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Authors

Hernandez, Rebecca R
Debenport, Spencer J
Leewis, Mary-Cathrine CE
et al.

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The native shrub, *Piliostigma reticulatum*, as an ecological "resource island" for mango trees in the Sahel

Rebecca R. Hernandez [a,b,*], Spencer J. Debenport [c], Mary-Cathrine C.E. Leewis [d], Fatou Ndoye [e], Inès E. Nkenmogne K. [f], Abdoulaye Soumare [e], Moses Thuita [g,1], Mariama Gueye [h], Edouard Miambi [i], Lydie Chapuis-Lardy [h,2], Ibrahima Diedhiou [j], Richard P. Dick [k]

[a] Department of Global Ecology, Carnegie Institution for Science, Stanford 94035, USA
[b] Department of Environmental Earth System Science, Stanford University, Stanford 94305, USA
[c] Department of Plant Pathology, The Ohio State University – OARDC, Wooster 44691, USA
[d] Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks 99775, USA
[e] Département de Biologie Végétale, Faculté des Sciences et Techniques, Université Cheikh Anta DIOP de Dakar, Centre de Recherche de Bel Air, BP 1386, CP 18524 Dakar, Senegal
[f] Department of Applied Chemistry, National School of Agro-Industrial Sciences, The University of Ngaoundéré, P.O. Box 455, Ngaoundéré, Cameroon
[g] Department of Soil Science, Chepkoilel University College, P.O. Box 1125, Eldoret, Kenya
[h] Institut de Recherche pour le Développement (IRD), Laboratoire Mixte International Intensification Ecologique des Sols cultivés en Afrique de l'Ouest (LMI IESOL), Campus IRD/ISRA Bel Air BP 1386, Dakar CP 18524, Senegal
[i] Université Paris Est Créteil, Avenue General de Gaulle, 94010 Créteil, France
[j] Department of Plant Biology, National School of Agriculture, University of Thiès, P.O. Box A 296, Thiès, Senegal
[k] School of Environment and Natural Resources, The Ohio State University, Columbus 43210, USA

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ABSTRACT

African farmers are increasingly adopting sustainable agricultural practices including use of native shrub intercropping approaches. In one village of Sénégal (near Thiès) it was reported that farmers planted mango (*Mangifera indica*) seedlings within the canopies of a native shrub (*Piliostigma reticulatum*). Anecdotal information and qualitative observations suggested that the presence of *P. reticulatum* promoted soil quality and a competitive advantage for establishing mango plantations. We hypothesized that soil chemical and microbial properties of mango rhizosphere soil growing in the presence of *P. reticulatum* would be significantly improved over soils associated with mango growing outside the influence of *P. reticulatum*. The results showed that mango-shrub interplanting significantly lowered pH, and increased arbuscular mycorrhizal fungi (AMF) colonization of mango roots, enzyme activities, and microbial biomass compared to mango alone. Phylogenetic analyses by PCR-denaturing gradient gel electrophoresis (DGGE) showed that community structures of fungi, bacteria, and bacterial genes responsible for denitrification (*nirK*) of the soil from the rooting zone of the mango-shrub intercropping system were distinct from all other soil outside the influence of *P. reticulatum*. It is concluded that *P. reticulatum* enhances soil biological functioning and that there is a synergistic effect of intercropping mango with the native shrub, *P. reticulatum*, in soil quality with a more diverse community, greater AMF infection rates, and greater potential to perform decomposition and mineralize nutrients.

1. Introduction

The Sahel is a fragile environment that has experienced severe environmental degradation due to increasing rural populations, overgrazing, intensive cropping, and scavenging for firewood (Lambin et al., 2014). As a result, soils are of poor quality in the Sahel, which contributes to low yields and food insecurity (Graaff et al., 2011; Niang and Ruppel, 2014). In sub-Saharan Africa, 98% of all cropland is rainfed (Bruinsma, 2003). Poverty and the

* Corresponding author. Present address: Energy and Resources Group, University of California, Berkeley, 310 Barrows Hall, Berkeley, CA 94720 USA; Earth Sciences Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Bldg. 84, Berkeley, CA 94720, USA. Tel.: +1 650 681 7457.
E-mail address: rebeccarhernandez@berkeley.edu (R.R. Hernandez).
[1] Present address: International Institute of Tropical agriculture, ICIPE-IITA projects, Duduville Campus, P.O. Box 30772-00100, Kasarani, Nairobi, Kenya.
[2] Present address: Institut de Recherche pour le Développement (IRD), UMR Eco&Sols, Campus Montpellier SupAgro, Place Viala, Bat 12, 34060 Montpellier Cedex 2, France.

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pervasive dependency on natural rainfall for agriculture are predicted to reduce the capacity for adaptation by sub-Saharan farmers to counteract the effects of climate change (Niang and Ruppel, 2014) while an increase in irrigation infrastructure and utilization in this area is not expected (Bruinsma, 2003; Calzadilla et al., 2009).

Agroforestry, where woody species are allowed to grow in cropped fields, is an approach that improves soils (Lorenz and Lal, 2014; Dossa et al., 2010, 2009, 2008), protects against water and wind erosion (Sileshi et al., 2011), and can increase crop yields (Dossa et al., 2013, 2012; Sileshi et al., 2012); benefits that collectively increase resilience to climate change, particularly in sub-Saharan Africa (Lasco et al., 2014). Here, farmers have long practiced what is called the parkland system where trees are randomly distributed in fields. Tree species including *Faidherbia albida, Parkia biglobosa,* and *Vitellaria paradoxa,* are well known for having positive ecological and agronomic impacts in these dryland ecosystems (Bayala et al., 2014).

However, recent research on a previously unrecognized component of cropped fields in the Sahel, native shrubs – e.g., *Guiera senegalensis* JF Gmelin (Combretaceae) and *Piliostigma reticulatum* (DC.) Hochst. (Fabaceae) – have been shown to have higher soil quality (Dossa et al., 2012, 2013) conferring improved crop yields (Dossa et al., 2012, 2013) compared to soils where shrubs are absent. Additionally, Kizito et al. (2012) showed that *G. senegalensis* and *P. reticulatum* perform hydraulic lift; the passive movement of water from relatively moist areas to drier areas in the soil (Caldwell et al., 1998). Although previous research in natural arid environments such as deserts has shown that various plant species create "islands of fertility" (Bonanomi et al., 2008; Schlesinger et al., 1996), recent research has shown that *G. senegalensis* and *P. reticulatum* deliver many more services than just nutrient cycling. This includes providing water through hydraulic lift, increased microorganism and beneficial nematode diversity in shrub rhizospheres, promotion of decomposition and nutrient mineralization, decreased P sorption, and increased nutrients for crops (Diedhiou et al., 2009; Diedhiou-Sall et al., 2013; Dossa et al., 2008, 2010; Kizito et al., 2012; Diakhaté et al., 2013). This research has shown that these two species serve as resource islands for groundnut (*Arachis hypogaea*) and pearl millet (*Pennisetum glaucum*) crops.

