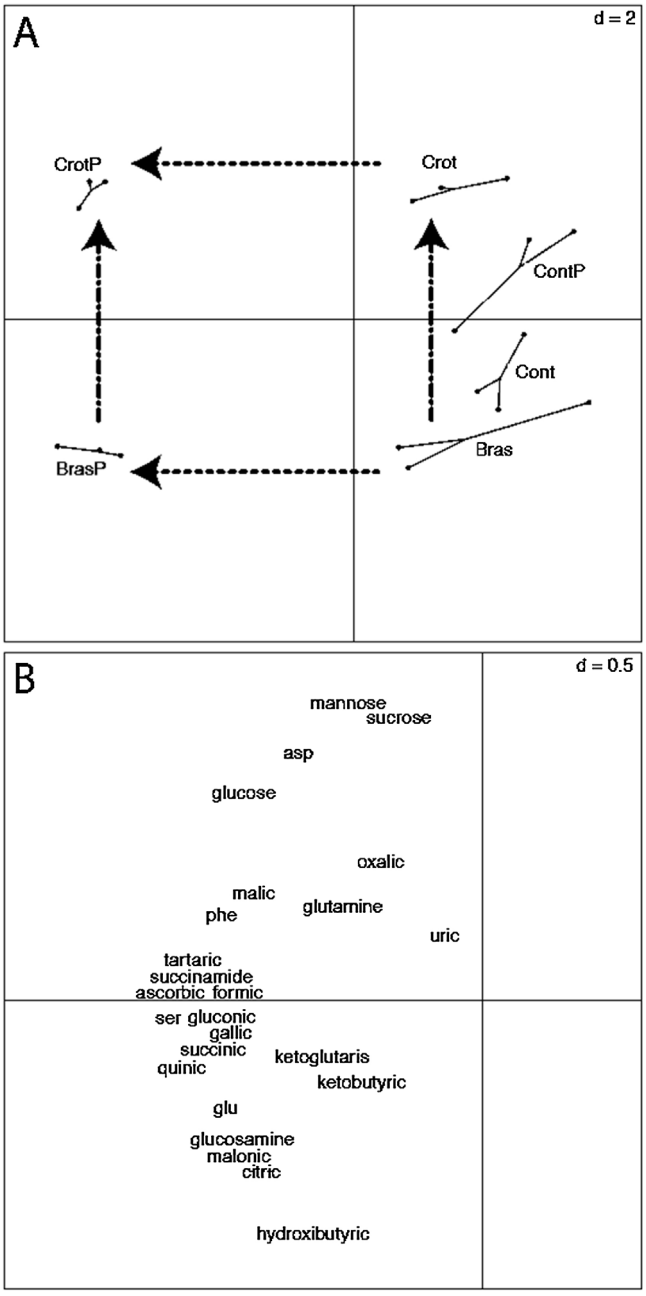

Fig. 1. Between-group analysis (BGA) of the SIR responses with respect to the soil treatments. Un-planted soil without (Cont) or with KRP amendment (ContP), soil cultivated with *B. oleracea*without (Bras) or with KRP amendment (BrasP), soil cultivated with *C. ochroleuca* without (Crot) or with KRP amendment (CrotP).

(iii) Whether the efficiency of rock phosphate amendment on plant growth depended on the level of mycorrhizal soil potential and more particularly on the extent of the mycorrhizal network.

Crotalaria spp. are known to be mycorrhizal plants (Colozzi and Cardoso, 2000). Using *G. intraradices* as AM fungal inoculant, Germani and Plenchette (2004) showed that with 17 *Crotalaria* species, all were highly colonized by the AM fungus and that mycorrhizal inoculation provided significant enhancements of phosphorus content in the shoot tissues and plant growth. For instance the relative mycorrhizal dependency of *C. ochroleuca* was of 66% showing that this plant species was highly mycorrhiza dependent for its development. In addition they demonstrated that mycorrhizal soil infectivity was maintained after cultivation of *Crotalaria* species inoculated with AM fungi. In soils, AM fungi are found as spores, hyphae or infected root pieces and all these propagules are sources of fungal inoculum (Duponnois et al., 2001). The extraradical mycelium is though to be the main source of mycorrhizal infective propagules (Sylvia and Jarstfer, 1992). In the present study,*C. ochroleuca* cultivation has significantly improve the mycorrhizal soil potential and, more particularly, the extent of the mycorrhizal network compared to *B. oleracea*, a non mycorrhizal plant species that did not exert any effect on AM fungal development. These mycelial networks are of particular importance in stressful environments (Barea et al., 2005). The soil colonization of the mycelial networkincreases the ability of the plants for the acquisition of water and mineral nutrients (more particularly those with a poor mobility or in low concentration in the soil solution) (Smith and Read, 2008). Hence and from the present study, the results show that *C. ochroleuca* cultivation led to a significant improvement of the mycorrhizal soil infectivity by promoting in particularthe development of the mycelial network that is considered as a main component in the AM symbiosis functioning (Van der Heijden et al., 1998).

