Pedobiologia **52** (2009) 273—286

Available online at www.sciencedirect.com
ScienceDirect

Pedobiologia
www.elsevier.de/pedobi

Decomposition and spatial microbial heterogeneity associated with native shrubs in soils of agroecosystems in semi-arid Senegal

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Received 9 November 2008; accepted 14 November 2008

KEYWORDS
Sahel;
Enzymes;
PLFA;
Shrubs;
Rhizopshere;
Decomposition

Summary

The interactions of the tree component with soils in the parkland systems of Africa have been investigated but little research has been done on unrecognized native woody shrubs that coexist with crops in farmers' fields, particularly in the Sahel. The two most important species are *Guiera senegalensis* and *Piliostigma reticulatum*, which are coppiced and burned prior to crop planting in Senegal and throughout the Sahel. The litter inputs and year-round presence of the shrub rhizosphere should have implications for soil quality and crop productivity, but the interactions of these shrubs with microbial communities and biogeochemical processes are uninvestigated. Hence, the objective was to determine the influence of shrub rhizospheric soil and residue type on microbial community composition and activity during decomposition as a first step towards developing a non-thermal residue management system. Two experiments, one for each shrub species, had a 2×3 factorial design with two soil treatments (0–10 cm depth beneath and outside the influence of the shrub) and three residue amendments (leaf, stem+leaf and control). The amended soil microcosms were incubated for 105 days in the lab and destructively sampled at 0, 7, 14, 45, 75, or 105 days. At each sampling, the soil microbial communities were profiled by phospholipids fatty acid (PLFA) analysis and analyzed for the activity of two C hydrolyzing enzymes (β-glucosidase and cellulase). Interestingly, the shrub

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0031-4056/$ - see front matter
doi:10.1016/j.pedobi.2008.11.002

canopy effect on the microbial communities was stronger than the residue type. The fungal biomarkers were more closely correlated than other microbial groups to residue chemistry whereas, Gram-positive bacterial and fungal markers (18:2ω6c, 18:1ω9c) were highly correlated with enzyme activities. In contrast, the actinomycete marker (10 Me 16:0) was poorly correlated with enzyme activities. We conclude that shrub rhizospheres in Sahaliean agroecosystems are distinct components in controlling microbial community composition, promoting fungi, microbial diversity, and litter decomposition.

Introduction

A common land-use practice in the Sahel is the parkland agroforestry system where tree and shrub species are randomly allowed to establish and grow in farmers' fields. The tree component in these agroforestry systems has received considerable attention with regard to their biophysical interactions with soil and crops (Young, 1989; Rhoades, 1997; Samba, 2001). However, an overlooked component of these parkland systems is the shrub vegetation.

Two native shrubs (*Guiera senegalensis* and *Piliostigma reticulatum*) are commonly found in farmers' fields in Senegal. Traditional management involves coppicing and burning aboveground residue in the spring, prior to the planting of row crops to clear fields. Non-thermal management of these organic materials holds potential to add organic matter and stimulate diversity and mass of microorganisms of soils. However, to accomplish this goal, burning of aboveground pruning materials needs to be replaced by biological degradation of residues at rates fast enough so that litter does not interfere with the establishment of summer row crops. A preliminary study by Diack et al. (2000) suggested non-thermal management may be possible because they showed fairly rapid decomposition of *P. reticulatum* residues in Senegal (80% mass loss after ~9 months in a cropped field).

Shrubs in arid and semi-arid environments create spatial heterogeneity of soil chemical properties (Van Miegroet et al., 2000); the so-called "island of fertility" (Garner and Steinberger, 1989; Schlesinger et al., 1990; Kieft et al., 1998; Wezel et al., 2000; Whitford, 2002). Additionally, it would be expected that litter fall, root exudates, and root turnover of woody perennial species would stimulate and shift microbial communities. Indeed, Gallardo and Schlesinger (1995) reported elevated microbial biomass beneath shrubs in a desert soil but otherwise there is virtually no information about the influence of these shrub generated "islands of fertility" on the microbial community during decomposition in semi-arid regions. Furthermore, that *P. reticulatum* and *G. senegalensis* are found in farmers' field, such information is needed as a foundation for developing optimized, non-thermal shrubs systems where residue burning is replaced by biological degradation.

Understanding how shrubs influence the soil microbial communities is important because they partition plant litter C among CO_2 losses, microbial biomass, and incorporation into soil organic C pools. Decomposition is a succession of processes at different trophic levels (Wardle and Lavelle, 1997; Adl, 2003); fungi and bacteria are the most important litter decomposers; directly attacking residues over soil fauna that largely assist decomposition by shredding of residues that increases surface area (Petersen and Luxton, 1982; Adl, 2003). The increased surface area from shredding, allows more rapid colonization and access of microorganism to substrates. However, no such soil biological information is available about the dominant Parkland shrub species (*G. senegalensis* and *P. reticulatum*) in the Sahel.

Therefore, the objective of the study was to determine shifts in microbial communities and enzyme activity responses during decomposition of *G. senegalensis* and *P. reticulatum* residues amended to soils originating from inside or outside the influence of shrub canopies/rhizospheres.

To accomplish this key C cycle, enzyme assays were done along with microbial profiling of microbial communities during shrub residue decomposition. Changes in phospholipid fatty acids (PLFA) profiles were used as they are indicative of changes in the overall structure of microbial communities (Frostegård et al., 1996) and "signature" PLFA can provide information on specific functional groups of microorganisms present in a community (Frostegård et al., 1993). The composition of PLFA from samples has been sensitive for detecting shifts in soil microbial communities during decomposition due to management and vegetation effects (Bossio and Scow, 1998; Drijber et al., 2000; Schutter and Dick, 2002; Petersen et al., 2002; Hackl et al.,

2005; McMahon et al., 2005; Moore-Kucera and Dick, 2008).

