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Soil microbial functional capacity and diversity in a millet-shrub intercropping system of semi-arid Senegal

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ABSTRACT

A few species of shrubs grow with dryland row crops in farmers' fields throughout the Sahel and can significantly increase crop yield. The presence of shrub roots and litter inputs should have implications for soil nutrient pool sizes but there is limited information on the interactions of these shrubs with microbial communities involved in biogeochemical processes. Therefore, the objective of this study was to determine the microbial composition and functional capacity of soil from the rooting zone of pearl millet (*Pennisetum glaucum*) grown in the presence or absence of the shrub *Piliostigma reticulatum* in Senegal. Soil samples were collected from a long-term field study where millet was cultivated alone or intercropped with *P. reticulatum* with annual incorporation of coppiced shrub residues. Higher nutrient contents and distinct differences in microbial communities (DGGE profiles) were found between soils from beneath the canopy compared to soil outside the influence of shrubs. The catabolic response profile (MicroResp™) showed that the soil microbial community at both shrub and non-shrub sampling locations, metabolized a wide range of substrates. Trehalose that can work as a signaling molecule was more rapidly degraded in the rooting zone of millet growing in the presence of *P. reticulatum* over millet alone. Urease, arylsulfatase and dehydrogenase activities in the millet root zone soil were higher when intercropped with *P. reticulatum* which indicates enhanced potential of biogeochemical processes to proceed in the presence of this shrub. It is concluded that the native shrub *P. reticulatum* promotes a more diverse and active microbial community in the rooting zone of millet and further indicates greater potential to perform decomposition and mineralize nutrients.

1. Introduction

Several indigenous perennial woody shrubs in the semi-arid landscape of West Africa are found in farmers' fields to various degrees but are largely unmanaged and historically unrecognized as beneficial for dryland crops. However, recent research has found two species, *Guiera senegalensis* and *Piliostigma reticulatum* to promote crop growth and improve soil quality (Dossa et al., 2009, 2013). In cropped fields these species are ecologically similar to woody species in unmanaged semi-arid environments which through litter inputs and root activity/turnover create "fertility islands" (Ridolfi et al., 2008). This effect is now more broadly termed "resource islands" because of the array of beneficial services such as improved water relations (Kizito et al., 2012), nutrient pools and availability, microbial habitat, and soil physical properties (Hernandez et al., 2015; Diedhiou et al., 2009; Dossa et al., 2009).

An important discovery of shrubs in semi-arid environments is their ability to perform hydraulic lift, also known as hydraulic redistribution (Caldwell et al., 1998) which can improve soil water relations in the upper soil profile (Kizito et al., 2006, 2012). Hydraulic redistribution occurs at night when stomata close and allows movement of water through roots along a water potential

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gradient from the wet (high potential) subsoil to dry (low potential) surface soil (Scholz et al., 2002; Kizito et al., 2012). Hydraulic redistribution provides a continuous water supply that maintains microbial diversity and activity (Diédhiou et al., 2009, 2013) and drives biogeochemical processes (Dossa et al., 2009) year around which does not happen for soil outside the influence of woody species in the dry season.

In Senegal, *P. reticulatum* is found to varying degrees in farmers' fields (Lufafa et al., 2008) and traditionally the shrub stands are largely unmanaged except for coppicing and burning aboveground residues in the spring, prior to the planting of row crops. An alternative, non-thermal management of shrub residues includes pruning and mulching and can improve crop productivity significantly (Dossa et al., 2013). The shrub-based cropping system promotes carbon storage and nutrient cycling, and higher moisture in soil that results in improved cereal yields (Dossa et al., 2009, 2013; Lahmar et al., 2012; Lufafa et al., 2008). Previous studies showed a shift in microbial communities beneath the shrub compared to soil outside the influence of this shrub (Diedhiou et al., 2009, 2013).

However, little information is available on the *P. reticulatum* intercrop system relative to direct effects on the millet (*Pennisetum glaucum* (L.) R. Br.) rhizosphere community composition and functional diversity. We hypothesized that the soil in the rooting zone of millet growing in the presence of *P. reticulatum* would have a more diverse and active soil microbial communities than millet alone. Therefore, the objective of this study was to characterize the genetic diversity and functional capacity of soil microorganisms in the millet root zone growing in the presence or absence of the shrub.

2. Materials and methods

The experimental site was near Nioro, Senegal, West Africa which is in the southern region of the Peanut Basin (13°45 N, 15°47 W) at 18 m above sea level. The two major crops cultivated at the site are millet (*Pennisetum glaucum* (L.) R. Br.) and peanut (*Arachis hypogaea* L.). The mean annual precipitation is 750 mm and mainly comes between July and September. The mean air temperatures range from 20.0 to 35.7 °C.

The soil is a Deck-Dior (Badiane et al., 2000) loamy-sand [fine-sandy, mixed Haplic Ferric Lixisol (FAO, 2006)], a leached ferruginous tropical soil. Top soil (0–10 cm) has sand content of >90%, organic matter and total N contents of 0.52 and 0.03% respectively, total P content of 70 mg kg^{-1}, and mean pH (water) of 6.2.

A field of approximately 0.5 ha with pre-existing *P. reticulatum* shrubs that had been under local farmer management for at least the last 50 years was selected. The site had been cropped continuously with a peanut (*A. hypogaea*) - millet (*Pennisetum glaucum*) rotation prior to the experiment, but left fallow for three years prior to starting the experiment. The experimental design was a split-plot with presence or absence of shrub as the main plot and fertilizer rate as the subplot with four replicates. The main plots were established in the winter of 2003 by manually removing existing shrubs from no shrub plots. For the plus shrub plot existing shrubs were augmented with seedlings that resulted in density of 888–1555 shrubs ha^{-1}. Shrubs were randomly but relatively evenly distributed. Main plot sizes were 46 × 4.5 m and subplot sizes were 10 × 4.5 m. There was a 2-m gap between sub-plots and 3-m gap between main blocks. All plots had a crop rotation of peanut (*A. hypogaea* var 55–437) and millet (*P. glaucum* var Souna 3) since 2004 following local farmer practices in the region.

