AN ABSTRACT OF THE DISSERTATION OF

Sire Diedhiou for the degree of Doctor of Philosophy in Soil Science presented on March 21, 2007

Title: Activity, Composition and Structure of Soil Microbial Communities in Savannah Shrubs of Agroecosystems in Semi-arid Senegal

Abstract approved:

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Richard P. Dick

Piliostigma reticulatum and *Guiera senegalensis* are two native shrubs that coexist with row crops in parkland systems of the Sahel of Africa. Although permanently green all year around in soil depleted in nutrients and dry for a nine-month period, these shrubs have been largely overlooked. Conventional management of these shrubs involves coppicing and burning of aboveground biomass each spring to prepare for the summer cropping season. Previous research has shown these shubs can provide high amount of biomass carbon at landscape levels and that they can, through their roots, move water from the wet subsoil to the dry surface soil at night when photosynthesis stops (hydraulic redistribution). However, the influence of these shrubs on the soil microbial communities and their role in biogeochemical processes is largely unknown. This dissertation reports studies that have been carried out to investigate the impact of these shrubs on the soil microbial communities. The rhizosphere effect of these two dominant shrubs was investigated during both the rainy season and dry season

by studying the soil microbial structure, composition and activity. This was done by: 1) profiling microbial communities through phospholipids fatty acid (PLFA) analysis and denaturing gradient gel electrophoresis (DGGE); 2) assaying enzymes (acid phosphatase, β-glucosidase, cellulase, chitinase, urease); and 3) measuring microbial biomass carbon (MBC) and mineral nitrogen dynamics.

Decomposition studies were conducted to determine the potential of non-thermal management of shrub residues. This was done by determining the influence of shrub canopy, macrofauna, and residue type on shrub litter decomposition and microbial dynamics under field and laboratory conditions.

Microbial communities were more diverse, more active and had greater biomass in shrub rhizospheres. The rhizosphere communities during the dry season were similar to the rhizosphere and bulk communities during the wet season. This suggests that shrub rhizosphere provide root exudates and/or water via hydraulic redistribution that supports microbial communities during the dry season. PLFA and enzyme activities were highly correlated and were more sensitive than DGGE in distinguishing the communities temporally and spatially. PLFA profiling showed that the rhizosphere effect was dominated by fungi and Gram-positive bacteria communities, and stimulated acid phosphatase and β-glucosidase activities.

Macrofauna access to shrub residue resulted in higher decomposition rates with 70 to 90% loss of mass after 210 days. There was an increase of MBC and enzyme activities when macrofauna were allowed, and beneath shrub canopy as opposed to outside of the canopy. In a laboratory incubation study, the influence of shrub

rhizosphere/canopy on soil was greater than the residue effect on the activity of soil microbial communities.

Decomposition of residues showed that leaf litter had a greater impact on soil microbial communities and enzyme activities than did stem materials or stem/leaf mixes. Cellulase and β-glucosidase were highly correlated with the fungal markers and Gram-positive bacteria markers.

In conclusion, the results showed that *Piliostigma reticulatum* and *Guiera senegalensis* are stimulating microbial activity and communities even in the dry season after six or more months without rainfall. Besides C inputs through litter fall, root turnover and exudates, this suggests that shrubs maintain moisture levels in the dry season for microorganisms by performing hydraulic redistribution of water from wet subsoils to dry surface soils at night through a passive water potential gradient. Consequently, shrubs are maintaining soil health and can drive biogeochemical processes year round which has not been previously recognized. These results provide a foundation to actively manage these shrubs to conserve Sahelian landscapes and to optimize agricultural productivity.

Activity, Composition and Structure of Soil Microbial Communities in Savannah Shrubs of Agroecosystems in Semi-arid Senegal

by

Sire Diedhiou

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

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CONTRIBUTION OF AUTHORS

Sire Diedhiou conducted research, analyzed data and wrote each manuscript.
Dr. Richard Dick and Dr. Aminata Niane-Badiane assisted with research design, data analyses and reviewed each manuscript.