African farmers are increasingly adopting sustainable agricultural practices including use of native shrub intercropping approaches (Diakhaté et al., 2013; Lahmar et al., 2012). Indeed, in collecting indigenous information we recently discovered a village in Senegal that has been using *P. reticulatum* as a nurse plant to assist the establishment of mango (*Mangifera indica* L. [Anacardiaceae]) seedlings (personal communication, Ibrahima Diedhiou). The village resides in the Peanut Basin of Senegal where the cultivation of peanut and millet was once abundant. Today, owing to the decline of peanut production, land degradation, and worsening climatic conditions, farmers have introduced cassava cultivation and growing of fruit (e.g., mango, cashew, jujube) to improve their income (Ouédraogo et al., 2006; Ræbild et al., 2010). A preliminary study showed that mango seedlings grow faster in the presence of *P. reticulatum* with no supplemental water over irrigated seedlings grown outside the influence of the shrub (personal communication, Ibrahima Diedhiou).

In Senegal and throughout Africa, *P. reticulatum* is a dominant shrub in famers' fields. This native evergreen has a deep-rooted taproot and exhibits hydraulic redistribution (Kizito et al., 2012). Also throughout Africa, mango is a subsistence and economic crop for both individual farmers and farming cooperatives (Van Melle and Buschmann, 2013). Thus, intercropping of shrubs with mango could provide benefits to subsistence farmers as a local and low cost resource. However, there is no information on how a shrub-mango intercropping system affects soil chemical and biological properties.

We hypothesized that a shrub + mango intercropping system will have a synergistic effect on soil microbial and chemical soil properties compared to mangoes planted outside the influence of the native shrub. Specifically, our objectives were to determine the effect of native shrub + mango intercropping on: (1) soil chemistry, (2) arbuscular mycorrhizal fungi (AMF) symbiosis, (3) soil enzyme activity, (4) soil microbial biomass, and (5) soil microbial community structure.

2. Material and methods

2.1. Study site

The research was conducted at a cooperative farm in Senegal (West Africa) in the village of Keur Ndiogou Ndiaye (14°45N, 16°51W; 43 m above sea level). The region is semiarid with a tropical Sudanian climate (Köppen climate classification BSh) with mean temperatures ranging from 20 °C in December through January to 33 °C in April through June. Annual precipitation is 450 mm – where 250–500 mm is semiarid – distributed from July to October, generally as intense, short-duration showers. The soil (rubic arenosol (FAO, 2006)), known locally as Dior is 95% sand, mainly originating from aeolian deposits and has no distinct horizonation in the top 1-m layer (Badiane et al., 2000).

In this region, the vegetation is savanna, with *P. reticulatum* and *G. senegalensis* as the dominant shrubs, including in farmers' fields at a mean density of 240 ha^{-1} (Kizito et al., 2006; Lufafa et al., 2008). Other woody species include *Combretum aculeatum* Vent., *Faidherbia albida* (Del.) A. Chev., *Ziziphus mauritiana* Lam. and *Balanites aegyptiaca* (L.) Del. Herbaceous vegetation in the interspace between shrubs consists of annuals dominated by *Alysicarpus ovalifolius* (Schum.) J. Leonard, *Cenchrus biflorus* Roxb., *Dactyloctenium aegyptium* (L.) Willd. *Eragrostis pilosa* (L.) P. Beauv. and *Merremia tridentata* (L.) Hall (Dossa et al., 2010).

2.2. Soil and root sampling

In July 2012, soils were collected within the village cooperative farm from (1) the rooting zone of *P. reticulatum* seedlings grown in plastic containers within the cooperative nursery (nursery shrub), (2) bare soil in the interspaces adjacent to intercropped mango trees (bare soil, approximately 5 m away), (3) the rooting zone of established *P. reticulatum* without intercropped mango trees (shrub alone), (4) the rooting zone of established *M. indica* trees (approximately 4 years old) without native shrubs (mango alone), and (5) the rooting zone of established *M. indica* trees (i.e., approximately 4 years old) intercropped with native *P. reticulatum* (mango + shrub). For each soil treatment types, there were four replicates (Supplementary Fig. 1).

For each of the four nursery shrub seedlings (1), we opened the container, removed all roots, put each replicate's roots in individual, sterile bags, and permanently stored them at 4 °C until analysis. The soil of each nursery shrub replicate was removed from the container and stored in individual bags. For treatments (2) through (5), three soil subsamples from each replicate were removed with a soil auger (2.5 cm radius) and to a depth of 10 cm. Subsamples for treatments (3) through (5) were collected equidistant around the base and within the canopies of individual plants, deposited in a sterile, plastic bag, and thoroughly mixed. Subsamples for treatment (2) were collected in the interspaces between individual plants, deposited in a sterile, plastic bag, and thoroughly mixed. After each sample, we cleaned the auger with alcohol. Immediately after sampling, soils were placed on ice. All soil samples were permanently stored the same day at 4 °C at IRD

(LMI IESOL, Dakar, Senegal). Root samples (≥3 per replicate) were destructively collected to a depth of approximately 15 cm. For each replicate of mango + shrub, both mango and shrub roots were collected. Roots were placed on ice and permanently stored at 4 °C at IRD laboratory in Dakar. Soil water content was determined gravimetrically by drying at 105 °C for 24 h and reported as a percent of g H_2O g^{-1} soil. Soil pH was measured in a 5:1 (w:w) water:soil slurry.

2.3. Arbsucular mycorrhizal fungi colonization and spore counts

Mango and shrub roots were washed with sterile water, and placed in a 10% KOH solution in a 90 °C water bath for 25 min. After incubation in the water bath, roots were rinsed over a 100 μm sieve under tap water for 1 min. Roots were then placed in tubes containing trypan blue solution (0.5 g trypan blue powder, 100 mL vinegar, and 900 mL distilled water. Roots were then incubated at 90 °C for 20 min. After incubation, roots were rinsed with water, and transferred to microscope slides. Approximately 10 root fragments were placed on each slide for each sample. Slides were examined using a compound microscope at 200×. Slides were placed over a printed grid, and intersections of grid line and root fragment were checked for mycorrhizal structures. Percent colonization was determined by dividing the number of mycorrhizal structures by the total number of root/grid intersections.