The subplot fertilizer treatments were 0, 0.5, 1.0 or 1.5 times the recommended fertilizer rate for each crop. The plots used for this study were the zero fertilizer plots (for details of crop management see Dossa et al., 2013). The treatments (four replications) sampled from this experiment were the no shrub plots (millet alone) (M), plus shrub plots (millet plus shrub) (M + S) both of which received no fertilizer. Two additional treatments with four spatially separated replications were randomly selected adjacent to the experimental site which was shrub alone (S) (canopy diam. ~2 m similar to S treatment shrubs but had not been cropped for >10 years) and a control (C) bare soil (5 m^2 each), absent of shrubs and crops for >10 years. So, neither the S or C treatments had millet growing prior to or during soil sampling. All treatments had four replications.

The M + S treatment was annually coppiced in the spring prior to crop planting following the local farmers' practices. The coppiced biomass was chopped to approximately 5 cm length and then applied as a mulch on the soil surface of the plot from which it was harvested. The subsequent shrub regrowth during the cropping season was coppiced and placed on the surface of the soil. At the end of the growing season the shrubs were allowed to regrow and continued to produce aboveground biomass over the dry season. The S treatment was not coppiced and had year around foliage, more or less as an evergreen and some litter fall. No fertilizer was applied to any of the treatments.

The millet grain yields in 2011 were 500 kg ha^{-1} in millet plots (M) and 886 kg ha^{-1} when millet was associated with *P. reticulatum* (M + S).

Soils samples were collected in August 2011 when millet panicles were emerging in the M and M + S plots. Ten soil cores (3 cm in diameter) (10 cm depth) were randomly sampled in the C treatment soil; shrub root zone of the S treatment; and the millet root soil of the M and M + S treatments. The cores were homogenized to make a composite sample per treatment, placed in a cooler, and transported back to the lab where they were stored at 4 °C for a maximum of 5 days before analysis.

2.1. Soil chemical analyses

Soil moisture was determined gravimetrically by drying fresh soil samples at 105 °C for 48 h. Total carbon and nitrogen were determined on an elemental analyzer (Flash EA 1112 series, ThermoFinnigan, France) and total phosphorus by colorimetry after acid digestion (Dabin, 1967). Soil pH was measured in 1:2.5 soil-to-water suspensions. Soil inorganic N content was determined colorimetrically from KCl 1 M extracts by flow injection analysis according to the method of Bremner (1965).

2.2. Microbial community characterization

Soil DNA was extracted from 0.25 g soil using the soil Fast DNA SPIN Kit (MP Biomedicals, Santa Ana, CA) following the manufacturer's instructions. The bacterial community diversity was determined by the PCR-DGGE method based on the V3 region of the 16S rDNA gene while the internal transcribed spacer (ITS) region was used for the fungal community characterization.

Touchdown PCR targeting total soil 16S rDNA bacterial community was performed with bacterial primers pair, 338f GC and 518r, as described by Assigbetse et al. (2005). PCR was done on a gene Amp PCR System 2400 thermal cycler (Applied Biosystems, USA) using pure Taq Ready-To-Go beads (Amersham Biosciences), 2.5 ng of DNA, and 1.25 μL of each primers (20 μM) in 25 μL PCR mixtures. The thermocycling regime was: 5 min at 94 °C (Denaturation), 1 min at 65 °C (Annealing), and 1 min at 72 °C (elongation) with a 0.5 °C touchdown every cycle during annealing for 20 cycles, followed by 10 cycles with annealing 55 °C, and a final cycle of 10 min at 72 °C. PCR products of 200 pb were generated after amplification and were loaded on agarose gel 1.5% (w/v) and stained for 30 min with ethidium bromide (1 mg L^{-1}).

The ITS region for the fungal community was targeted using a nested PCR approach to enhance the sensitivity as described by Hernandez et al. (2015). For the first round PCR, the primers ITS1F and ITS4 were used for this amplification. PCR was carried out with pure Taq Ready-To-Go beads (Amersham Biosciences) with 2.5 μL of each primers and 2.5 ng of DNA. Amplification was performed in GeneAmp PCR System 2400 thermal Cycler (Applied Biosystems, USA). The 25 μL of mixture were submitted to 95 °C for 5 min (Denaturation) was followed by 30 cycles of 95 °C for 30 s, 55 °C for 30 s (annealing), 72 °C for 1 min (elongation) and a final extension at 72 °C for 10 min. DNA was excised and eluted in 100 μL of deionized water over night at 4 °C. Second PCR was performed in a final volume of 25 μL using ITS1F-GC and ITS2 primers. PCR was carried out with 2.5 μL of each primers (25 μM) and 1 μL of the first PCR amplification served as template for the second reaction following this program: 95 °C for 5 min (initial denaturation), followed by 30 cycles of 95 °C for 30 s, 55 °C for 30 s (annealing), 72 °C for 1 min (annealing) and a final extension at 72 °C for 10 min. PCR products were separated by gel electrophoresis on 1.5% (w/v) agarose and DNA was visualized after staining with ethidium bromide (1 mg L^{-1}). The PCR products were quantified and denaturing gradient gel electrophoresis (DGGE) performed in 8% acrylamide/bis-acrylamide [40% (37.5: 1 v/v)] gels with a denaturant gradient ranging from 45 to 70% and 22–58% for the bacterial and the fungal communities respectively. Electrophoresis were performed in TAE 1× buffer at 60 °C at constant voltage of 100 V for 18 h using IngenyPhorU system (Ingeny, Goes, The Netherlands). The gels were stained for 30 min with ethidium bromide (1 mg L^{-1}) and washed for 10 min. The banding patterns were then digitized using a VilberLourmat gel imaging system (VilberLourmat, France). Band detection and their intensity quantification were performed using Phoretix 1D Software V10 (Nonlinear Dynamics, Newcastle upon Tyne, UK).