Materials and methods

Site description

The experimental location was the semi-arid agro-ecological zone in the peanut (*Arachis hypogea*) production zone of Senegal. The region is characterized by a tropical Sudanian climate with an annual rainfall of 700 mm and potential evapotranspiration of 1800 mm yr^{-1}.

Soils were collected from two sites. The first site was at Keur Mata Arame which is located in the northern region of Senegal (14°45N, 16°51W, and 43 m above sea level) with mean annual precipitation of 450 mm. Temperatures range from 20.3 °C in December–January to 33.4 °C in April–June. The soil has 95% sand, mainly of aeolian deposits, and is classified as Rubic Arenosol (FAO, 2006), locally referred to as a Dior soil (Badiane et al., 2000). *G. senegalensis* is the dominant shrub vegetation. Shrub stand density at the site is about 240 shrubs ha^{-1} (average canopy diameter ~2 m). The second site (Nioro du Rip) is located (13°45N, 15°47W) at 18 m above sea level with mean annual precipitation of 750 mm distributed from July to September and mean air temperatures ranging from 20 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 ferrugineous tropical soil. The dominant shrub species at the site is *P. reticulatum* with stand density of 185 shrubs ha^{-1}.

All sites were in farmers' fields that were under a peanut (*Arachis hypogea*) and pearl millet (*Pennisetum thyphoides*) crop rotation where farmers coppice and burn aboveground shrub residue every spring season prior to planting of row crops.

Laboratory incubation study

A lab incubation, done for each species, had a completely randomized 2×3 factorial design for each shrub type where there were two soil treatments (soil beneath or outside the shrub canopy) and three residue treatments: (1) leaf, (2) a proportional mix (wt/wt) as found in the field of 60% stem plus 40% leaf, and (3) control soil with no residue. The shrub residues were collected in March, 2002 (shrubs were ~1 m in height at harvest). Leaves and stems of woody species were separated and all plant residues were dried at 35 °C for 5 days then individually chopped to pass a 1 cm sieve and kept in sealed plastic bags. Soil was collected in August, 2002 randomly with a coring device (approximately 30 cores of 2.5 cm diameter) in the Ap horizon (0–10 cm depth) in the area beneath and outside the canopy (3 m distance from the canopy edge). This was replicated on three shrubs and this spatial replication was maintained for subsequent laboratory incubations. Composite soil cores, within a shrub replicate, were homogenized and then crushed to pass 2-mm mesh screen, air-dried and stored at 22 °C.

The incubation study was started by first bringing soils to two-thirds field capacity and incubating at 25 °C for 3 days to allow for equilibration. Residues were then mixed with 100 g of soil (0.7% w/w) and placed in a 0.25 L plastic cup and incubated at 25 °C. Soil moisture was maintained gravimetrically every 2–3 days. Soils were sampled destructively at days 7, 15, 45, 75, and 105 of the incubation period.

Analytical procedures

Phospholipids fatty acids analysis

Microbial community structure was determined by analysis of PLFAs using the method of Bligh and Dyer (1959) with slight modification. Briefly, fatty acids were extracted in three steps from 3 g of soil in triplicate with a one-phase chloroform–methanol–phosphate buffer solvent. The extracted lipids were fractionated into neutral lipids, glycolipids, and polar lipids using silicilic acid columns (Supelco, Bellefonte, PA, USA). The polar lipid fraction was trans-esterified with mild alkali to recover the PLFA as methyl esters in 300 μL of hexane (Guckert et al., 1985).

Tridecanoic FAME (13:0, Supelco, Inc.) was added at various concentrations as internal standard and PLFA were analyzed by gas chromatography (GC) (Agilent Ultra 2 column; temperature ramping 120–260 °C at a rate of 5 °C min^{-1}). Helium was used as the carrier gas and peaks were detected by flame ionization detector (Frostegård and Bååth, 1996). Individual fatty acid methyl esters were identified and quantified using the MIDI Sherlock Microbial Identification System (MIDI, Newark, Delaware, USA) and in addition with a mixture of 37 FAME (FAME 37 47885-4; Supelco, Inc), 24 bacterial FAME mixture (P-BAME 24 47080-U; Supelco, Inc.). Quantification of FAMES was accomplished by using varying concentrations of tridecanoic FAME (Supelco, Inc., Bellefonte, PA) and allowed peak areas to be converted to a molar

basis. Fatty acids with less than 0.5% of the total relative abundance were not included in the data set.

A total of 38 PLFA were detected and identified in the different soil samples. PLFA biomass was estimated by adding the masses of all fatty acids detected and was expressed in nanomoles of PLFA g^{-1} of dry weight of soil (nmol g^{-1} soil) (White et al., 1979; Frostegård et al., 1991; Bossio et al., 1998). For the multivariate analysis, results for each individual fatty acid were expressed as a percentage of the total amount of fatty acids (mol%) found in a given sample. Total percentages of PLFA identified for each microbial group were calculated to represent their relative contribution to the total microbial biomass. For all the remaining analyses, the absolute values were expressed in nmol g^{-1} C of PLFA g^{-1} g of soil was used.

A total of 30 PLFA markers out of 34 identified which represent 89% of the total PLFA were used for the multivariate analyses for *Guiera*. For *Piliostigma*, 32 PLFA out of 38 identified (92%) were used for multivariate analysis.

Biomarkers for major microbial groups were calculated by summing PLFAs as follows: Gram-positive bacteria (GM+) (sum of i15:0, a15:0, i16:0, i17:0, and a17:0) (O'Leary, 1988); Gram-negative bacteria (GM−) (sum of cy17:0, 16:1ω7, 18:1ω7, and 17:1ω9) (Wilkinson, 1988); actinomycetes (actinos); and bacteria (sum of i15:0, a15:0, i16:0, i17:0, a17:0, cy17:0, cy19:0, 16:1ω7, 18:1ω7, and 17:1ω9) (Frostegård and Bååth, 1996). The 18:2ω6,9 is used as a measure of fungal biomass. Total PLFA ($PLFA_{tot}$) was summed within a sampling date and used as an indicator of microbial biomass.