Mycorrhizal spores were extracted from soils by placing 100 g of soil into 1 L of tap water, thoroughly mixing, and pouring over a stacked set of 4 sieves (400 μm, 200 μm, 100 μm, and 50 μm from top to bottom). Spores were collected from all but the 400 μm sieve and placed into separate 30 mL centrifuge tube containing 5 mL of 20% sucrose solution and 5 mL of 60% sucrose solution. Tubes were centrifuged at 3000 rpm for 3 min at 4 °C. Supernatant, excluding the pellet, was then washed over a 50 μm sieve. Spores were transferred to a petri dish with water, and observed under a stereoscope.

2.4. Soil enzyme activity

The potential activities of three extracellular enzymes involved in C and P cycling were determined according to established procedures (Adam and Duncan, 2001; Hayano, 1973; Tabatabai and Bremner, 1969). Acid (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2) (AcP) and alkaline phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1) (AlkP), and β-glucosidase (EC 3.2.1.21 ß-D-glucoside glucohydrolase) (releases glucose from cellobiose) were determined on 1.0 g soil samples incubated at 37 °C using *p*-nitrophenyl phosphate (buffered at pH 6.5 for AcP and 11.0 for AlkP) and *p*-nitrophenyl-β -D-glucoside, as a substrate, respectively. After one hour of incubation, the product *p*-nitrophenol (*p*NP) was measured colormetrically at 410 nm after stopping the reaction. Fluorescein diacetate hydrolysis is mediated by non-specific enzymes such as proteases, lipases, and esterases and therefore represents a broad-spectrum assay. For the hydrolysis of fluorescein diacetate (3′,6′-diacetylfluorescein in pH 7.6 buffer; FDA) we added 1.0 g of soil in 15 mL of buffer to 200 μL FDA solution (4.8 mM) and incubated under shaking for 1 h at 30 °C. The reaction was stopped by addition of 1 mL acetone and the suspension was centrifuged (1000× min^{-1}) for 5 min, filtered, and absorbance of the filtrate was measured with at 490 nm.

Results of enzyme activities are reported on an oven-dry-weight basis, determined by drying the soils for 24 h at 105 °C. With the exception of cellulase, the assays were done on the air-dried soil. The soil suspension was shaken for an additional 1.75 h. The reaction was stopped by addition of 20 mL acetone. The suspension was centrifuged for 5 min at 4300 × *g* and filtered through Whatman No. 4 filter paper; absorbance of the filtrate was measured with a visible light spectrophotometer at 499 nm.

2.5. Soil microbial biomass

Microbial biomass was estimated using substrate induced respiration method (Anderson and Domsch, 1978). Microbial CO_2 respiration was determined by direct injection into a micro GC Analytical Instruments SRA (MTI P200, Microsensor Technology Inc., Fremont, CA) equipped with a thermal conductivity detector using helium as the carrier gas. The soil microbial content was estimated from the maximum rate of glucose-induced respiration by applying the formula: $x = 40.04y + 0.37$, where y = the maximum initial rate of respiration (in mL CO_2 100 g $soil^{-1}$ h^{-1}), and x = mg microbial-C 100 g $soil^{-1}$.

2.6. Soil microbial community structure

To determine differences in the fungal, bacterial, and denitrifying bacterial community structure among the four soil treatment types, we subsampled 0.5 g of soil from each replicate and extracted genomic DNA according to the manufacturer's protocol for the FastDNA Spin Kit for Soil (MP Biomedicals, LLC; Solon, Ohio). Cell lysis was performed by vigorous shaking in a bead beater at a speed of 25 revolutions s^{-1} for two minutes. DNA was eluted with 50 μL of the DNA elution solution included in the kit. DNA extraction was done in duplicates on each sample and stored at −20 °C until PCR–DGGE analysis.

For total bacterial community, the eubacterial primer pair 338f-GC (Øvreås et al., 1997) and 518r (Muyzer et al., 1993) were used to amplify the targeting total 16S rDNA bacterial community. Analysis of total soil bacterial community was performed as described by Assigbetse et al. (2005). The polymerase chain reaction (PCR) amplifications were performed in 25 μL mixtures using pureTaq™ Ready-To-Go™ PCR beads (Amersham-Biosciences, Orsay, France) with 5 ng of template DNA, 25 μM of each primer and supplemented by 0.5 μL of BSA (10 mg/mL). Thermal profile for the amplification of bacterial community consisted of an initial denaturation of 5 min at 94 °C followed by 20 cycles as follows: 30 s at 94 °C, 30 s at 65 °C, 1 min at 72 °C followed by 10 other cycles: 30 s at 94 °C, 30 s at 55 °C, 1 min at 72 °C and a final elongation step of 15 min at 72 °C.

For the community of Cu-containing bacterial genes encoding the key enzyme nitrite reductase (*nirK*) implicated in the denitrifying process that leads ultimately to the release of gaseous N products into the atmosphere, the PCR amplification was carried out with f1acu and r3cu-GC primers (Throbäck et al., 2004). These primers generate fragments of approximately 472 bp. Thermal profile for the amplification community consisted of an initial denaturation of 5 min at 94 °C followed by 10 cycles as follows: 30 s at 94 °C, 30 s at 62 °C, 45 s at 72 °C followed by 25 other cycles as follows: 30 s at 94 °C, 30 s at 57 °C, 45 s at 72 °C and a final elongation step of 15 min at 72 °C.

Fungal community in soils was determined by nested PCR amplification of the targeted ITS rDNA with ITS1f and ITS4 primers for the first PCR and the primer pair ITS1f-GC and l'ITS2 for the second round (Anderson et al., 2003). Nested-PCR amplifications were carried out with Ready-To-Go PCR beads. The first PCR had a total reaction volume of 25 μL with 2 μL DNA. Final concentration of primers was 25 μM and thermal cycling conditions for the amplification were as follows: an initial denaturation of 5 min at 95 °C followed by 30 cycles as follows: 30 s at 95 °C, 30 s at 57 °C, 1 min at 72 °C and a final elongation step of 10 min at 72 °C. Specificity of amplification products was checked by agarose (1.5%) electrophoresis. Bands were excised on UV and each band was eluted in 100 μL ultra pure water. One μL of the eluted DNA was used as template for

the second PCR. This second PCR reaction was conducted in a final volume of 25 μL with pureTaq™ Ready-To-Go™ PCR beads (Amersham-Biosciences, Orsay, France) and 25 μM of each primer. After an initial denaturation of 5 min at 95 °C, DNA was amplified over 25 cycles of 30 s at 95 °C, 30 s at 57 °C, 1 min at 72 °C and extension of 10 min at 72 °C. Specificity of amplification products was checked by agarose (2%) electrophoresis.