2.3. Microbial catabolic capacity using MicroResp™

The MicroResp™ method was done to determine the ability of the soil microbial community to metabolize a range of carbon sources (Campbell et al., 2003). The method was followed as described by Campbell et al. (2003) and what follows is a brief description of the procedures. Field moist soil samples were sieved at 2 mm to remove coarse plant debris and stones before analysis. Soil was placed in a 96-deep-well micro-plate by filling 96 holes of container with soil which a piece of paper beneath it that was pulled out, allowing soil to fill each well and then all wells were leveled. The weight of the microplate plus soil was recorded and subtracted from the microplate tare weight. Water was added to each soil sample to reach a water potential of −0.01 MPa (pF2).

Each well received separate organic substrates which were carbohydrates (D-glucose, saccharose, trehalose, cellulose, maize dextrin), carboxylic acids (oxalic acid, malic acid), phenolic acid (protocatechuic acid), amino acid (alanine), and proteins (bovine serum albumin, known as BSA). These carbon sources are ecologically relevant as: components typically found in or added to soils such as plant residues, root exudates, trehalose (induces desiccation tolerance in plants), and as sources of mineralized nutrients (Campbell et al., 1997). The control was water and soil only to determine basal respiration. The catabolic profiling was carried out with 4 replicates per substrate per soil sample. The deep-well micro-plates were sealed individually to a colorimetric CO_2-trap microplate and incubated in the dark at 29 °C for 6 h. CO_2-trap absorbance was measured at 570 nm with a Victor3 multilabel counter (PerkinElmer) immediately before and after 6 h incubation. A calibration curve of absorbance versus headspace equilibrium CO_2 concentration was fitted to regression model. Values were expressed in μg C–CO_2 $gsoil^{-1}$ h^{-1} (Campbell et al., 2003).

Glucose-induced respiration was assumed to be proportional to active microbial biomass using the conversion factor of 40 of Anderson and Domsch (1978).

2.4. Enzyme activities

β-glucosidase was assayed following the method of Hayano (1973), with minor modifications. Briefly 100 mg of fresh soil were placed in a centrifuged flask and the substrate, 5 mM para-nitrophenyl β-D-glucopyranoside (Sigma) was added in a citrate phosphate buffer (pH 5.8) solution, mixed, incubated for 2 h (37 °C). After incubation, the reaction was stopped with Na_2CO_3 followed by centrifugation (5 min), and supernatant absorbance of the supernatant measured (400 nm) on a spectrophotometer (Ultrospec 3000, Pharmacia-Biotech). Three replicates were prepared for each sample. Values were corrected for a blank (substrate added immediately after the addition of Na_2CO_3) and for adsorption of released para-nitrophenol (pNP) in the soil. Results were expressed as μg pNP $gsoil^{-1}$ h^{-1}.

Phosphatase activity was determined as described by Tabatabai and Bremner (1969). Briefly, 100 mg of fresh soil was placed in a centrifuged flask and then the substrate 5 mM *p*-nitrophenyl phosphate (Sigma) in a buffered (pH 6.5) solution was added followed by a 1 h incubation (37 °C). After incubation, 0.5 M $CaCl_2$ and 0.5 M NaOH solutions were added followed by centrifugation and measurement of the product para-nitrophenol (*p*NP) (400 nm) in the supernatant on a spectrophotometer (Ultrospec 3000, Pharmacia-Biotech). Assays were performed in triplicate along with a control. Results were expressed as μg *p*NP $gsoil^{-1}$ h^{-1}.

Urease activity was assayed following a method described by Kandeler and Gerber (1988), incubating 1 g of soil with the substrate 1.2 M urea with a borate buffer (pH 7.0) for 2 h (37 °C). The reaction was stopped by adding 3 ml of potassium chloride (2 M KCl) and the product ammonium was determined colorimetrically (660 nm) by a modified Berthelot reaction and results were expressed as μg N–NH_4 soil g^{-1} h^{-1}. Assays were performed on 3 replicates with a control per soil sample. The activity of arylsulfatase was determined using the method described by Klose et al. (2011). One gram of fresh soil was placed in centrifuged flask with the substrate, 50 mM*p*-nitrophenyl sulfate in 0.5 M acetate buffer (pH = 5.8) and 0.25 ml of toluene followed by a 1 h (37 °C). After incubation, 0.5 M $CaCl_2$ and 0.5 M NaOH solutions were added followed by centrifugation and measurement of the product para-nitrophenol (*p*NP) (400 nm). The results were expressed as μg *p*NP g $soil^{-1}$ h^{-1}.

Dehydrogenase activity (DHA) was assayed as an indication of the overall microbial activity according to the method of Prosser et al. (2011). Briefly, this procedure utilizes 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride (INT) that serves as the electron acceptor and is reduced during the assay by microbial cells to form the product, ethanol-soluble, *p*-iodonitrotetrazolium formazan (INTF). Fresh soil (1 g) was mixed with 3.5 ml of INT solution in 3.5 ml of THAM buffer (pH 7) and incubated at 37 °C for 2 h. After incubation ethanol:N,N-dimethylformamide solution (1:1 v/v) was added to extract INTF, which was colorimetrically determined at 464 nm after centrifugation. The assay measurements were done in 3 replicates with a control for each sample. Results were expressed as μg INTF soil g^{-1} h^{-1}.

Fluorescein diacetate (FDA) hydrolysis is a broad spectrum assay that is carried out by a range of enzymes and was done as described by Adam and Duncan (2001). In brief, 1 g soil sample was mixed with the substrate (2 mg FDA mL^{-1}) along with a 60 mM potassium phosphate buffer (pH 7.6) and incubated 1 h (30 °C) followed by the addition of acetone. The suspension was centrifuged and

absorbance of fluorescein was measured (490 nm) in replicated samples and a control against the reagent blank. The FDA hydrolysis activity was expressed as μg fluorescein soil g^{-1} h^{-1}.

2.5. Data analysis

The four soil sampling location or treatment data (C, S, M and M + S) was analyzed as a one-way ANOVA followed by means separation using the LSD test at 0.05 probability level with XLStat-Pro (V 2010 AddinSoft®). Principal component analysis (PCA) was applied using mean values data of soil enzyme activities (XLStat-Pro; V 2010 AddinSoft®).