The ratio of the sums of the fungal and bacterial signature fatty acids (FUN/BACT) was included in the data analysis as it is an indicator of overall changes in the soil microbial community structure (Bardgett et al., 1998; Olsson, 1999; Zelles, 1999; Fierer et al., 2003). We calculated and analyzed two stress indicators; the saturated to monosaturated PLFA ratio (SAT/MONO) and the cy19:0/18:1ω7c ratio. A decrease in the SAT/MONO ratio is indicative of decreasing substrate availability and/or increasing anaerobic conditions (Bossio and Scow, 1998; Larkin, 2003). Cyclopropyl fatty acids (cy17:0, cy19:0) are produced from the corresponding monounsaturated fatty acids (16:1ω7c, 18:1ω7c) by many Gram-negative bacteria in response to depletion of substrate or stress (Guckert et al., 1986; Petersen et al., 1997; Lundquist et al., 1999a) and therefore the ratio of cy19:0/18:1ω7c is used as a stress indicator.

Enzyme activities

Activities of two enzymes related to the C cycle and decomposition (β-glucosidase and cellulase) were measured. Cellulase activity was determined by incubating 1 g of soil with 10 mL of 2 M acetate buffer (pH 5.5) containing the substrates, carboxymethyl cellulose sodium salt (0.7% w/v) (Schinner and von Mersi, 1990). Reducing sugars released during incubation reduced alkaline potassium hexacyanoferrate (III) to potassium hexacyanoferrate (II), which was measured spectrophotometrically at 690 nm (Deng and Tabatabai, 1994). Results were expressed as mg glucose released g^{-1} dry soil h^{-1}. Analyses were done in triplicate for each sample. A control was done with a soil and buffer mixture in the absence of the substrate to account for the background levels of reducing sugars in the soil.

β-Glucosidase activity was determined by measuring the product *para*-nitrophenol (ρNP) after incubation of fresh soil in the presence of the substrate ρNP-glucopyranoside for 1 h at 25 °C as described by Tabatabai (1994). The quantity of ρNP released was determined colorimetrically on a spectrophotometer at 420 nm and was reported as μg ρNP g^{-1} dry soil h^{-1}.

Soil pH was determined with a glass electrode in 1:2.5 soil:water ratio.

Soil and plant analyses

Total soil and plant C was determined by combustion on a LECO WR-12 C autoanalyzer (LECO Corp., St. Joseph, Missouri). Total N in soils and organic residues was determined by Kjeldahl digestion followed by steam distillation according to Bremner and Mulvaney (1982). Total P in plant residues and soils was determined by a modified Kjeldahl Li_2SO_4–H_2SO_4 procedure (Parkinson and Allen, 1975). Lignin, cellulose, and hemicellulose were determined by the method of Goering and Van Soest (1970). Total polyphenolic content was determined in the diluted hot water extracts (Valachovic et al., 2004) with the Folin Ciocalteau reagent as described by Ohno and First (1998) using tannic acid as standards. Reactive polyphenols were estimated as the polyphenols precipitated by shaking the diluted hot water extract samples with Sigma purified casein (Valachovic et al., 2004).

Nitrate-N and ammonium-N in a 1 M KCl soil extract were determined, respectively, by the salicylate-nitroprusside and the hydrazine-sulfanilamide methods (Mulvaney, 1996). Results shown in Table 1 for inorganic N are the summation of nitrate and ammonium N. Resin P in brief (Hedley et al., 1982) was done by shaking 2 g soil with a 2.5 cm^2 anion exchange resin strip (AR-204UZR-4R Ionics) for 16 h and then desorption of P from the strips

Table 1. Chemical properties of the soils. ($n = 4$).

Soil location	Total C ($g\,kg^{-1}$)	Total N ($g\,kg^{-1}$)	Total P ($mg\,kg^{-1}$)	Inorganic N ($mg\,kg^{-1}$)	Extractable P ($mg\,kg^{-1}$)	pH
Nioro (*P. reticulatum*)						
Beneath canopy	5.77a*	0.21a	65a	4.9^{NS}	1.5^{NS}	6.4a
Outside canopy	3.23 b	0.19b	55b	4.4^{NS}	2.2^{NS}	5.8b
Keur Mata (*G. senegalensis*)						
Beneath canopy	3.35a	0.20a	59b	3.9a	5.5a	5.2^{NS}
Outside canopy	2.51ab	0.18b	63a	2.4b	4.1b	5.4^{NS}

*Means within a column for each location (residue and shrub species) followed by the same letter are not significantly different at $p > 0.05$.

with 0.5 M HCl solution after 16 h shaking. The solution PO_4–P was determined colorimetrically by the ascorbic acid method (Murphy and Riley, 1962). The resin-P extracts PO_4 from the soil solution and represents biologically available P (Tiessen and Moir, 1993).

Statistical analysis

Effects of residue amendment and soil source, and incubation time on microbial PLFA groups (amount of PLFA $nmol\,g^{-1}$ soil) were analyzed as repeated measures ANOVA (r.m. ANOVA) (SAS Institute Inc., 1996). Shifts in PLFA profiles over time were analyzed by non-metric multidimensional scaling (NMS) using the PC-ORD package (MjM Software Design, Gleneden Beach, OR) (McCune and Grace, 2002) on PLFA data converted to mol% of total peaks.

To assess whether community PLFA profiles differed according to location, substrate amendment and time of incubation, permutational multivariate analysis of variance (PerMANOVA) (Anderson, 2001) was performed on the coordinates of soil communities along axes 1 and 2. In addition, correlations between PLFAs relative concentrations and NMS coordinates were calculated to identify PLFA whose gradients were represented by axes 1 and 2. The PLFA data was correlated with enzyme activities as well as some specific PLFA groups using SAS correlation software (SAS Institute Inc., 1996).