Denaturing gradient gel electrophoresis (DGGE) analysis was carried out as described by Assigbetse et al. (2005) using 8% acrylamide gels (acrylamide–bisacrylamide 40% (37.5:1)) with a 45–70% denaturant gradient, where 100% denaturant was defined as 7 M urea plus 40% formamide. Phoretix 1D v10 was used to calculate the percentage of similarity among lanes by taking into account the migration distance and the relative intensity of bands.

2.7. Statistical analysis

To determine statistical differences in the means of variables (or whether samples originate from the same distribution) – i.e., soil pH, soil water content, AMF colonization and spore count, enzymes, and microbial biomass – among the five soil sampling locations, we used an analysis of variance or a Kruskal–Wallis rank sum test. The latter nonparametric test was employed when data did not meet the assumption of normality. All data sets were tested for normality using the Shapiro–Wilk test. We used the parametric (Tukey's range test) and nonparametric (Mann–Whitney *U*) post-hoc tests to determine differences between all pairwise comparisons, as appropriate. All statistics were conducted in R (Version 2.12.0, Vienna, Austria).

To compare the activity of all four enzymes among soil treatments, we ordinated the activity data using nonmetric multidimensional scaling (NMS) that uses rank orders to compare groups of samples based on count-based distance metrics, in this case, Sørensen (Bray–Curtis) distance measures. Multiple response permutation procedure (MRPP) tests were used to evaluate the relationships between treatment type and potential soil enzymatic activity. Community structure data was also subject to NMS analysis. Calculations were carried out using PC-Ord 6.0 (Mccune et al., 2002).

Unweighted pair group method cluster analysis was used to produce DGGE fingerprint dendrograms for each community structure type (i.e., fungal, bacterial, and *nirK*), which were subsequently compared. Richness of DGGE gels were calculated from the number of bands present (Vivas et al., 2009). The structural diversity of the total and *NirK* bacterial and fungal communities was examined with the Shannon index of general diversity H0 (Shannon and Weaver, 1963). The intensity of the bands was reflected as peak heights in the densitometry curve.

Differences in bacteria, fungi, and denitrifier community structure were determined using NMS implemented using PC-Ord 6.0 and MRPP tests were conducted to determine if community structure and categorical environmental variables such as "treatment" were significantly different (Mccune et al., 2002). Diversity measurements were calculated in PAST (Hammer et al., 2001).

3. Results

Our study provided a comprehensive characterization (for details see Supplementary Table 1) of the effect of native shrub intercropping with mango trees and the implications of this agricultural technique for sub-Saharan Africa.

3.1. Soil moisture and pH

Soil water content varied significantly across soil treatments ($p = 0.036$, Kruskal–Wallis rank sum test; Supplementary Fig. 2). Soil water content was lowest in the soil found in bare interspaces ($3.7 \pm 0.2\%$ [95% CI]), which is significantly different than soils from the rooting zone of nursery shrubs ($7.4 \pm 1.0\%$) and individual mature shrubs ($5.5 \pm 1.7\%$). Soil water content of *P. reticulatum* soils intercropped with mango was not significantly different than the soils from individual shrubs or individual mango trees. Soil pH also varied significantly across soil treatments ($p < 0.0001$, analysis of variance [ANOVA] test; Supplementary Fig. 2). Soils ranged from 5.8 ± 0.2 (bare soil) to 7.0 ± 0.3 (nursery shrub). Bare soils and soils from mangos intercropped with shrubs had significantly lower pH than shrubs and mangos cultivated individually.

3.2. Arbuscular mycorrhizal fungi

We observed AMF hyphae, vesicles, and arbuscules in the roots of all treatments, including (Supplementary Fig. 3, Fig. 1A and B) young *P. reticulatum* root, (C) mature *P. reticulatum* root, and (D) mature *M. indica* root. Spore density was significantly different across rooting soil treatment types ($p = 0.014$, Kruskal–Wallis rank sum test; Fig. 1A). Densities were greatest for soils from mature mango trees (242 ± 132 spores $100\,g^{-1}$ [90% CI]) and lowest for soils from mango intercropped with shrubs (11 ± 18 spores $100\,g^{-1}$ [90% CI]). Arbuscular mycorrhizal fungi colonization, both frequency and intensity, significantly varied across soil treatments ($p = 0.033$; $p = 0.08901$; Kruskal–Wallis rank sum test, respectively; Fig. 1B). Mean frequency ($100.0 \pm 0.0\%$ [90% CI]) and intensity ($55.5 \pm 41.2\%$ [90% CI]) was greatest in the intercropped shrub roots.

3.3. Soil enzyme activity

Soil enzyme activity (μg product release dry $soil^{-1}\,h^{-1}$) was quantified in the rooting zone of all treatment types. Enzymes acid phosphatase ($p = 0.060$), alkaline phosphatase ($p < 0.0001$), β-glucosidase ($p = 0.043$), and fluorescein diacetate hydrolysis ($p = 0.012$)

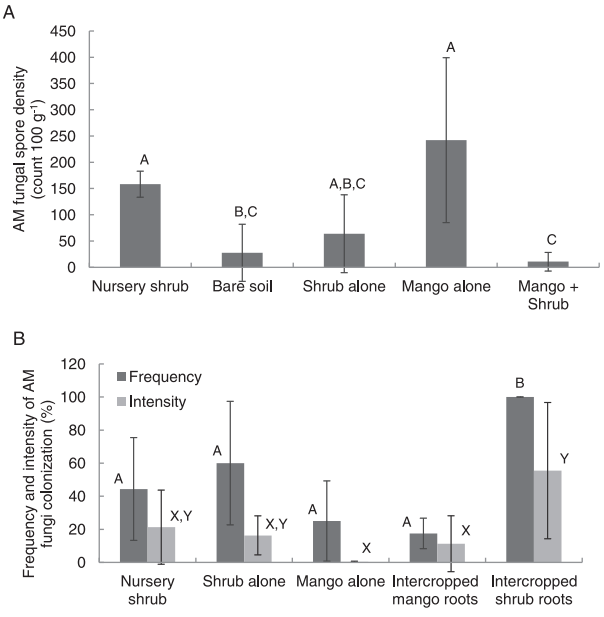

Fig. 1. (A) Arbuscular mycorrhizal fungal spore density (count $100\,g^{-1}$) varied significantly across soil types ($p = 0.014$, df = 4, chi-squared = 12.432, Kruskal–Wallis rank sum test). Spore density varied significantly across soil types ($p = 0.014$, df = 4, chi-squared = 12.432, Kruskal–Wallis rank sum test). (B) Arbuscular mycorrhizal fungi colonization, both frequency and intensity, significantly varied across soil types ($p = 0.0335$, df = 4, chi-squared = 10.451; $p = 0.089$, df = 4, chi-squared = 8.071; Kruskal–Wallis rank sum test). Soil types that do not share a similar letter are significantly different. Error bars are 90% confidence intervals.