To evaluate relationships between microbial community composition and environmental variables, we first ordinated the DGGE presence/absence data using nonmetric multidimensional scaling (nMDS) and then looked for significant correlations between the ordination axes and environmental variables (McCune and Grace, 2002). The nMDS ordination was performed using a Sorensen distance measure and the 'slow and through' autopilot mode implemented in the PC-ORD software Version V6.0 (MjM Software Design, Gleneden Beach, OR, USA). This setting used multiple runs ($n = 250$) and significance tests to find the best solution for the optimum number of axes with the lowest final stress (McCune and Grace, 2002). Although a 3-dimensional solution was proposed to be optimal for bacterial community, nMDS was presented using the 2 first axes. Statistical significance of treatment clustering for nMDS ordination was done using Multi Response Permutation Procedure (MRPP) with the Sorensen distance measure (Mielke and Berry, 2001). Indicator values (IV) were established to determine which DGGE bands best show treatment differences (McCune and Grace, 2002), ranging from 0 (no indication) to 100 (perfect indication). The Monte Carlo test was performed with 9999 randomizations to determine the relevance of each band ($P < 0.05$) and bands with value <25 were considered to be a good indicator (Dufrêne and Legendre, 1997).

DGGE fingerprints data were used to estimate three diversity indices. The number of DGGE bands present in each sample was used to measure the Richness index (R) (Vivas et al., 2009). The Shannon-Weaver index of general diversity (H′) (Shannon and Weaver, 1962) and the Simpson index of dominance (D) (Simpson, 1949) were calculated from the number of bands present and the relative intensities of each band (Pi) in each lane.

The Shannon-Weaver diversity (H') was calculated using the following equation:

$$H' = -\sum \mathrm{Pi}\ \ln(\mathrm{Pi})$$

The Simpson index (D) was calculated with the formula

$$D = \sum P_i^2$$

In both equations Pi (the proportion of abundances of the intensities bands) was measured as the ratio ni/N, ni being the peak height of a band and N the sum of all peak heights in the profiles.

For the MicroResp™ data, the respiration rates were determined considering the same number of added substrates for each soil sample. Therefore, the catabolic diversity can be regarded as a simple measure of catabolic evenness, meaning the equality among the catabolic attributes (i.e., substrate utilization) of microbial communities (Oren and Steinberger, 2008). The Simpson's reciprocal index of evenness (E) was calculated from the respiration response profiles as $E = 1/\Sigma Pi^2$ where Pi is the proportion of the respiration rates measured as the ratio between *Ri* the respiration response of each substrate and ΣR_i the respiration rates summed for all substrates (Oren and Steinberger, 2008).

3. Results

3.1. Soil chemical properties

Total carbon, nitrogen and phosphorus contents were significantly higher in soil under the shrub canopy (S) than in bare soil (C) (Table 1). However total C, N and P contents were not significantly different when comparing millet cultivated alone (M) or in the presence of the shrub (M + S) (Table 1). Similar trends were observed for soil NH_4^+–N while soil NO_3^--N content was significantly increased from 1.5 to 4.1 μg NO_3^--N g^{-1} soil in M and M + S, respectively. Soils were acidic but there was no significant treatment effect on pH (Table 1).

3.2. PCR-DGGE analysis of the microbial community structure

3.2.1. Soil bacterial community

The bacterial DGGE profiles of all treatments were complex, with a total of 66 bands (data not shown). Seven bands showed a high indicator value (IV > 67) ($p < 0.05$) for individual treatments (Table 2). The bands 10, 18, 26 and 41 were related to the C treatment (IV > 67) while they disappear (IV = 0) for treatments with the shrub. On the other hand, the bands 12 and 66 were dominant when the shrub was present. The band 13 was only found for millet root zone soil with or without shrub presence (Table 2).

Bacterial diversity indices are shown in Table 3. Both H' and R (Supplemental data) indices increased in millet soil when intercropped with the shrub compared to millet alone (Table 3) while the opposite trend was observed for the dominance index of Simpson (D; (Supplemental data)).

The differences in DGGE profiles between treatments were best visualized using a two dimensional nMDS ordination method (Fig. 1). The first axis accounted for 75% of the total variance. Nonmetric MDS ordination resulted in close clustering of replicates within a treatment but distinct separation of the treatments as shown in Fig. 1. Axis 1 shows clustering by shrub presence, or absence indicating that soil bacterial communities associated with *P. reticulatum* were distinct from those collected from treatments without the shrub.

The Multi Response Permutation Procedure (MRPP) analysis showed that the treatments in nMDS ordination were significantly distinct ($p < 0.001$). Several factors correlated with the nMDS ordination. In particular, the inorganic N (NO_3^- and NH_4^+) was strongly correlated with the bacterial community structures of shrub soil.

3.2.2. Soil fungal community

The PCR DGGE of soil fungal community targeting the ITS region revealed 85 bands in the DGGE fingerprints (data not shown). The indicator species analysis of the DGGE profiles of ITS gene fragments showed that several bands discriminate between treatments (IV > 67, $p < 0.05$; Table 2). The bands 53 and 64 in the profiles were prevalent in the absence of shrubs (C and M treatments). Conversely, bands 68, 73 and 80 were significantly found in soil with *P. reticulatum* (S and M + S) (Table 2). On the other hand, none of the three diversity and dominance indices (H', R, and D) were significantly affected by treatments (Table 3). The nMDS ordination obtained from the fungal gene fragment profiles displayed 4 distinct treatment clusters (Fig. 2).

Furthermore, as shown by Axis 1 (representing 70% of the total variance), the fungal communities responded significantly to the presence of the shrub (MRPP analysis; $p < 0.001$) while the dendrogram analysis revealed 30% of dissimilarity between treatments with and without the shrub, (C and M vs. S and M + S, respectively; data not shown). A strong correlation was observed

Table 1
Soil chemical properties (0–10 cm in depth) at Nioro-du Rip, Senegal. Data are mean values followed by the standard deviation of the mean in parentheses (n = 4).