Results

Chemical analysis of soils and plant residues

Table 1 shows that presence of both *G. senegalensis* and *P. reticulatum* shrubs resulted in greater amounts of organic C with 33% and 78% more C, respectively, over soil outside the influence of the canopy. However, the canopy effect was much lower for total N with <11% increases over that of non-canopy soil. C:N ratios of the residues were relatively low (20–27) for both species, suggesting there should be net N released during decomposition (Sylvia et al., 2005). The *G. senegalensis* canopy/rhizosphere had minimal effects on soil pH whereas *P. reticulatum* caused significantly higher soil pH in soil beneath than outside the canopy.

Residue chemistry in Table 2 shows that lignin content was high for *P. reticulatum* leaves and for the *G. senegalensis* mix, whereas polyphenolic content was highest in *P. reticulatum leaf/stem* compared with all other residues.

Temporal and residue amendment responses

Guiera senegalensis

The highest amount of $PLFA_{tot}$, fungal, bacterial and actinomycetal PLFA occurred at day 15. The amount of $PLFA_{tot}$ was the same at days 7 and 45; the fungal PLFA at day 7 was significantly higher than at day 45 ($p < 0.01$). Soils amended with leaf material had a higher amount of $PLFA_{tot}$, fungal and actinomycetal PLFAs than soil amended with leaf/stem mix up to day 45 regardless of source of soil (Figure 1). There was a residue-type effect for all communities groups ($p < 0.03$) except for the bacterial community PLFA at day15 with soil beneath the canopy having higher PLFA levels, regardless of the residue amended.

With respect to the residue amendments, $PLFA_{tot}$, bacterial, fungal, and actinomycetal PLFA were higher beneath the canopy than outside the canopy up to day 45. There was a strong effect of time and soil source ($p < 0.01$). Unamended soil from beneath the canopy had higher $PLFA_{tot}$ than did soil outside the canopy at days 15 and 45 (15 and 12 $nmol\,g^{-1}$ soil, respectively).

Table 2. Chemistry of shrub residues (*G. senegalensis* (GS), *P. reticulatum* (PR)) added to soils ($n = 4$).

		P (mg g^{-1})	C (%)	N (%)	LG (%)[a]	CL (%)[b]	HM (%)[c]	PP (%)[d]	RP (%)[e]	C:N	C:P	LG:N	PP:N	(PP+LG):N	RP:N
GS	Leaf	1.0	35.4	1.6	10.3	21.6	12.8	6.4	5.0	21	347	6.3	3.9	10.2	3
	Leaf+stem	0.64	33.3	1.3	18.1	45.2	13.3	3.9	3.6	26	520	14.2	3.1	17.3	2.8
PR	Leaf	1.0	35.2	1.8	13.1	19.8	13	5.3	4.2	20	348	7.4	3.0	10.4	2.4
	Leaf+stem	0.67	33.7	1.2	13.6	44.4	13.2	7.3	6.2	27	502	10.8	5.8	16.7	5

$p < 0.05$.
[a]LG, lignin.
[b]CL, cellulose.
[c]HM, hemicellulose.
[d]PP, total polyphenols.
[e]RP, reactive polyphenols.

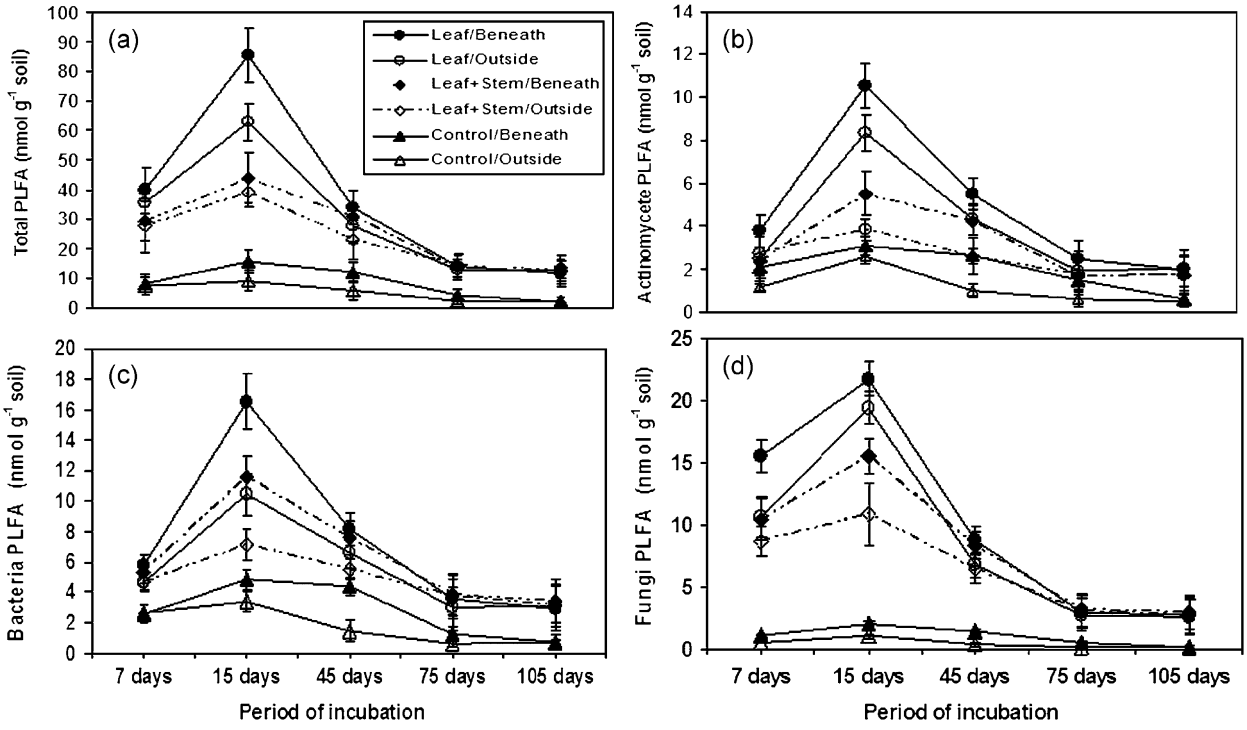

Figure 1. Total PLFA (a), actinomycete (b), bacterial (c), and fungal (d) PLFAs of soil sampled beneath and outside the canopy of *G. senegalensis* and amended with different residues. Bars are standard of deviations.