There are various authors who made substantial contributions to the three manuscripts presented in this dissertation. The contributing authors for these manuscripts are presented below.

Manuscript 1: Spatial and temporal distribution of soil microbial community composition and activity in rhizospheres of indigenous shrubs of semi-arid Senegal

Authors: S. Diedhiou, A.N. Badiane, K.B. Assigbetsee, I. Diedhiou and R.P. Dick

Manuscript 2: Microbiology and macrofaunal activity relative to rates of indigenous shrub residue decomposition in farmers' fields of Senegal

Authors: S. Diedhiou, E.L. Dossa, A.N. Badiane, K.B. Assigbetsee, I. Diedhiou, A.N.S. Samba, M. Khouma, M. Sène and R.P. Dick

Manuscript 3: Succession of soil microbial communities during decomposition of native shrub litter of Sahelian agro-ecosystems

Authors: S. Diedhiou, A.N. Badiane, I. Diedhiou and R.P. Dick

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CHAPTER 1

GENERAL INTRODUCTION

Sub-Sahelian Africa is part of semi-arid Savanna landscape that has parkland cropping systems. The Sahel is characterized by a low and sporadic rainfall regime which occurs within three months during the summer wet rainy season. Temperatures are high throughout the year averaging 22.8°C in December-February to 32°C in April-June. As a result of the semi-arid ecosystem, productivity is primarily controlled by water availability. Shrubs, associated with vast expanses of seasonal grass cover during the summer rainy season or bare soil, are the components that dominate in this region (Dancette and Sarr, 1985; Breman and Kessler, 1995).

With the increasing rural populations, the accessibility of land and fallowing periods are reduced (Floret and Pontanier, 1993). Soils are no longer given time to replenish nutrients to improve their quality, and consequently, they are being depleted in nutrients (Pieri, 1989; Feller, 1995; Bationo et al., 1998; Badiane et al., 2000a; Bationo and Buerkert, 2001). To meet the food demands of rapidly increasing populations, the area of cultivation has increased in the last years by clearing savanna shrubs for cropping (Ramankutty, 2004). The combined effects of drought, overpopulation and land use change are likely to increase the depletion of soil nutrients and degradation of the vegetation (Breman and Traoré, 1986; Bationo and Buerkert, 2001). This leads to a decrease in soil organic matter, which causes a reduction in soil structure and thus increases susceptibility to wind and water erosion, and reduced crop productivity (Bationo and Buerkert, 2001; Dossa, 2007).

A substantial increase in yield would be expected from correcting soil nutrient deficiencies which has been confirmed experimentally (Pieri, 1989). The standard approach is to use commercial fertilizers to correct nutrient deficiencies. However, socio-economic conditions limit the use of fertilizers and machinery which are unavailable and expensive in the Sahel. Thus, to maximize yields and fertilizer efficiency, regular inputs of organic residues are needed (Bationo et al., 1998; Badiane et al., 2000b). Green manure and organic amendments in crop rotations provide an alternative and less expensive solution to increasing soil organic matter quality and fertility (Wani et al., 1995; Samba, 2001).

Agroforestry systems with trees have shown potential for improving crop production in low-input systems (Ganry et al., 1989; Breman and Kessler, 1995). In semi-arid climates it is common to find higher fertility in the topsoil under tree canopies than in adjacent open land, as indicated by the increased availability of nutrients and organic matter; soil fertility generally decreases significantly from the center to outside the tree canopy (Young, 1989; Belsky et al., 1990; Dossa, 2007).

In Senegal and throughout neighboring Sahelian countries there are shrubs with the characteristic of regrowing every year in farmers' fields after the cropping season. In some areas two predominant shrubs *Guiera senegalensis* and *Piliostigma reticulatum* nearly cover the landscape but in others there is a less dense distribution (Lufafa, 2006). These species provide leaf/stem litter, root mass and root activity, which provides organic matter, conserves nutrients, builds structure, and stimulates

biological activity in soils. In farmers' fields, the shrubs are cut at the soil surface and burned just prior to the rainy season.