Property	Control	Shrub	Millet	Millet + Shrub
Total C (mg C g^{-1})	2.5 (0.1) a†	4.0 (0.4) b	2.6 (0.6) a	3.2 (0.4) ab
Total N (mg N g^{-1})	0.22 (0.01) a	0.36 (0.04) b	0.26 (0.03) a	0.29 (0.03) ab
Total P (μg P g^{-1})	42.3 (2.5) a	59.5 (5.2) b	48.8 (4.1) ab	51.5 (3.9) ab
NH_4^+–N (μg N g^{-1})	1.5 (0.3) a	5.6 (1.0) c	3.2 (0.3) ab	4.0 (0.7) bc
NO_3^--N (μg N g^{-1})	1.0 (0.1) a	5.6 (0.9) b	1.5 (0.2) a	4.1 (1.4) b
pH (H_2O)	5.5 (0.1) a	5.5 (0.1) a	5.2 (0.1) a	5.6 (0.1) a

†Means followed by the same letters within a row are not significantly different at $p < 0.05$.

Table 2
The indicator values (IV) (and its associated p-value) from DGGE analyses of the 16S DNA region (bacterial communities) and the ITS region (fungal communities).

16S rDNA region			ITS region		
Bands	IV	*p* value[a]	Bands	IV	*p* value
10	67	0.036	53	100	0.002
12	67	0.039	64	67	0.031
13	100	0.002	68	67	0.033
18	100	0.003	73	67	0.033
26	80	0.013	80	67	0.033
41	80	0.011			
66	80	0.011			

[a] The *p*-value is based on Monte Carlo test using 999 permutations.

Table 3
Shannon-Weaver diversity (H') indice of bacterial (16S rDNA DGGE) and fungal (ITS DGGE) communities in soils of the different treatments. Number in parentheses are standard deviations of the mean (n = 4).

	Control	Shrub	Millet	Millet + Shrub
Bacteria	3.74 (0.02) b†	3.59 (0.01) a	3.52 (0.04) a	3.70 (0.03) b
Fungi	3.74 (0.05) a	3.77 (0.06) a	3.77 (0.08) a	3.88 (0.02) a

†Means followed by the same letters within a row are not significantly different at $p < 0.05$.

3.3. *MicroResp*™

The catabolic profiling assesses the functional capacity of the soil microbial communities to perform hydrolytic reactions related to organic matter decomposition and mineralization of nutrients and respiration as an indicator of microbial activity. Respiration rates higher than basal respiration (no added substrate) were observed for all C sources except cellulose and protocatechuic acid ($p < 0.05$; F = 5.490; Fig. 3). Each C substrate added to soil was used in a similar manner whatever the treatment, only the intensity of the substrate consumption varied among treatments.

The highest respiration rate was found after addition of saccharose (2.40 μg C–CO_2 $gsoil^{-1}$ h^{-1}), glucose (2.29 μg C–CO_2 g^{-1} soil h^{-1}) or malic acid (2.05 μg C–CO_2 g^{-1} soil h^{-1}) for the shrub-millet intercropping treatment. The lowest values were recorded for oxalic acid and then BSA substrate in the control treatment. The addition of alanine, glucose or trehalose substrate generated higher CO_2 emissions from soil collected under the shrub than from the control ($p < 0.05$; F = 3.565). The respiratory rate of glucose provides an estimate of the microbial biomass: 36.6 μg microbial C g $soil^{-1}$ in C, 47.5 μg microbial C g $soil^{-1}$ in M, 79.5 μg microbial C g $soil^{-1}$ in M + S and 92.7 μg microbial C g $soil^{-1}$ in S, respectively. However due to a high interval of confidence, the difference between millet cultivated alone (M) and millet cultivated in the shrub presence (M + S) was not significant ($p = 0.167$; F = 3.565). The trehalose substrate had highest respiration rates in the S treatment (Fig. 3). The MicroResp™ analysis showed a general trend for higher

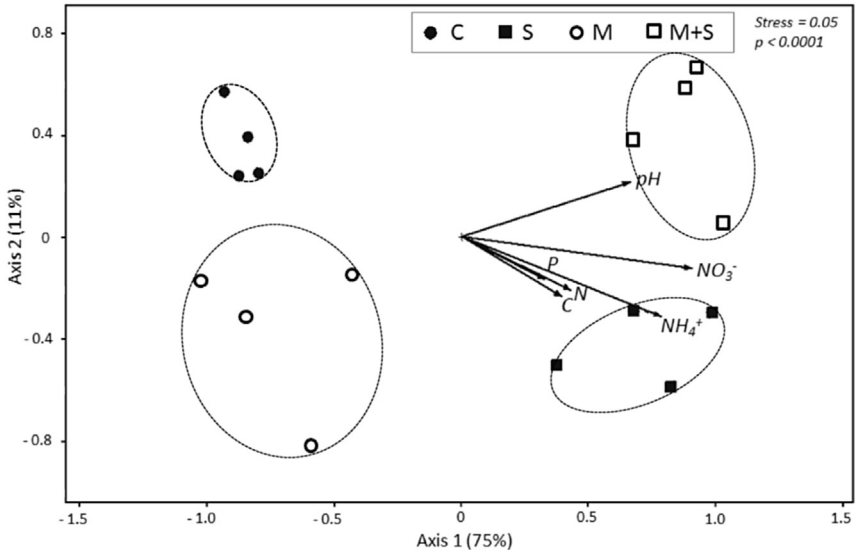

Fig. 1. Nonmetric MDS ordination of the DGGE profiles of 16S rRNA gene fragments (total bacteria) of the treatments (C, Control; S, Shrub; M, Millet; M + S, Millet + Shrub) with superimposed vectors derived from soil chemistry data.

between some soil chemical properties (mainly NO_3^- and NH_4^+) with the two soil fungal community groups according to the presence or absence of the shrub (Fig. 2).

