PLFA and Enzyme Activities to assess the Impact of Shrub Canopy and Residue Type on Microbial Community during shrub decomposition in semi-arid Senegal

Sire Diedhiou[A], Aminata Badiane[B], Ibrahima Diedhiou[C] and Richard Dick[D]

[A]CIRAD, French Agricultural Research Center for International Development, Martinique
[B]USAID, United States Agency for International Development, Dakar, Senegal
[C]University of Thies, Thies, Senegal
[D]Ohio State University, Columbus OH USA Email: dick.78@osu.edu

Abstract
In semi-arid Senegal two shrubs dominate in farmers' fields, *Guiera senegalensis* and *Piliostigma reticulatum*, which are coppiced and burned prior to crop planting. The interactions of these shrubs with the microbial communities are uninvestigated. Our objective was to determine the influence of shrub rhizosphere and residue chemistry on the microbial community during the decomposition process. The experimental design was a 2X3 factorial design with two soil treatments (beneath and outside the influence of the shrub) and three residue amendments (leaf, leaf+stem and control). The samples were incubated in laboratory conditions with destructive samplings at days 7, 14, 45, 75, or 105 after incubation. Shrub rhizosphere on the microbial communities was stronger than the residue type effect. The fungal biomarkers were more closely correlated than other microbial groups to residue chemistry. Furthermore, Gram-positive bacteria and the fungal markers 18:2ω6c and 18:1ω9c were highly correlated with both cellulase and β-glucosidase activities. This study showed PLFA profiling of microbial communities was sensitive to temporal dynamics and residue amendments during residue decomposition and that correlation of PLFA markers with hydrolytic enzyme activities provides a means of inferring the functional role of microbial groups that dominate over time during decomposition.

Key Words
PLFA, Fungi markers, enzymes, rhizosphere, shrub litter.

Introduction
Shrubs and trees in semi-arid environments are known as islands of fertility where soil beneath the canopy is characterized by a high C and N content, as well as high microbial biomass and activity (Gallardo and Schlesinger 1995). In the semi-arid Senegal, two native and dominant shrubs, *Piliostigma reticulatum* and *Guiera senegalensis* are coppiced and burned in the spring to prepare for the next cropping period. Relatively little is known about *P. reticulatum* and *G. senegalensis* as islands of fertility and their role in decomposition. Nor has there been an attempt to develop non-thermal management systems to optimize the use of the litter for improving soil quality and crop productivity. Fundamental studies examining the shrub canopy influence on the microbial community structure and decomposition are needed. The objectives of this research were to study the structure and activity of the microbial communities during litter decomposition for *G. senegalensis* and *P. reticulatum* with respect to 1) impacts of soils from beneath or outside the shrub canopies and 2) shrub residue chemistry using PLFA and enzymes activities methods.

Materials and methods
Site Description and Laboratory Incubation Study
The experimental location was the semiarid agro-ecological zone in the semi-arid Senegal characterized by a tropical sudanian climate with potential evapotranspiration of 1800mm/yr. A Dior loamy sand was collected for *G. senegalensis* near Bambey (precipitation of 400-600 mm/yr) and similarly another set of sandy loam soil samples was collected for *P. reticulatum* near Kaolack (precipitation of 700-1000 mm/yr). Soils are low in C and N. The incubation study had a completely randomized 2 x 3 factorial design for each shrub type with two soil treatments (0-5 cm depth soil beneath or outside the shrub canopy) and three residue treatments (leaf, 60% stem plus 40% leaf, and control soil with no residue). Residues were mixed with 100 g of soil and placed in a 0.25 L plastic cup and incubated at 25°C (0.7% w/w). Soils were sampled destructively at days 7, 15, 45, 75 and 105 of the incubation period.

Phospholipids fatty acids analysis
Microbial community structure was determined by analysis of PLFA using a modified method described by Bligh and Dyer (1959). Fatty acids were extracted in three steps from 3g of triplicate sub-samples soil with a one-phase chloroform-methanol- phosphate buffer solvent. The polar lipid fraction was trans-esterified with mild alkali to recover the PLFA as methyl esters in 300 µl of hexane. PLFA were analyzed by gas chromatography (GC) (temperature ramping 120°C to 260°C at a rate of 5ºC per min). Total PLFA ($PLFA_{tot}$) was summed across each sampling date and was used as an indicator of microbial biomass.