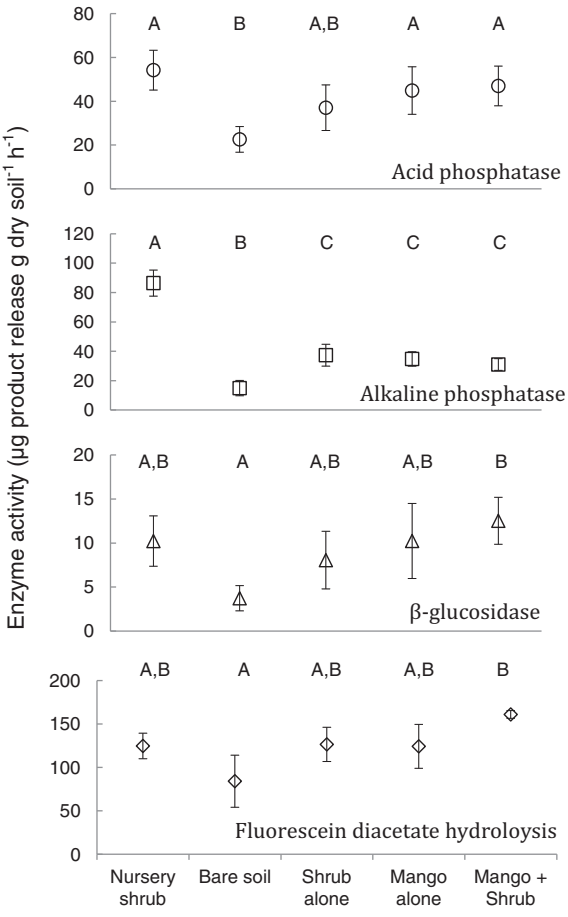

Fig. 2. Soil enzyme activity expressed as the amount of product release (µg fluorescein soil g $^{-1}$ h^{-1}) sampled in the rooting zone of all treatment types. Enzymes acid phosphate ($p = 0.060$, df = 4, chi-squared = 9.029), alkaline phosphate ($p < 0.0001$, df = 4, F value = 48.773), β-glucosidase ($p = 0.043$, df = 4, F value = 3.227), and fluorescein diacetate ($p = 0.012$, df = 4, F value = 4.630) all varied significantly across soil types (Kruskal–Wallis rank sum test). Soil types that do not share a similar letter are significantly different. Error bars are 90% confidence intervals.

all varied significantly across soil treatments (Kruskal–Wallis rank sum test; Fig. 2). In general, significant differences in enzyme activity were observed between bare soil and soils from the rooting zone of mango + shrub or the nursery seedlings of *P. reticulatum*.

Nonmetric multidimensional scaling (NMS) ordination and multiple response permutation procedure (MRPP) analyses detected differences in enzymatic activity across treatment types (Supplementary Fig. 4, Table 1). The enzymatic activity of the mango + shrub treatment was significantly different than bare and nursery shrub soil. However, the MRPP did not detect differences in enzyme activity between the mango with shrub and either mango alone or shrub alone. Enzyme activity in bare soil was different from all other treatments, as was the enzyme activity associated with nursery shrubs. The multivariate NMS ordination analysis of the enzyme activities showed a clear separation of the treatments ($R^2 = 0.89$, stress = 0.076; Fig. S4).

Table 1
Multiple-response permutation procedure tests for differentiation of bacterial community enzymatic activity compared for all four soil treatments in the study.

Treatment	Effect size, *A*	Significance, *p*
Bacterial community structure (16S)	0.42855258	<0.001
Fungal community structure (ITS)	0.32570032	<0.001
Denitrifying community structure(*nirK*)	0.41081677	<0.001
Total enzyme activity	0.33194337	<0.0001
Mango + shrub vs. bare soil	0.37536744	0.008
Mango + shrub vs. shrub alone	0.16187042	0.051
Mango + shrub vs. mango alone	0.15963747	0.079
Mango + shrub vs. nursery shrub	0.46598742	0.006
Bare soil vs. shrub alone	0.14904427	0.045
Bare soil vs. mango alone	0.16680065	0.030
Bare soil vs. nursery shrub	0.40309807	0.007
Shrub alone vs. mango alone	−0.1027356	0.893
Shrub alone vs. nursery shrub	0.27003659	0.010
Mango alone vs. nursery shrub	0.26714522	0.012

3.4. Soil microbial biomass

Microbial biomass-C was significantly greater in the mango + shrub soil treatment (183 ± 4 µg microbial-C g soil^{-1}) than all other treatment types ($p = 0.026$, Kruskal–Wallis rank sum test; Fig. 3). All other treatments ranged from 78 ± 18 (bare soil) to 112 ± 29 (shrub alone) µg microbial-C g soil^{-1} and were not significantly different from each other.

3.5. Microbial community structure

We found significant differences ($p < 0.001$) among the soil microbial communities across soil treatments (Fig. 4, Tables 1 and 2). The DGGE fingerprint dendrograms showed high similarity in structure for total (16S) and *nirK* bacteria community structure between the mango + shrub and shrub alone and mango + shrub and nursery shrub, respectively. Bare soil was only approximately 30% similar in total bacterial community structure with other soil treatments.

Bacterial communities associated with the shrub alone had the most number of individual bands and greatest diversity, followed by communities (in decreasing order of diversity) associated with the nursery shrubs, mango + shrub, mango alone, and bare soils (Table 2). In fungal communities, the shrub alone also had the highest number of individual bands and diversity, followed by communities associated with mango alone, nurse shrub, mango + shrub, and finally bare soils. The denitrifying bacterial community (*nirK*) associated with nurse shrubs had the greatest number of individual bands, however, the diversity of shrub-associated denitrifying bacteria was greater than that of the nurse shrub communities.

The relationship between the measured community structure (16S, ITS and *nirK*) was assessed using NMS of community composition data using the Bray–Curtis similarity measure. Nonmetric multidimensional scaling ordination and MRPP analyses detected differences in microbial community structure across all measured genes and treatment types (Table 1). In all MRPP tests associated with community structure as measured by DGGE, communities were significantly different from each other (16S: $A = 0.429$, $p < 0.001$; ITS: $A = 0.326$, $p < 0.001$; *nirK*: $A = 0.411$, $p < 0.001$). The differentiations in community structure across

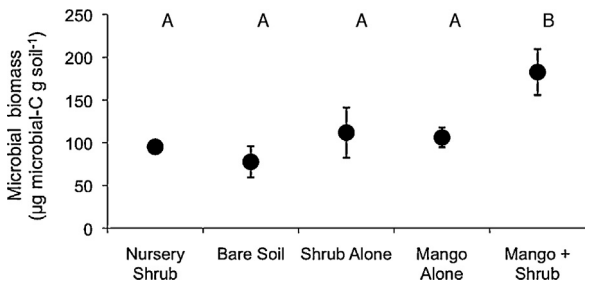

Fig. 3. Soil microbial biomass (mg microbial-C 100 g soil^{-1}) varied significantly across soil types ($p = 0.026$, df = 4, chi-squared = 11.071, Kruskal–Wallis rank sum test). Soil types that do not share a similar letter are significantly different. Error bars are 90% confidence intervals.