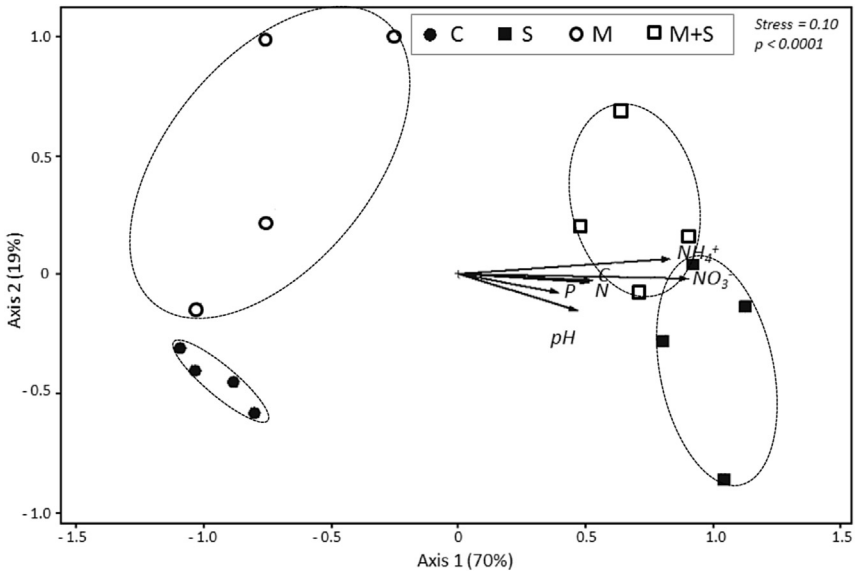

Fig. 2. Nonmetric MDS ordination of the DGGE profiles of ITS gene fragments (fungi) of the treatments (C, Control; S, Shrub; M, Millet; M + S, Millet + Shrub) with superimposed vectors derived from soil chemistry data.

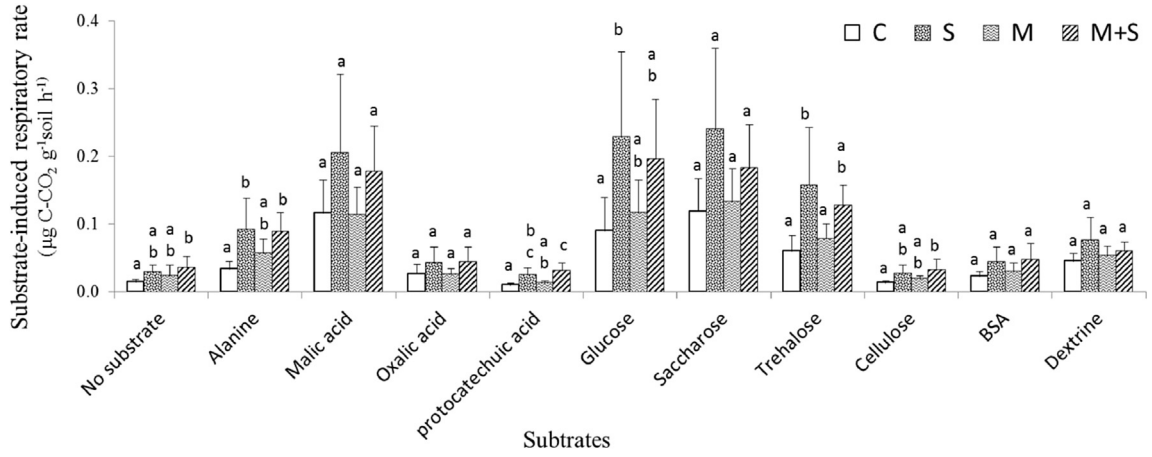

Fig. 3. Catabolic profiling obtained with MicroResp™ assay in response to the different treatments (C, Control; S, Shrub; M, Millet; M + S, Millet + Shrub). Data are mean values +SD (n = 3). Bars with different letters within a substrate are significantly different at $p < 0.05$.

respiration rate across all substrates from soil when millet was intercropped with the shrub than cultivated alone, although the differences were only statistically significant for the protocatechuic acid. The non-significant effects for the other substrates may be due to high variability. For intercropping M + S treatment was characterized by high mineralization of s phenolic acid.

Taking into account all the catabolic data, Evenness index showed no significant relative variability ($P > 0.05$; $F = 1.625$). E varied between 6.46 for the control to 7.03 for M + S treatment (Sup materiel).

3.4. Soil enzymes activities

Except for the β-glucosidase activity, the highest values of enzymatic activities in soil were recorded for the M + S intercropping treatment. No statistical difference was observed when comparing results in soil beneath shrub stand to millet alone regardless the enzyme assay type. However, the M + S was significantly higher than millet alone for arylsulfatase, dehydrogenase and urease activities (Table 4). For β-glucosidase and acid phosphatase activities, and for FDA hydrolysis, values were slightly higher in soil when millet was intercropped to *P. reticulatum* rather than cultivated alone but the differences were not statistically significant at a probability level of 0.05.

Principal component analysis of enzyme activities showed a distinct separation of clusters along the first axis (60.1% of the total variance) based on the presence of the shrub. Although the second axis accounted for only 12.8% of the variability, the score of variables (mainly acid phosphatase, dehydrogenase and β-glucosidase activities) resulted in the most distinct clustering for the M and S treatments (Fig. 4).

4. Discussion

4.1. Shrub effect: bacterial and fungal community structure

The DGGE profiling (Figs. 1, 2 and 4) showed that microbial communities of soils with the presence of shrub (S and M + S

Table 4
Enzymatic potential activities in top soil of the different treatments. Data are mean values followed by associated standard deviation in parentheses (n = 4).