Enzyme activities
Activities of two enzymes related to the C cycle (β-glucosidase and cellulase) were measured. Cellulase were 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). The β-glucosidase activity was determined by measuring the product *para*-nitrophenol (*p*NP) after incubation of fresh soil in the presence of the substrate, pNP-glucopyranoside as described by Tabatabai (1994).

Statistical Analysis
Effects of residue amendment and shrub canopy on microbial PLFA groups were analyzed using SAS. Shifts in PLFA profiles over time were analyzed by non-metric multidimensional scaling (NMS) using the PC-ORD. To assess the difference in community PLFA profiles according to location, substrate amendment and time of incubation, permutational multivariate analysis of variance (PerMANOVA) was performed. Amount of PLFA have been also correlated with enzyme activities as well as some specific PLFA groups using S-plus.

Results
Guiera senegalensis
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. A share of 95% of the data was explained by the first two axes using NMS analysis. The fungal marker 18:2ω6c was highly correlated with axis one ($r = 0.94$) whereas the actinomycete marker 10Me 16:0 was negatively correlated with axis one ($r = -0.91$). Correlation among microbial PLFA, showed that the fungal to bacterial ratio was highly correlated with both axis 1 ($r = 0.85$) and axis 2 ($r = 0.79$). A significant difference was found between control and amended soils ($p < 0.001$) (Fig. 1). However, the difference between soils amended with leaf vs. leaf/stem mix was insignificant. 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 soil ($p < 0.01$).

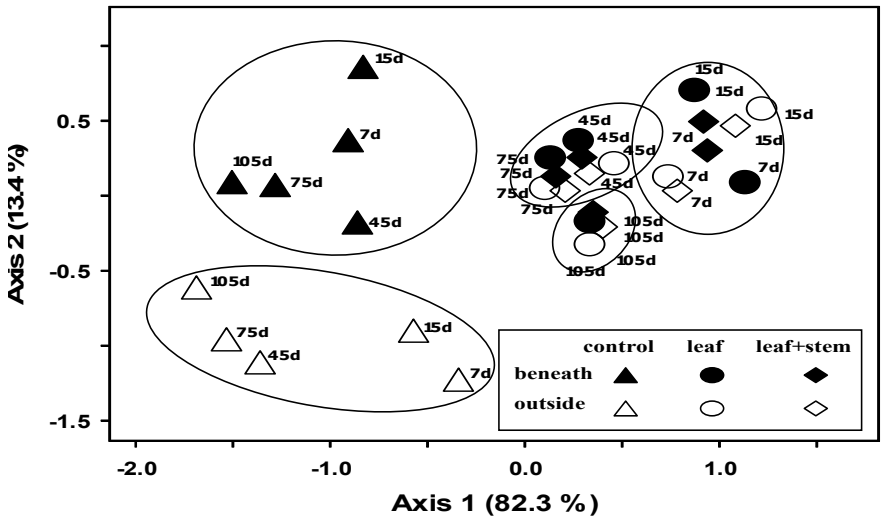

Figure 1. NMS representation of soil sample distances based on the mol % of 30 PLFA peaks extracted from soil associated with *Guiera senegalensis* amended with different residues and incubated over time (d = days of

Piliostigma reticulatum

The residue effect was significant for all samples ($p < 0.03$) with higher PLFA amount with soil amended with leaves (Table 1). $PLFA_{tot}$, bacterial, fungal and actinomycetal PLFA were higher beneath than outside canopy up to day 45. The location effect as well as the time effect was strong for all samples ($p < 0.001$). The marker 18:0 2OH and the fungal markers 18:2ω6c and 18:1ω9c had the highest positive correlation with axis 1 respectively ($r = 0.78$, $r = 0.77$ and $r = 0.76$). FUN/BACT ratio was strongly correlated with axis 1 ($r = 0.86$) whereas Gram-negative bacteria and the fungal group were highly correlated with axis 2 respectively. There were a strong difference between control soil and amended soil ($p<0.0001$) and a stronger difference between soil beneath canopy and outside canopy with respect to sampling date ($p<0.001$).