The AM symbiosis influences the structure and functioning of surrounding bacterial communities (Marshner et al., 2001; Marshner and Timonen, 2005) by modifying root functions (i.e. root exudation) that involves a selective influence onrhizosphere populations (Andrade et al., 1998). As microbial communities around AM rootsdiffer from those of the rhizosphere, this microbialcompartment is commonly named “mycorrhizosphere”(Wahbi et al., 2016a,b). Two different zones are usually distinguished in the mycorrhizosphere, one under the dual influence ofroot and fungal partners (mycorrhizosphere) and the other influencedby the AM hyphae (the mycosphere and hyphosphere) (Marshner and Timonen 2005). In addition, fungalactivities could also modify the functional diversity of microbialcommunities (Schreiner et al., 2003; Sanon et al., 2006; Ouahmane et al., 2006).Soil microbial activities are involved in numerous soil biological processes such as decomposition, nutrient transformations, plant growth promotion/suppression and modification of soil physical processes (Wardle et al., 1999). Measuring catabolic response profiles can provide a pattern of this microbial functional diversity (Degens et al., 2000). It is well known that plant species greatly influence the characteristics of soil microflora (abundance, genetic and functional diversity) (Marshner et al., 2001). Our results are in accordance with these previous studiesby reporting different catabolic response profiles with the soils cultivated with two different plant species.

KRP amendment has induced strong modifications in the catabolic response profiles among the treatments.Specificplant species are known for their ability to chemically alter P speciation in the rhizosphere and mobilize soluble P (Pypers et al., 2005). The main mechanisms resulting in enhanced utilization of rock phosphate (RP) are acidification of the rhizosphere (Hinsinger and Gilkes, 1997) and exudation of organic acids (Hoffland, 1992). It has been also demonstrated that RP amendment could promote the establishment of mycorrhizal symbiosis in the cultural soil(Cardoso and Kuyper, 2006). Our results are in accordance with this RP influence on mycorrhiza with, in particular, a higher development of the hyphal network in the *C. ochroleuca* treatment. In addition, the efficiency of the inorganic phosphate amendment (impact on plant growth and on P nutrition) was larger with the legume species than with the non-mycotrophic plant species (*B. oleracea*). It has been demonstrated that the hyphal network is largely involved in the solubilization processes by contributing to phosphatase activity in the root-free soil though direct exudation of phosphatases into the soil (Joner and Johansen, 2000). It has been also demonstrated that P solubilising ability of AM fungi could result from a multitrophicmicrobial association including hyphosphere and mycorrhizospherecommunities which were selected by

AMfungi and potentially beneficial to the plant growth and thefungal symbiosis (Ouahmane et al., 2007). This multitrophicmicrobial association could be also involved in theprocess of mineralization of P from inorganic material.

In the present study, a significant correlation has been calculated between the length of hyphal network and the soil catabolic eveness ($R^2 = 0.75$. $p < 0.001$). As it hasbeen related that microbial catabolic diversity in soils with lowcatabolic evenness was less resistant to stress or disturbancethan in soils with high catabolic evenness (Degens et al., 2001), the better development of the hyphal network could also contribute to a better resistance of the soil microbiota to natural or anthropogenic impacts.

The present study confirms that the AM symbiosis contributes to the stability of the soil microbial functionalities and has an important role in the processes of inorganic phosphate solubilization. It also argues that it is possible to promote the mycorrhizal potential through a high mycotrophic plant species such as *C. ochroleuca*. However and since it has been reported that different mycorrhizal ecotypes are more beneficial tosome plant species than others (Sanders, 2002) and that an extensive extra-radical mycelium allows a more efficient exploitation of soil nutrients and water (Allen, 2007), emphasis has to be on agricultural practices that promote the occurrence and functioning of AM symbiosis. It has been suggested that the best agricultural strategy is to imitate natural ecosystems. Hence fundamental and technical studies are need to (i) determine the mycorrhizalstatus and dependency of the native plants from the cultural areas and (ii) to identify the composition of mixtures of native plant species that usually allow a larger diversity and/or abundance of AM fungi.

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