Microbial properties	Control	Shrub	Millet	Millet + shrub
β-glucosidase (μg pNP g soil^{-1} h^{-1})	23.2 (4.4) a	45.6 (16.9) b	30.1 (6.5) ab	42.0 (9.8) b
Urease (μg N–NH_4^+ g soil^{-1} h^{-1})	5.5 (0.9) a	9.1 (0.8) b	7.3 (1.6) ab	11.9 (2.0) c
Acid phosphatase (μg *p*NP g soil^{-1} h^{-1})	49.3 (10.2) a	59.3 (15.0) ab	72.6 (5.0) b	75.6 (15.1) b
Arylsulfatase (μg *p*NP g soil^{-1} h^{-1})	0.3 (0.06) a	0.6 (0.19) bc	0.5 (0.12) b	0.8 (0.05) c
Dehydrogenase (μg INTF g soil^{-1} h^{-1})	0.10 (0.03) a	0.15 (0.05) ab	0.10 (0.02) a	0.16 (0.04) b
FDA (μg Fluorescein g soil^{-1} h^{-1})	108.4 (10.7) a	193.0 (16.4) b	180.0 (66.4) b	209.7 (44.8) b

Values followed by the same letters within a row are not significantly different at $p < 0.05$.

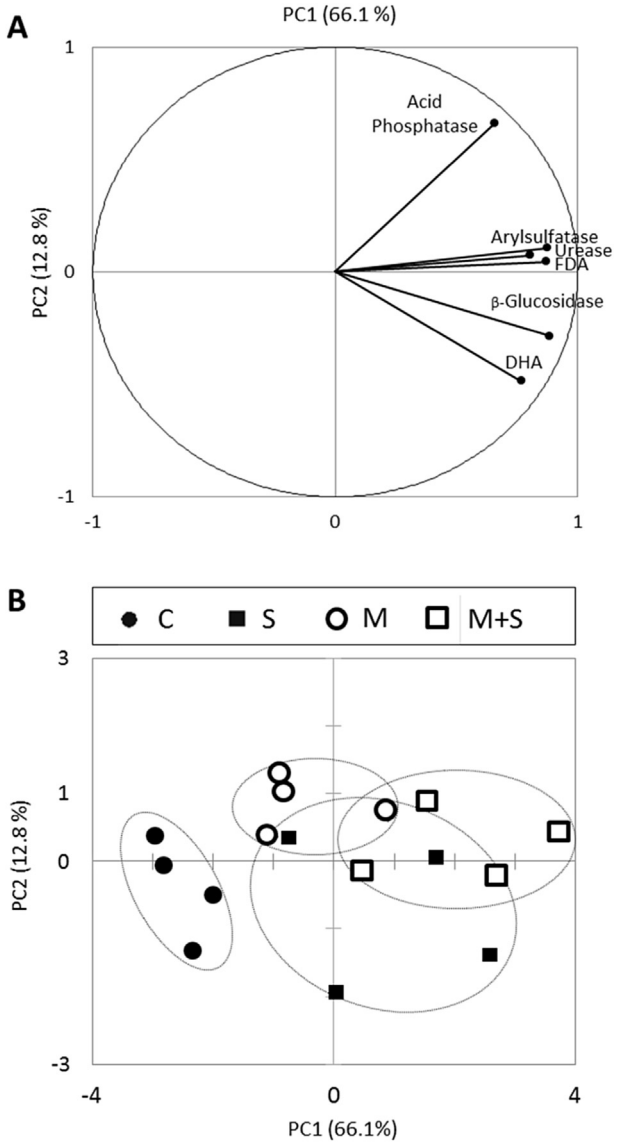

Fig. 4. Principal component analysis of soil enzyme activities according to the different treatments (C, Control; S, Shrub; M, Millet; M + S, Millet + Shrub). A: Correlation circle of the variables. B: projection of samples on the first factorial plane.

treatments) had a significantly different structure than in soil with no shrubs (C and M treatments). These results for *P. reticulatum* were consistent with other shrub species in arid or semi-arid environments for both bacterial and fungal communities (e.g., Bachar et al., 2012; Hernandez et al., 2015). Similarly, Debenport et al. (2015) found that soil of the millet root zone had a more diverse bacterial community when millet was intercropped with *P. reticulatum* or *G. senegalensis* than when grown outside the influence of these shrubs.

Although the number of DGGE bands did not differ among treatments, the intensity of certain bands from bacterial and fungal communities were increased in soil beneath millet plants grown in the presence of *P. reticulatum*. The diversity index that combines the number of bands and the intensity of the bands was significantly affected by treatments but only for bacteria; it being more diverse in soil collected from the rooting zone of millet growing in the presence of the shrub than root soil of millet alone. These findings suggested that changes in the microbial community structure may not lead to shifts in the diversity index values (as we observed for the fungal community), since some taxonomic groups may be compensated by the presence of other species (Hartmann and Widmer, 2006).

Multivariate analysis showed that there was a positive correlation of soil chemical properties with clustering of bacterial and fungal communities in soils beneath shrubs. This reflects that conditions beneath shrubs over time through litter input and root turnover improve soil quality for microbial communities by increasing the total carbon, nitrogen, and phosphorus as well as inorganic N content, especially nitrate as shown in Table 1. This would be consistent with the concept of shrubs in semi-arid or arid regions creating resource islands of nutrients and improved soil structure (Dossa et al., 2013).

Another mechanism for this response in arid savannas is root exudation due to the year-round presence of shrub roots (e.g., Kaplan et al., 2013). Urbanova et al. (2015) reported that changes in microbial community composition, especially for root-symbiotic and saprotrophic fungi are tree species-specific, suggesting that root exudates were controlling fungal diversity. In contrast they found that bacteria were more affected by litter input or soil pH associated with each tree species.

The presence of shrubs year-round in cropped fields provides an opportunity for biomass inputs to soils if coppiced residues are not burned. This practice then improves soil structure, providing habitat and increases nutrients and soil organic matter (SOM) (Lufafa et al., 2008). This is consistent with our results where shrub intercropped soils had greater total C that corresponded to greater microbial biomass and diversity. Contributing to this microbial response is that shrub litter changes the chemistry of SOM (Bending et al., 2004) and the chemistry of soluble organic C due to root exudates and leachates from litter. In turn this affects rates of decomposition, N mineralization, and the structure of the microbial community (Berthrong et al., 2013; Diakhaté et al., 2013).