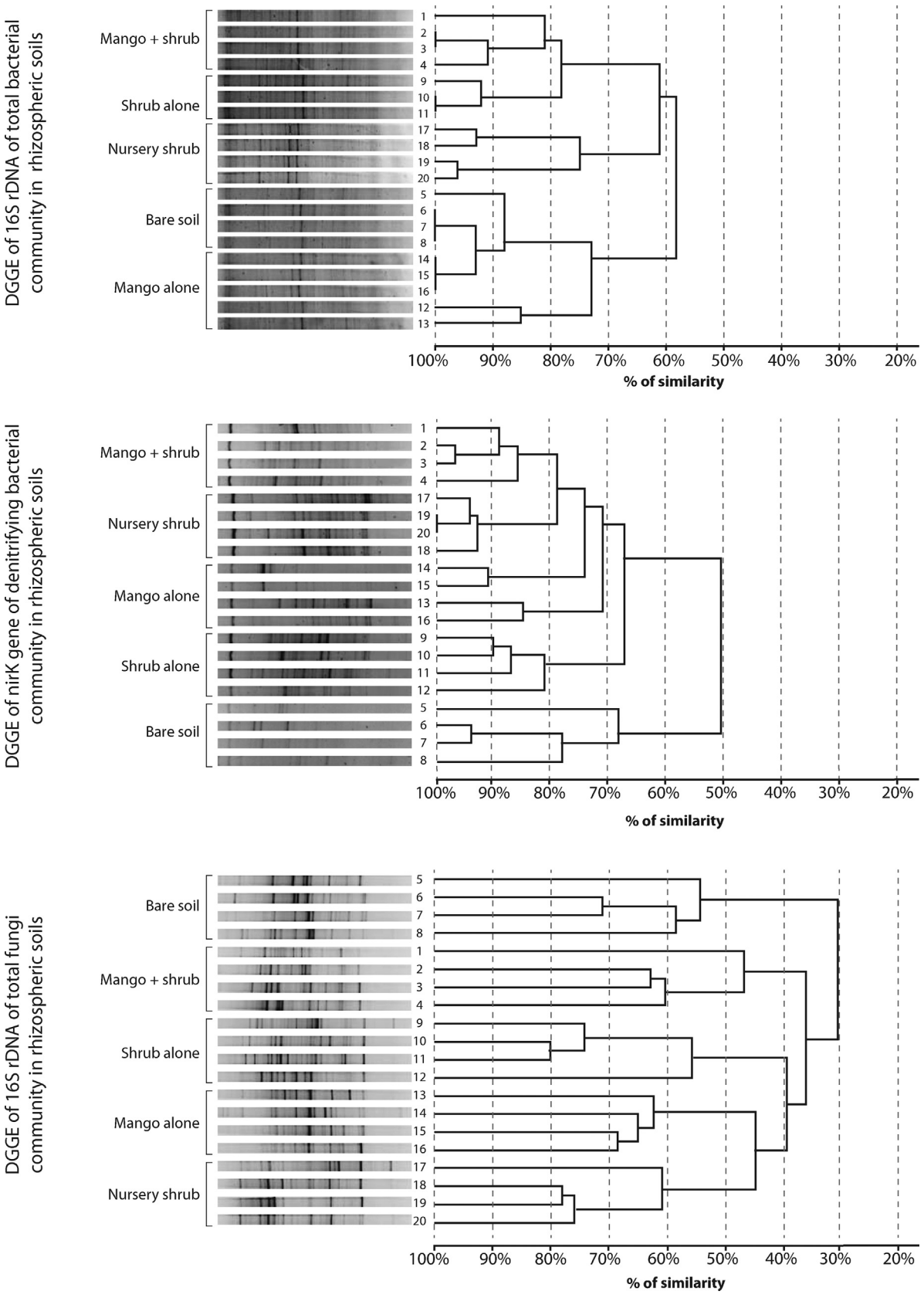

Fig. 4. Phylogenetic analyses (and percent similarity) by PCR-denaturing gradient gel electrophoresis (DGGE) of (top) 16S rDNA of bacteria, (middle) *nirK* gene of denitrifying bacteria, (bottom) 16S rDNA of fungi across soil treatment types.

Table 2
Diversity indices for bacteria, *nirK* gene of denitrifying bacteria, and fungi in the rooting zone of soil in the nursery shrub, shrub (alone), mango (alone), and mango + shrub intercropping treatments, and bare soil.

Diversity index	Nursery shrub	Bare soil	Shrub alone	Mango alone	Mango + shrub
Richness (S)					
Bacteria (16S)	34.00	25.0	34.25	25.50	28.75
Fungi (ITS)	18.50	13.75	20.50	18.75	15.75
nirK	31.00	17.50	30.50	25.50	22.25
Shannon's diversity index (H')					
Bacteria (16S)	3.47	3.19	3.50	3.22	3.33
Fungi (ITS)	2.72	2.46	2.86	2.79	2.65
nirK	3.39	2.84	3.39	3.21	3.04
Simpson's diversity index (D)					
Bacteria (16S)	0.97	0.96	0.97	0.96	0.96
Fungi (ITS)	0.92	0.90	0.93	0.93	0.92
nirK	0.96	0.94	0.97	0.96	0.95

treatment types were reflected in the NMS ordinations (16S: $R^2 = 0.85$, stress = 12.68; ITS: $R^2 = 0.87$, stress = 9.13454; *nirK*: $R^2 = 0.61$, stress = 13.49).

4. Discussion

4.1. Mango + shrub soil moisture and pH

Precipitation is the most limiting resource for sub-Saharan African agriculture (Bruinsma, 2003). Previous studies have shown that resource islands, compared to interspaces, modify soil water properties that can lead to changes that serve as a positive biophysical feedback to plant establishment and growth (Bhark and Small, 2003; Kizito et al., 2012; Rango et al., 2006). Plant–soil-hydrological processes in resource islands are complex, varying greatly across spatiotemporal scales, by depth, and an area of incipient research (Aguilera et al., 1999; Jury et al., 2011; Li et al., 2013; Schade and Hobbie, 2005). In our study, soils of native shrubs had significantly greater soil water content (SWC) than bare soil at 10 cm depth, however, no significant difference in SWC was found among soils from interspaces, mango alone, and intercropped mango + shrub. Our samples were collected during the wet season, however, in 2014, soil samples were re-collected (i.e., same site and soil treatments) during the dry season and we found SWC of intercropped mango + shrub was significantly greater than bare soil (personal communication, R.R. Hernandez, 2014). Given these limitations, future research should elucidate the role of soil depth, seasonal changes, and hydraulic lift on SWC for shrubs intercropped with mangos.