Specific markers, functional groups and stress indicators averaged for day 15 or averaged across all sample dates (data not shown) revealed that the MONO, SAT and 18:2ω6c PLFA and the SAT/MONO ratio were significantly different with respect to the residue treatments at day 15. The monosaturated PLFA and stress indicators cy19:0/18:1ω7c were also significantly different with respect to soil source ($p < 0.05$). The sum of all PLFA across days of incubation resulted in greater amounts of PLFA beneath than outside the canopy and also greater amounts of PLFA for amended soil than for non-amended soil.

Piliostigma reticulatum

As an index of microbial biomass, $PLFA_{tot}$ was higher at the first sampling date at day 7 for all treatments except for the control (Figure 2). Fungal and bacterial PLFA were higher at day 7 with 30 nmol of PLFA g^{-1} soil and 25 nmol of PLFA g^{-1} soil, respectively; whereas, the actinomycetal PLFA was highest at day 15 (15 nmol g^{-1} soil). Control soil had much lower $PLFA_{tot}$ compared with amended soil. The residue effect was significant for all samples ($p < 0.05$). PLFAs for the control soil were higher at day 15 than at day 7 for all microbial groups with the exception of the actinomycetal groups. $PLFA_{tot}$, bacterial, fungal, and

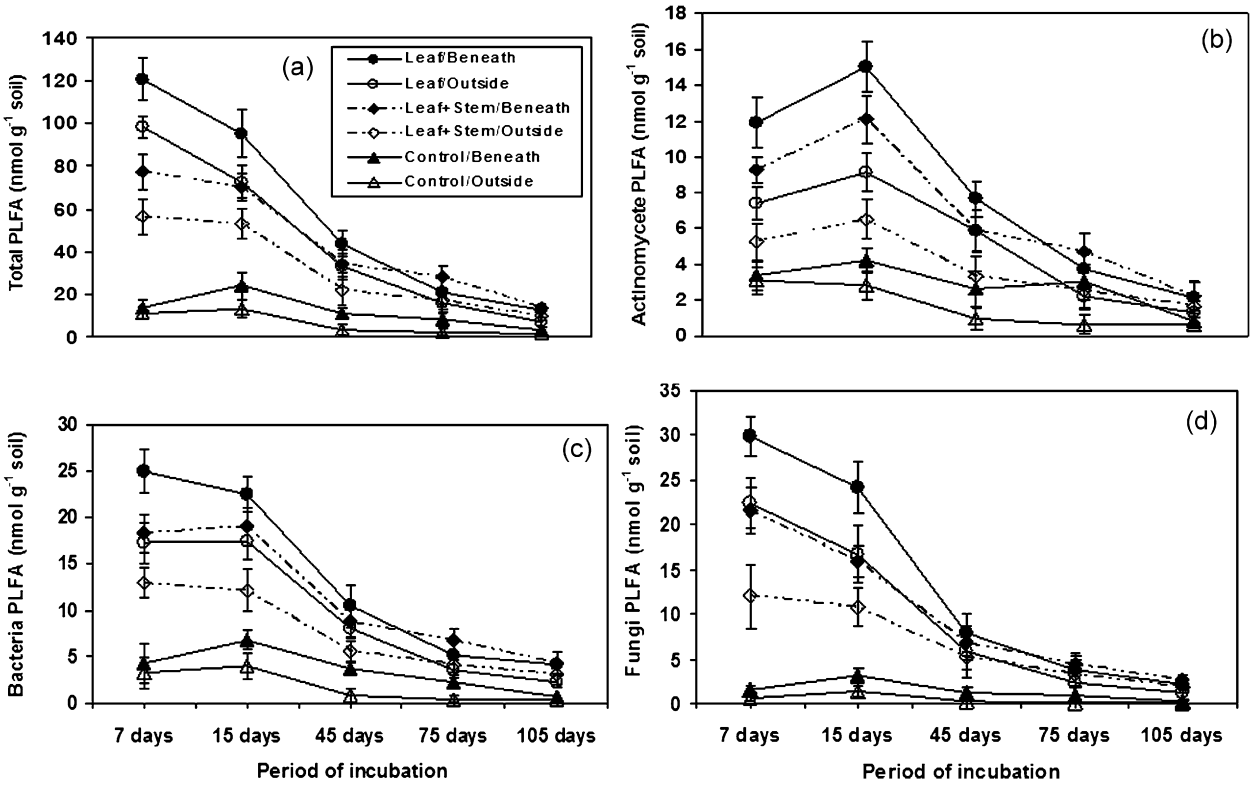

Figure 2. Total PLFA (a), actinomycetes (b), bacterial (c), and fungal (d) PLFAs of soil sampled beneath and outside the canopy of *P. reticulatum* and amended with different residues. Bars are standard of deviations.

actinomycetal PLFAs were higher beneath than outside canopy up to day 45. The location effect as well as the time effect was strong for all samples ($p < 0.001$).

Soil beneath the canopy amended with leaf litter (120 nmol g^{-1} soil) or outside the canopy (98 nmol g^{-1} soil) at days 7 and 15 had the highest amount of $PLFA_{tot}$. Overall, $PLFA_{tot}$ was significantly higher beneath than outside canopy up to day 45.

Physiological stress markers and microbial PLFA markers (nmol g^{-1} soil) were averaged at day 15 or averaged across all sample dates (data not shown). With respect to site location, SAT, MONO, and 18:2ω6c PLFA were higher from soil beneath than outside the canopy at day 15 and for $PLFA_{tot}$. PLFA markers 10 Me 16:0, 18:2ω6c and SAT and MONO PLFAs were higher on soil amended with leaf litter compared with soil amended with leaf+stem at day 15 and for $PLFA_{tot}$.