4.2. Soil microbial catabolic and enzymatic activity

The impact of shrub-intercropping on the activity of soil microorganisms to perform biogeochemical processes was determined by both enzymatic activities and catabolic profiling. Dehydrogenase more directly provides information on the capacity of the microbial community to perform an oxidation reaction and

reflects overall microbial activity (Dick, 1997; Salazar et al., 2011). Dehydrogenase activity was significantly higher in the millet rooting zone soil of the M + S compared to millet alone. This result indicates there is greater availability of labile substrates for M + S soil to support the overall microbial community because this is a non-specific oxidative reaction that all living cells perform.

The C sources used in MicroResp™ consisted of organic compounds, including sugars, amino acids, organic acids and phenolics that may occur in soil after root exudation or litter/mulch decomposition. Substrate utilization was quite homogeneous. The relative utilization of the substrates in the four soils was fairly similar for most of the C sources used for establishing the catabolic profile by MicroResp™. Our finding showed no significant catabolic profile response due to M + S intercropping. This may because of the low number of substrates used in our study. Creamer et al. (2015) used seven substrates to measure microbial respiration profiles across Europe using MicroResp™, also showing no significant differences between their treatments.

We assumed edaphic constraints would explain these observations. Several studies (Oren and Steinberg, 2008; Tian et al., 2007) of semi-arid and arid soils with low organic matter have shown that polymeric sugars (like saccharose) are the second most utilized substrate group after simple sugars (like glucose), followed by carboxylic and aromatic carboxylic acids. The utilization levels of carboxylic acids (like malic acid, oxalic acid) were equal for the four soils, regardless the treatment. Root exudation of carboxylic acids is a well-known plant mechanism for increasing the availability of nutrients, especially in P-deficient soils (Wrage et al., 2010).

Conversely, MicroResp™ showed a distinct response for two of the substrates in the shrub presence (trehalose and protocatechuic acid), reflecting stimulation of microbial subpopulations that are capable of degrading these particular compounds.

The MicroResp™ assay was significantly greater for trehalose in the shrub soil compared to the control soil or millet root soil, which was probably due to a subpopulation adapted to degrade this substrate. Trehalose is a widely distributed carbohydrate that stimulates microbial and plant resistance to abiotic stresses. In plants this compound through signaling regulates stomatal conductance and water-use efficiency (Iordachescu and Imai, 2008).

Trehalose may also play a role in interactions of plants with other organisms, including mycorrhizae, rhizobia, non-symbiotic rhizobacteria, other fungal and bacterial species (Fernandez et al., 2010), and pathogenic nematodes (Hofmann et al., 2010). Extracellular trehalose is essential for the infectivity of several pathogens but at the same time stimulates plant resistance to biotic and abiotic stresses (Fernandez et al., 2010). Diakhaté et al. (2013) showed a decrease in plant-feeding nematodes in the *P. reticulatum* intercropping system. Since soil in the presence of *P reticulatum* exhibited greater potential to hydrolyze trehalose, this would suggest that, indeed, *P reticulatum* does produce this compound and caused this microbial response.

MicroResp™ assay detected a higher respiratory rate for protocatechuic acid in shrub-millet soil than in millet alone or shrub alone. Protocatechuic acid is an aromatic compound that is a key intermediate metabolite of lignin degradation. The coppiced residues of the shrub applied to soil as mulch in the intercropping system is composed of a mixture of chopped leaf and stem with a C:N ratio of 27 and a high concentration of lignin (Dossa et al., 2009). Shrub residues provided a greater availability of C compounds whose consumption by microbes requires additional acquisition of N compounds. Thus, woody inputs in the shrub-based system may have stimulated a community or subpopulation that more efficient in the use of phenolic compounds. Interestingly, in a companion study, bacterial OTUs in the genus *Chitinophaga* and fungal OTUs in various genera were found to be enriched in the soil from the millet root zone when grown in the presence of *P. reticulatum* (Debenport et al., 2015).

The activity of arylsulfatase was significantly higher in the root zone soil of the shrub-millet system than the millet alone. Most arylsulfatases are not constitutive enzymes, and their synthesis by bacteria and fungi is needed to mineralize S to meet microbial sulfur and is controlled by the C and sulfur contents in organic inputs (Dick, 1997). This reflects better growth conditions under shrub canopies for microorganisms that produce extracellular enzymes and for stabilizing enzymes in the soil matrix. Several soil properties are affected by shrub-based cropping systems which is increased soil organic matter, nutrient availability and soil moisture (Kizito et al., 2012; Diakhate et al., 2013; Dossa et al., 2013) and are often highly correlated with enzyme activities (Geisseler et al., 2011; Li and Sarah, 2003).

β-glucosidase and urease activities tended to be higher in the M + S intercropping system ($p < 0.1$). Urease acts on C–N bonds and is used in agricultural studies to predict N mineralization when organic amendments are added to soils (Klose and Tabatabai, 2000; Hernandez et al., 2015).

The enzymes we studied are hydrolytic and involved in organic matter decomposition. Thus the higher potential activity in soils receiving shrub residues as mulch indicates a greater potential to decompose organic residues.

4.3. Perspectives

In summary, *P. reticulatum* significantly altered soil microbial community structure and activity of enzymes in soils in shrub-based cropping systems. This is likely due to root exudates and root turnover, litter inputs of this system, and hydraulic redistribution (Kizito et al., 2012) that provide some water and resources year-round for microorganisms in the shrub rhizosphere. Our research suggests that the microbial communities are more diverse and potentially active in the millet root zone soil when grown with *P. reticulatum* rather than in monoculture of millet. These results would suggest that shrubs could harbor beneficial microorganisms to promote crop growth and yields. However, more in-depth research is needed to understand the microbial interactions between shrub rhizospheres and crop rhizospheres as a basis for greater and more stable yields of dryland crops in the Sahel when grown in the presence of shrubs. Recent advances in sequencing technology, analytical tools, and microbiological techniques offer new opportunities to identify beneficial organisms harbored in the intercropping (and mulching) system. New research directions should target defining optimal conditions of their support to the intercrop and studying their behavior facing climate change.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jaridenv.2016.01.010.

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