Species-specific effects of root mediated changes of soil pH are well studied (Hinsinger et al., 2003), however, relatively little is known about the mechanisms underlying pH dynamics in soils of shrub intercropping (Dossa et al., 2010; Hinsinger et al., 2011). Across studies, the effect of shrub intercropping on pH remains ambiguous (Diakhaté et al., 2013; Wezel et al., 2000); for example, Diakhaté et al. (2013) found no difference in soil pH among control soils, bulk soil from *P. reticulatum*, millet, or *P. reticulatum* intercropped with millet. We found no significant difference in pH between bare soils and intercropped mango + shrub, however, individual shrubs and mangos showed significantly greater pH than both aforementioned soil treatment types. This is consistent with the findings on *P. reticulatum* by Dossa et al. (2010). However, one might expect a leguminous shrub like *P. reticulatum* to acidify their environment (Hinsinger et al., 2003), which Dossa et al. (2010) did observe in the co-occurring, albeit non-legume shrub, *G. senegalensis*. Broadly speaking, plant roots taking up more anions (e.g., nitrate, NO_3^-) may cause the bulk soil to be more alkaline (i.e., to maintain electrical neutrality at the root-soil interface) at the individual plant-scale (Li et al., 2014; Nye, 1981), which may explain the higher pH observed in mango and shrub soil treatments. Although, this does not explain why intercropped mango + shrub would not do the same and this observation cannot, at least in this study, be attributed simply to higher respiration rates (Lambers et al., 1996), changes in litter chemistry, or observed higher microbial biomass-C, without further investigation (Dossa et al., 2010, 2008).

4.2. Mango + shrub and arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi serve a critical role in resource acquisition for aridland plants (Bashan and De-Bashan, 2010). In this study, we found that spore densities for individual mango plants were significantly greater than intercropped mango + shrub and bare soil. Several studies in aridlands have found that spore counts are greater for bulk soil derived within the resource island than in plant interspaces (Aguilera et al., 1999; Carrillo-Garcia et al., 1999) but no difference has also been observed (Titus et al., 2002) as we found for shrubs and intercropped mango + shrub.

We found significantly greater rates of AMF colonization for intercropped shrub roots than all other root treatments. Additionally, intercropped shrub roots had significantly greater intensity of AMF colonization than all mango roots, regardless of whether it was intercropped or as a sole plant. Shi et al. (2007) found that when an herbaceous plant was growing within an established resource woody species canopy, AMF colonization was higher than when these same plant species were grown as a sole plant. Our results suggest that *P. reticulatum* when acting as a "nurse plant" does not reduce AMF colonization for intercropped mango, as we observed.

4.3. Mango + shrub and soil enzyme activity

The most notable effect on soil enzyme activity was that, outside the influence of plant canopies and roots, activity levels were consistently lower across all soil enzymes assayed and significantly different than the mango + shrub treatment. This reflects the most extreme growth conditions for microorganisms that produce extracellular enzymes and for stabilizing enzymes in the soil matrix. The soil outside the influence of plants would have had the lowest organic C inputs and root turnover from crops only – whereas the mango + shrub treatment would have the most litter input and more root biomass turnover than any of the treatments, thus stimulating the decomposers to produce hydrolytic enzymes. Whereas for the nursery, sole mango and sole shrub soils, they had intermediate activity that was not significantly different than either soils outside the influence of plants or from the mango + shrub treatment.

The results show that the mango + shrub treatment has the most potential to perform decomposition reactions and mineralization of nutrients (in our case phosphatase activity from organic compounds) based on enzymes that were assayed. In the case of β-glucosidase, the low molecular weight sugar, glucose, is released that is a readily available energy source for microorganisms. Unlike β-glucosidase and phosphatase that each performs unique reactions, fluorescein diacetate (FDA) hydrolysis is performed by a number of enzymes (lipases, proteses and esterases). Therefore, the origin of enzymes could come from a range microbial species and is a broad-spectrum indicator of enzyme accumulation in the mango + shrub treatment.

These results are similar but somewhat in contrast to the study of Diedhiou-Sall et al. (2013) who found elevated enzyme activities for soil beneath the canopies of *P. reticulatum* and *G. senegalensis* over soil outside the influence of the shrub in Senegal. For all enzymes we assayed, although empirically higher than the shrub alone treatment, they were not statistically higher than the bare soil.

Enzymes in soil are primarily of microbial origin (Ladd, 1978) but the activity measured at the time of sampling is not solely from enzymes associated with viable cells (Kandeler and Dick, 2006). This is because a large percentage of the activity for many enzymes and particularly for hydrolytic enzymes assayed in our study comes from free enzymes no longer associated with viable cells but complexed with humic colloids and stabilized on clay surfaces (Boyd and Mortland, 1990). This was first confirmed by Douglas McLaren who used irradiation to show that sterile soil can have