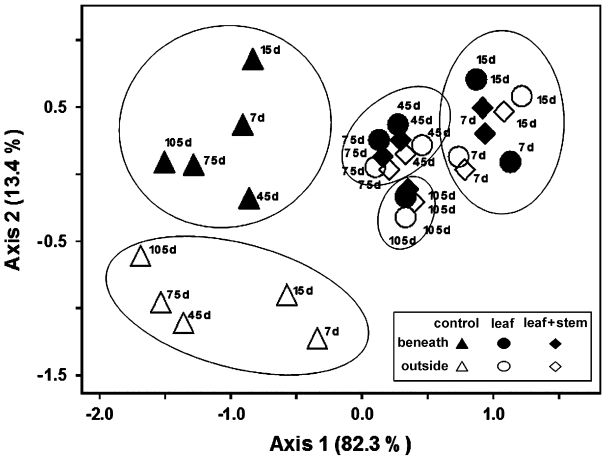

Figure 3. NMS representation of soil sample distances based on the mol% of 30 PLFA peaks extracted from soil associated with *G. senegalensis* amended with different residues and incubated over time (d = days of incubation).

Non-metric multidimensional scale (NMS) analysis

Guiera senegalensis

Non-metric multidimensional scale analysis of the data showed that 98% of the data was explained by the first two axes with the first axis explaining 82% and the second axis 13% (Figure 3). The fungal marker 18:2ω6c was highly correlated with axis one ($r = 0.94$) whereas the actinomycete marker 10 Me 16:0 was negatively correlated with axis one ($r = -0.91$). Axis two was highly correlated with the branched bacterial marker 16:1ω5c ($r = 0.72$).

Correlation among microbial PLFAs, showed that the fungal to bacterial ratio was highly correlated with both axis 1 ($r = 0.85$) and axis 2 ($r = 0.79$). The NMS scores of PLFA profiles were further analyzed using PerMANOVA. A significant difference was found between control and amended soils ($p < 0.001$). However, the difference between soils amended with leaf vs. leaf/stem mix was not significant ($p < 0.05$). The amended soil beneath the canopy tended to separate from soil outside the canopy with respect to sampling date. There was a strong difference between soil taken beneath shrubs and outside shrubs for the control soil ($p < 0.001$) and within each sampling date for amended soils ($p < 0.01$). Within unamended soil, each sampling date was strongly different from one another ($p < 0.005$).

Piliostigma reticulatum

The variation explained by analyzing PLFA (as mol%) using NMS was 87% for axis 1 and a total of 96% of the variability was explained by the first two axes (Figure 4). The marker 18:0 2OH and the fungal markers 18:2ω6c and 18:1ω9c had the highest positive correlation with axis 1, respectively ($r = 0.78$, 0.77, and 0.76), whereas the actinomycete marker 10 Me 18:0 had the strongest negative correlation ($r = -0.86$). For the second axis, the highest correlation was with the actinomycete marker 10 Me 16:0 ($r = 0.78$). FUN/BACT ratio was strongly correlated with axis 1 ($r = 0.86$) whereas Gram- bacteria and the fungal group were highly correlated with axis 2 respectively ($r = 0.67$ and 0.66, respectively).

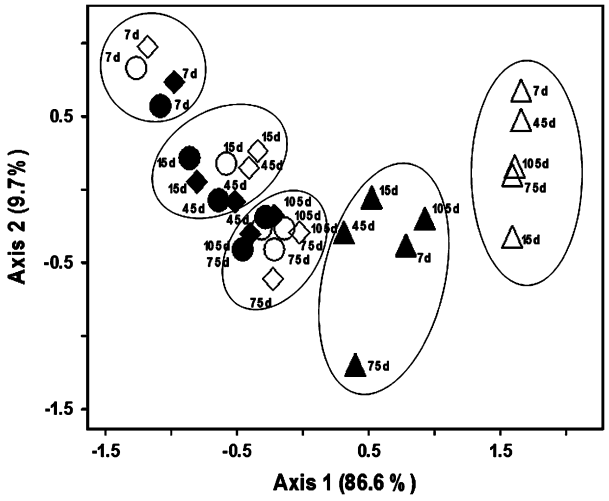

Figure 4. NMS representation of soil sample distances based on the mol% of 32 PLFA peaks extracted from soil associated with *P. reticulatum* amended with different residues and incubated over time (d = days of incubation).

The multivariate analysis (PerMANOVA) of NMS scores of all PLFA data showed a strong difference between control soil and amended soil ($p < 0.0001$). However, no difference was found within amended soils. PerMANOVA analysis showed a strong difference between soil beneath canopy and outside canopy with respect to sampling date ($p < 0.001$). For amended soil, the first sampling date was strongly different from all other sampling dates ($p < 0.001$). Conversely, no difference was found between the second and the third sampling ($p = 0.1$). Sampling dates were different from the 4th and 5th sampling ($p = 0.01$).

Simple correlations

For *G. senegalensis*, β-glucosidase activity was significantly correlated with the PLFA markers 17:0a, and 15:0 ($r = 0.68$*) and also with 15:0i ($r = 0.62$*). The fungal marker 18:2ω6c had a correlation of $r = 0.64$ with β-glucosidase activity. The marker 10 Me 16:0 had the weakest correlation ($r = 0.25$). Cellulase activity was more correlated with 17:0a ($r = 0.70$) and 15:0 ($r = 0.75$); the correlation with the fungal marker was $r = 0.64$. The weakest correlation was again obtained with the 15:1a ($r = 0.25$).