Under current management, this organic matter is not being utilized effectively. Burning the residue reduces the amount of C and N returned to soils, does not build organic matter, and would be largely unavailable for biological activity (i.e. as charcoal). Increasing the availability of nutrients by adding organic matter input, to avoid or correct reduction in soil fertility, is the primary technological solution to develop sustainable agricultural systems in semi-arid area. Keeping plant residues in the field is a critical component of soil management, not only for nutrient value, but also for soil protection from wind and water erosion. Incorporating plant residues increases the proportion of primary production returned to or retained by the soil.

Plant residues play a significant role in providing a positive environment for improving soil microbial communities, which in turn play a significant role during the decomposition process of organic materials (Sinsabaugh and Moorhead, 1994). Activities and diversity of soil microorganisms drive nutrient mineralization for plant uptake. Soil fauna also has been shown to improve the growth and conditions of plants (Beare et al., 1992; Tian et al., 1995). If farmers are to adopt non-thermal management of these residues to promote C sequestration and soil quality, alternative systems are needed that depend on biological decomposition. Otherwise residues may accumulate that interfere with cropping operations.

An advantage of *G. senegalensis* and *P. reticulatum* is that studies have shown these shrubs were able to redistribute water from the wet sub-soil to the dry surface

soil especially during the dry period (Kizito et al., 2006) which could affect microbial communities and biogeochemical processes. Fundamental information is needed on how these shrubs affect soil and ecosystem processes with an ultimate goal to develop more sustainable management systems, e.g. intercropping of perennial shrubs with food crop annuals.

The purpose of this thesis, which is composed of three main parts, was to investigate the impact of *G. senegalensis* and *P. reticulatum* on soil microbial community. Chapter 2 compares soil microbial communities in shrub rhizosphere, bulk soil and non-rhizosphere soil through denaturing gradient gel electrophoresis (DGGE) and phospholipids fatty acid (PLFA) as indicators of microbial community structure, enzyme activities, microbial biomass carbon (MBC) and mineral N. Chapter 3 is a litterbag field study on the influence of these shrubs, the residue type, soil microorganisms and macrofauna influence on residue decomposition rate. In the 4th chapter, the decomposition under laboratory conditions of shrubs residue was studied by characterization of PLFA and enzyme activities.

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CHAPTER 2

Spatial and Temporal Distribution of Soil Microbial Community Composition and Activity in Rhizospheres of Indigenous Shrubs of Sahelian Agro-ecosystems

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Abstract

The Sahel is dominated by the presence of permanent shrubs all year around regardless of the fact that they are coppiced many times during the year. In Senegal, shrub species, *Guiera senegalensis* and *Piliostigma reticulatum*, redistribute water from the deep soil to the surface which benefits other plants. The shrubs also have been shown to contribute to a higher nutrient content beneath their canopy. However, the influence of these shrubs through root exudates and hydraulic redistribution on the soil microbial communities remains unknown. This study was carried out to determine the impact of the shrubs on the activity, structure and composition of the microbial communities and how those communities are modified during the dry season as well as the wet season. There were two experimental locations: an area with 300 mm of rainfall a year and an area with 700 mm a year.

The experimental design was a 2 x 2 factorial design with three soil treatments (rhizosphere soil, bulk soil, and non-rhizosphere soil sampled two meters away from the shrub) and two seasons (wet season and dry season); the wet season lasts only 3 months. The rhizosphere soil was separated depending on the age of the roots for the denaturing gradient gel electrophoresis (DGGE) analysis of 16S DNA and 28S DNA. Additionally, the soil was subject to phospholipids fatty acid (PLFA) analysis, enzyme activities, microbial biomass carbon (MBC) and nitrogen (N) mineralization. For the DGGE profiling, the bacterial community responded more to the rhizosphere effect whereas the fungal community was more sensitive to the season. There were strong rhizosphere and seasonal effects on PLFA, MBC, enzyme activities and mineral N.