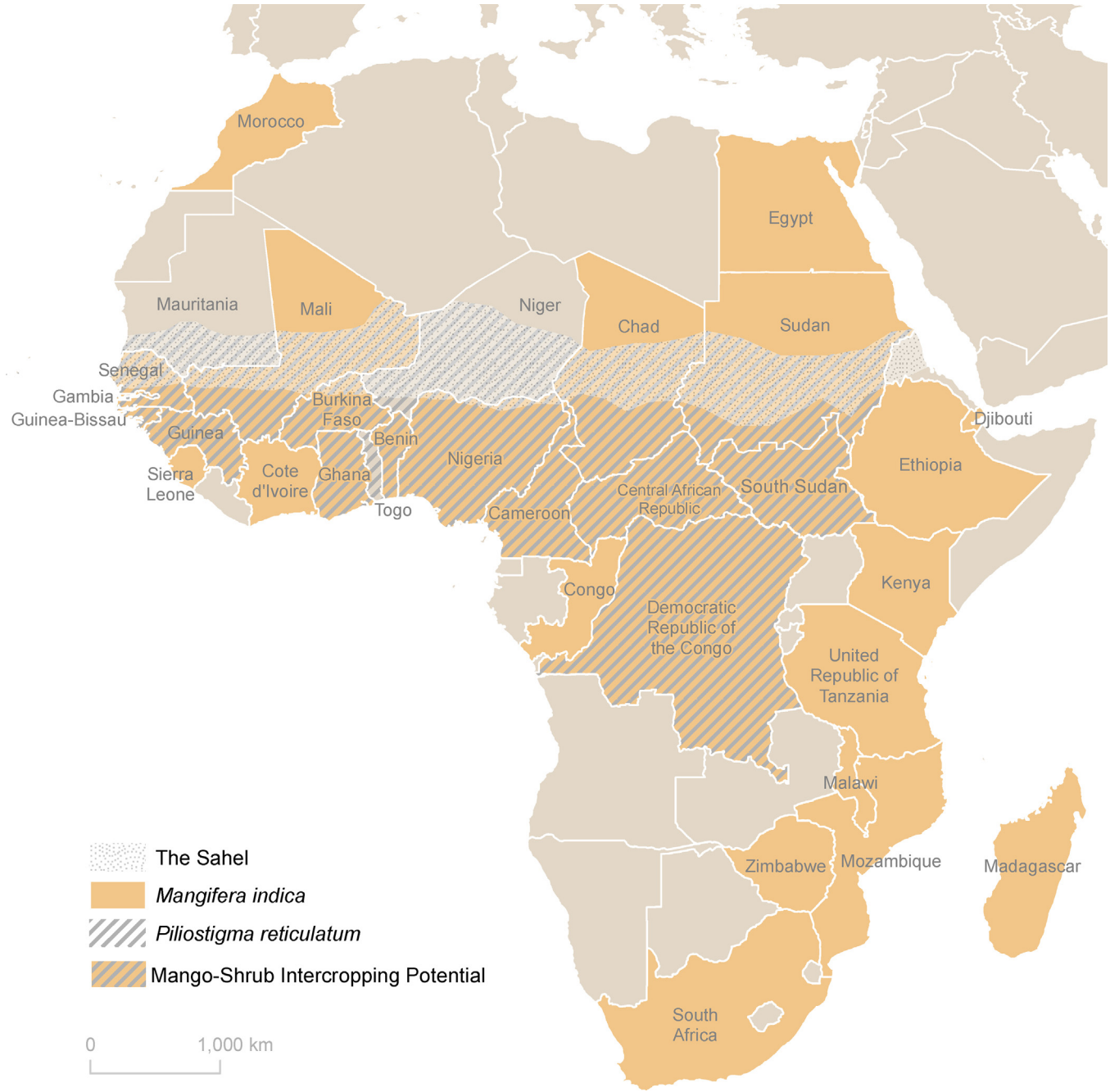

Fig. 5. Geographic distribution of mango cultivation (*Mangifera indica*), native shrub (*Piliostigma reticulatum*), and opportunities for mango + shrub intercropping where the two distributions intersect within Africa.

significant levels of enzyme activity (McLaren et al., 1957). So for the mango + shrub treatment the elevated levels of enzyme activity came not only from a larger and more active microbial community at the time of sampling but also represents a build-up residual enzymes stabilized in the soil matrix over the years since the plants were established. The latter phenomenon would be due the increased turnover of organic inputs of litter and roots that produces organic compounds and aggregates that protects and complexes enzymes that remain catalytic. Therefore, this provides an indirect indicator of a higher quality soil relative to organic matter and soil structure. The results also show the ability of soil enzyme activities to detect changes in management effects and their usefulness as a soil quality indicator.

4.4. Mango + shrub and microbial biomass

Microbial biomass, the amount of C held in living heterotrophic microorganisms, changes relatively rapidly, unlike total soil organic C and is therefore an excellent indicator of changes in land management (Hoyle and Murphy, 2006) including changes in agricultural applications (Chander et al., 1998). In this study, differences in microbial biomass results were particularly striking, with mango + shrub intercropping showing significantly greater soil microbial biomass than all other soil treatments. Bare soil, shrub alone, mango alone, and nursery shrub treatments were comparable to results from Diakhaté et al. (2013), which is consistent with our results on the mango + shrub intercropping system having higher biomass. Given that the mango shrub intercropping systems were very young (i.e., approximately 4 years), it is also possible that the soils have not reached equilibrium and that microbial biomass may still be increasing.

4.5. Mango + shrub and soil microbial community structure

We found that microbial communities in the rhizosphere soil of *P. reticulatum*-mango systems are significantly different from those of the rhizosphere soils of either species when grown alone. The NMS analysis of bacterial and fungal DGGE banding patterns showed that the mango-shrub intercrop communities clustered most closely with the shrub alone communities. This suggests that the mango plants in this intercropping system are able to benefit from the rhizosphere microbiome which is associated with *P. reticulatum.* Interestingly, MRPP analysis shows that while the mango-shrub and shrub alone communities are similar in diversity, the mango-shrub communities are significantly different from either mango or shrub plant rhizosphere microbiome communities alone. These findings are similar to those of Diakhaté et al. (2013), who showed that in a shrub-millet intercropping system, the nematode communities of the intercrop and the shrub alone were the most similar. This could be due to the shrub rhizosphere microbiome being dominant, with the selective recruitment of microorganisms from the mango rhizosphere microbiome.

5. Conclusions

We conclude that *P. reticulatum* has a synergistic effect on the microbial ecology when grown in combination with mango. This resulted in soil with a more diverse community and greater potential to perform decomposition and mineralize nutrients. It is noteworthy that AMF infection was found in both the mango and the *P. reticulatum* because this sets up the possibility of a common AMF hyphal network that could be a reason for the improved growth of mango seedlings when grown in the presence of *P. reticulatum* (Egerton-Warburton et al., 2007; Gosling et al., 2006).

5.1. Implications of mango + shrub intercropping system on sub-Saharan Africa

The results provide evidence to support wider campaigns to pilot test and demonstrate shrub-mango intercropping elsewhere and with other tree fruit crops. In Africa, *M. indica* is a relatively cosmopolitan commodity in over 25 countries from Morocco to South Africa (Fig. 5). *P. reticulatum* is also widely distributed, found throughout the Sudano-Sahelian savanna and from Senegal to Sudan (Dao, 2012). Its presence in the landscape already supports multiple uses by rural populations including traditional medicine, feed (leaves and fruit) for livestock, timber, and rope from the bark (Zoundi et al., 1996). Where mango production and native shrub distributions intersect, opportunities for mango + shrub intercropping may exist but are largely underutilized (Fig. 5). The results also suggest that this intercrop system would be valuable for increasing resilience to climate change, but this needs further research.

The research raises other questions that now should be answered (e.g., if the presence of *P. reticulatum* increases mango crop yields once mangoes reach the fruiting stage). Soil microbial community structure is different but higher resolution data is needed such as next-generation sequencing technology to enable phylogenetic characterization of the communities in shrub + mango intercrops and how seasonal dynamics change soil biochemical and nutrient properties.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.agee.2015.02.009.

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