For *P. reticulatum* the fungal marker 18:1ω9c was highly correlated with both β-glucosidase ($r = 0.72$) and cellulase activity ($r = 0.84$). The fungal marker 18:2ω6c also had a higher correlation with activity of both enzymes ($r = 0.62$ for β-glucosidase and $r = 0.81$ for cellulase). The 10 Me 16:0 marker had very low correlations with β-glucosidase activity ($r = 0.18$) and cellulase activity ($r = 0.25$).

Discussion

Temporal dynamics

The temporal patterns of $PLFA_{tot}$ and the other markers were different for the two shrub residues. The greatest response was at Day 7 for *P. reticulatum* compared with *G. senegalensis* that peaked at day 15 (Figures 1 and 2). This likely reflects the higher [lignin+polyphenols contents] to [N] ratio of *G. senegalensis* over *P. reticulatum* (20.3 and 16.7, respectively) which delayed decomposition, and microbial growth. Polyphenolics are known to inhibit biological activity, decomposition, and N mineralization (Eviner and Chapin, 2003; Krauss et al., 2004; Mafongoya et al., 2004). As the decomposition proceeds only recalcitrant

materials remain, which leads to a decrease in the microbial population and organisms adapted to decompose these materials (Boufalis and Pellissier, 1994; Bernhart-Reversat, 1999; Sall et al., 2003).

The actinomycete markers were an exception to the above response to the *P. reticulatum* amendment because they peaked later (day 15) than the other major functional groups. They also tended to be higher at later stages of decomposition than the other functional groups. Actinomycetes have been shown to thrive in general, under more stressful conditions than either bacteria or fungi (Morris and Boerner, 1999).

Compared to other functional microbial groups, the fungi biomarkers had generally greater biomass between days 7–15 for soils amended with either shrub species' residue. Fungi have hyphae that allow them to fully exploit nutrient reserves as compared with place bound bacterial communities (Holland and Coleman, 1987). Saprotrophic fungi play a central role in decomposition because some possess an array of enzyme systems that can attack simple, more labile compounds such as carbohydrates and proteins as well as more recalcitrant and complex compounds like cellulose and lignin (Carlile et al., 2001). Our results support these previous studies that fungi are the most responsive group for shrub residues and are particularly important in soil beneath the shrub canopies.

Effects of shrub canopy litter and rhizosphere

Biological properties of soil from beneath and outside canopy were related to soil chemistry. The soil beneath the canopy had higher total C, total N, and C:N ratios for both plant species than soil outside the canopy. This is due to the shrub litter input and, root turnover and exudates (Boerner and Koslowsky, 1989; Boettcher and Kalisz, 1990; Bolton et al., 1993). This was reflected in higher PLFA levels in soil beneath than soil outside the influence of shrubs across all residue treatments and sampling dates. Similarly, enzyme activities were higher beneath than outside the influence of canopy.

In our study, soils amended with leaves had moderately higher amounts of microbial PLFA and greater enzyme activities than did soils amended with a mixture of leaves and stems. Substrate chemistry may influence the composition of the decomposer communities (Swift et al., 1979; Heal et al., 1997) which in turn may affect rates of decomposition (Elliott and Elliott, 1993). This was particularly true for the fungi where fungal PLFAs were significantly higher in soils amended with leaf than soils amended with leaf+stem. Leaf litter had a lower C:N ratio (20–21) compared to the leaf+stem mixture (26–27). This is consistent with studies that have shown fungi to be more sensitive to C:N ratios and organic matter input chemistry than other microbial groups (Mafongoya et al., 1996).

In contrast to the $PLFA_{tot}$ that varied widely over the incubation period, the fungal markers were more stable over time but were strongly affected by residue type. This again shows the central role of fungi in decomposition regardless of substrate chemistry. This was shown by Broder and Wagner (1988) who reported elevated fungal responses over bacteria at most successional stages of wheat straw decomposition.

The shift to fungal dominance in soil beneath the canopy and with the addition of shrub residues provides justification to develop optimized shrub-crops systems with non-thermal residue management. First this shows that soil beneath the canopy may be the best location to facilitate decomposition (i.e. promotes removal of materials that might interfere with planting and establishing crops). Secondly, the results would suggest evidence that the presence of the shrubs and incorporation of their residues improves soil quality by stimulating fungi. This is because fungi are a critical factor in improving soil quality by forming macro aggregates (Gupta and Germida, 1988) – macro aggregates being critical for aeration, root penetration, protection of C, and water holding capacity (Brady and Weil, 2008).

SAT/MONO PLFA ratio is an indicator of microbial stress (Guckert et al., 1985; Bååth, 2003). This ratio was lower beneath than outside the canopy of both species, regardless of the residue amended. The ratio cy19:0 over 18:1ω7c, which is a stress indicator, was significantly lower in soil taken beneath the canopies of both species. The stress indicators, the cy19:0 over 18:1ω7c ratio and the MONO/SAT ratio have been shown to be sensitive to management. Several studies of soil PLFA have documented an increase in monounsaturated fatty acids with increased availability of organic substrates (Bossio and Scow, 1998; Peacock et al., 2001). These results suggest microbial communities in soil beneath the canopy are under less stress than those in soil outside the influence of shrubs.

Certain PLFAs are used as stress markers because lipid utilization by microorganism is preferential for the cis-monoenoic fatty acids, likely because of their faster turnover and ease of metabolism. Thus during starvation, the molar percentages of

saturated, cyclopropyl, and trans-monoenoic acids increase and this ability to modify the cis-monoenoic acids to the cyclopropyl acids or synthesize trans-monoenoic acids or both could be a survival mechanism to maintain membrane integrity during starvation (Guckert et al., 1986). An increased ratio of cyclopropyls to their precursors in an environmental sample is an indication of physiological stress within the Gram-negative microbial community (Balkwell et al., 1988).