Table 1. PLFA (nmol g^{-1} soil) for soil taken beneath and outside canopy of *P. reticulatum* (n=3).

	PLFA averaged across all sampling date				
		Beneath canopy		Outside canopy	
Residue	Taxonomic groups				
Control	10Me 16:0	7.3	(0.9)	5.3	(0.4)
	18:2ω6,9c	2.3	(0.5)	1.1	(0.5)
	FUN/BACT	2.0	(0.1)	1.6	(0.2)
Leaf	10Me 16:0	23	(2.1)	15	(2.7)
	18:2ω6,9c	34	(3.0)	19	(1.1)
	FUN/BACT	4.3	(0.3)	4.2	(1.0)
Leaf+stem	10Me 16:0	21	(1.1)	13	(1.1)
	18:2ω6,9c	28	(4.0)	17	(1.0)
	FUN/BACT	4.1	(0.5)	4.1	(0.8)

Correlation of PLFA with Enzyme Activities

For *G. senegalensis* the PLFA markers 17:0a, 15:0 and 15:0i had the strongest correlation with β-glucosidase with respectively ($r = 0.68$) for the first two markers and ($r = 0.62$) for 15:0i. The fungal marker 18:2ω6c had a correlation of $r = 0.6$ with β-glucosidase. The marker 10Me 16:0 had the weakest correlation ($r = 0.25$). Cellulase activity was more correlated with 17:0a ($r = 0.70$) and 15:0 ($r = 0.75$). 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 both enzymes $r = 0.6$ for β-glucosidase, and 0.8 with the cellulase activity. The marker 10Me 16:0 is the least correlated with the β-glucosidase activity ($r = 0.18$) and also its correlation is low with cellulase ($r = 0.25$).

Discussion

Amendment with leaves had a moderately higher amount of microbial PLFA than did soils amended with a mixture of leaves and stems. Substrate chemistry may strongly influence the composition of the decomposer communities which in turn may affect decomposition of plant material (Heal *et al*. 1997). This is particularly true for the fungi where fungal PLFA were significantly higher in soils amended with leaf than soils amended with leaf + stem. In this experiment, the amount of $PLFA_{tot}$ depended primarily on the time of incubation; for the fungi group it depended primarily on the residue type. This shows again the ubiquity of the fungi to respond readily to substrate availability and in relation to the chemistry of the residues added to soils. This was also shown by Broder and Wagner (1988) who reported fungal response to residue chemistry during successional stages of wheat straw decomposition. The highest correlation of the fungal markers (18:2ω6c, 18:1ω9c) with axis 1 may be due to the fact that fungi communities responded readily to the available C sources at early stages of decomposition and then decreased thereafter. 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 for both shrubs species. Our results are consistent with Schutter and Dick (2002) who showed that fungal markers, 18:2ω6c and 18:1ω9c were stimulated by the addition of cellulose to soils.

Gram-positive bacteria markers also had a strong correlation with cellulase and β-glucosidase activities in amended soils. Unlike Gram-negative bacteria which colonize readily decomposable compounds Gram-positive bacteria can thrive on more recalcitrant materials and under more stressed environments and, furthermore can dominate in agricultural soils that typically are under more stress (Haack *et al*. 1994).

Our results were consistent with other studies that showed the influence of soil organic matter content, vegetation type and soil management on soil microbial composition (Schutter and Dick 2002; Jackson *et al*. 2003; Jandl *et al*. 2005).

Conclusion

The dominant factor that resulted in the largest shift in microbial communities is the effect of shrub canopies/rhizosphere over non-rhizosphere soil. Correlation analysis provided indirect evidence that residue chemistry affected soil communities with fungi being the most responsive to type of litter added than any other functional groups. Strong correlations of cellulase and β-glucosidase activities with fungal PLFA 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. These results clearly show that microbial communities beneath shrubs are more important and distinctly different than soils outside the influence of the two shrub species. These results have practical implications in that it appears that the presence of shrubs did enhance the decomposition process. This is a reasonable basis to begin developing non-thermal residue management with a goal of replacing the current destructive farming practice of burning residue.

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