However, the ratio of cyclopropyl fatty acids to their precursor 16:1 or 18:1 fatty acids as a stress indicator (Knivett and Cullen, 1965; Law et al., 1963) is potentially confounded when a change in cyclopropyl fatty acid abundance occurs concurrently with a decrease in precursor fatty acids. In this case it may make it difficult to distinguish between taxonomic and physiologic changes in the community. Also the trans/cis ratio that is maintained or dominates during stress may be hindered by the occurrence of branched fatty acids in many Gram-negative bacteria (Harwood and Russell, 1984). Such inconsistent results were reported by Bossio and Scow (1998). However, our results showed stress markers were reflecting an expected stress differential between soil originating from inside and outside the shrub canopy which is consistent with many studies that utilized PLFA stress markers. For example, the higher cyclopropyl to cyclopropyl precursor ratio has been associated with a decrease in bacterial growth rates and/or an increase in C limitation or disturbance (Knivett and Cullen, 1965; Guckert et al., 1986; Kieft et al., 1997; Calderón et al., 2000; Fierer et al., 2003; Moore-Kucera and Dick, 2008). Similarly, the ratio of total saturated/total monounsaturated fatty acids has been shown to be higher in microbial communities that inhabit environments where organic C and/or nutrients are limiting (Zelles et al., 1992; Kieft et al., 1997; Fierer et al., 2003; Lundquist et al., 1999a, b; Moore-Kucera and Dick, 2008).

The higher correlations of the fungal markers (18:2ω6c, 18:1ω9c) and the actinomycete marker (10 Me 18:0) with axis 1 in NMS analysis, indicates that actinomycetal and fungal groups were important in structuring microbial communities in soil conditions of this study. However, the actinomycete markers 10 Me 16:0 and 10 Me 18:0 were correlated in opposite directions with axis 1 compared with the fungal markers. This may be due to the fact that fungi that respond to readily available C sources were higher at early stages of decomposition and decreased thereafter. Conversely actinomycetes started low and increased steadily over the course of the incubation.

The important role of fungi in the C cycle was shown by the generally high correlations of cellulase and β-glucosidase activity with the fungal markers 18:2ω6c and 18:1ω9c in soil amended with residues of either shrub species. Both of these enzymes are important in C mineralization with β-glucosidase degrading both labile and recalcitrant C forms (Bandick and Dick, 1999). Our results are consistent with Schutter and Dick (2001) who showed that fungal markers, 18:2ω6c and 18:1ω9c were stimulated by the addition of cellulose to soils.

Similar to fungi, Gram-positive bacteria markers also had a strong correlation with cellulase and β-glucosidase activities in *G. senegalensis*-amended soils. Unlike Gram-negative bacteria which colonize readily decomposable compounds, Gram-positive bacteria can thrive on more recalcitrant materials (Schutter and Dick, 2001) and under more stressed/C limited environments (Griffiths et al., 1999; Fierer et al., 2003).

In contrast to other functional groups, the actinomycete marker 10 Me 16:0 had the lowest correlation with enzyme activities. Nonetheless, as mentioned above, this marker was elevated on a relative basis over other functional groups during later stages of decomposition. This reflects their ability to dominate in periods of stress (Moore-Kucera and Dick, 2008; Killham, 1994) (this seemed to be the case in our study) and during decomposition of recalcitrant compounds such as chitin, cellulose (Killham, 1994), and hemicelluloses (Killham, 1994) which would be expected to be the materials remaining for later stages of decomposition.

Many studies have shown an increase in the relative amount of fungi vs. bacteria during decomposition (Neely et al., 1991; Beare et al., 1992; Lundquist et al., 1999b; Henriksen and Breland, 2002), but some have observed fairly constant or even declining fungal/bacterial ratios with time of decomposition (Broder and Wagner, 1988; Lundquist et al., 1999b). As decomposition proceeds, the chemical composition of the residue changes (Horwath and Elliott, 1996), this directly affects the succession of the soil microbial community. In this study, the fungal/bacterial ratio increased from 0.4–0.5 to 1–1.1 at day 15 with respect to amendment. This stands in contrast to Schutter et al. (2001) who found that fungal populations were not enhanced in soil amended with triticale or winter pea residues and studies by Lin and Brookes (1999) who found no changes in bacteria:fungi ratio in soil amended with ryegrass.

Even though the shrub residues had relatively low C:N ratio our results may be different to the

previous studies mentioned above for several reasons. In these studies, residues were from herbaceous species known to have a relatively low lignin content and to be less recalcitrant than woody species. Furthermore, our materials are high in polyphenolic compounds, which are known to inhibit decomposition.

Conclusions

Residue amendments affected microbial communities which had large temporal and successional microbial shifts during decomposition of shrub residues. Correlation analysis provided indirect evidence that residue chemistry (polyphenolic content) affected soil communities with fungi being the most closely related to litter chemistry than any other functional group.

Strong correlations of cellulase and β-glucosidase activities with fungal PLFAs provided evidence for the dominance and ability of fungi to degrade the two shrub species residues. Gram-positive bacteria had high correlations with enzyme activities and it was particularly stimulated by *G. senegalensis*-amended soils. Conversely, the actinomycetal marker (10 Me 16:0) had low correlations with enzyme activities. These results clearly show that microbial communities beneath shrubs are more diverse, less stressed, and distinctly different than communities from soil outside the influence of the two shrub species that dominate throughout the Sahel. Furthermore, these shrub soil communities appear to have more potential to drive decomposition. These results have practical implications in that the presence of shrubs enhances the decomposition process. This study provides a reasonable basis to begin developing non-thermal residue management with a goal of replacing the current destructive farming practice of burning coppiced residue every spring to prepare soils for the summer cropping season.

Acknowledgments

This study was conducted as part of the Coupled Biogeochemical Cycles/Biocomplexity Grant no. 0120732 funded by the NSF. The authors are grateful to the Senegalese National Research Institute (ISRA) and would like to thank Saliou Faye for his assistance in soil and shrub materials sampling. We are grateful to Dr. Rockie Yarwood for technical assistance, Joan Sandeno for editing and Stephanie Boyle for reviewing this manuscript.

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