Furthermore, the rhizosphere communities during the dry season were quite similar to the one during the wet season. This study showed the profound modification of the microbial communities due to the presence of shrubs through root exudate input and hydraulic redistribution.

2.1. Introduction

The close proximity of two native shrubs, *Guiera senegalensis* and *Piliostigma reticulatum,* to crop species in the Sahel has important implications for the functioning of these semi-arid agroecosytems. Data collected in Senegal showed that shrub roots can redistribute water from the wet subsoil to the surface, a phenomen known as hydraulic redistribution (Kizito et al., 2006). Hydraulic redistribution is the movement of water from regions of higher soil water potential to regions of lower one via plant roots, and typically it is characteristic of semi-arid to arid environments (Richards and Caldwell, 1987) as well as mesic environment during period of drought (Dawson, 1993; Caldwell et al., 1998). At some point the rhizosphere soil would be wetter than soil some distance from the root, due to the efflux of water from the root.

Water redistributed can be as high as 0.1 mm day^{-1} for *P. reticulatum* and 0.2 mm day^{-1} for *G. senegalensis* (Kizito et al., 2006). The magnitude of hydraulic redistribution has been debated. While some studies concluded that the quantities of water redistributed by this mechanism are minute (Song et al., 2000), others concluded that hydraulically redistributed water has a significant impact on plant drought tolerance and total water utilization; it may enhance seedling survival and maintain

overstory transpiration during summer drought (Wan et al., 2000; Brooks et al., 2002). Hydraulic redistribution could be important to drive microbially mediated biogeochemical processes during the long dry season because moisture conditions close to the root control the activity of rhizosphere micro-organisms (Parke et al., 1986).

Moisture, composition, amount and diversity of organic root exudates contribute in determining the composition, structure and activity of the rhizosphere communities (Lynch and Whipps, 1990). A large proportion of the rhizosphere carbon is in the form of water-soluble substances such as sugars, organic acids and amino acids. The differences in type and quantity of carbon available in different root zones thereby select for distinct rhizosphere community structure (Young, 1998; Yang and Crowley, 2000). In addition, these communities may vary with respect to the plant species (Westover et al., 1997). Rhizosphere microbial communities can be regarded as a subset of the soil microbial communities; therefore they are influenced by soil chemical and physical properties.

Suitable methods to characterize the rhizosphere communities have been developed from culturable to unculturable techniques, but they all have limitations. Molecular techniques based on PCR have been used to overcome the limitations of culture-based methods; however, they are not without their own limitations. Denaturing gradient gel electrophoresis (DGGE), which is a DNA-based technique, was first applied to bacterial communities by Muyzer et al. (1993) and is now widely used. DGGE analysis is run from amplified DNA of the whole communities, either

dead or alive; thus it does not allow a characterization of the living communities, but does allow a profiling of the dominant communities.

The living communities can be characterized using phospholipids fatty acid (PLFA) analysis. The utility of PLFA as a measure of viable biomass is based upon their presence in the membranes of all living cells, with the exception of the Archaea (Langworthy, 1985), and their rapid turnover upon cell death (White et al., 1979). Each species in the microbial community contributes to the PLFA profile in proportion to its biomass. Furthermore, total PLFA content has been shown to correlate well with other measurements of microbial biomass in mineral soils and forest floors (Zelles et al., 1992; Bailey et al., 2002; Leckie et al., 2004). However, PLFA profiles do not give any information on species composition, rather, they divulge the fingerprint of community structure and have been widely used to study community structure in a varied range of systems including rhizosphere soil (Buyer et al., 2002; Butler et al., 2003).

PLFA analysis is considered to be a robust tool that consistently discriminates among communities of different origin with different activities, and land management strategies (Zelles, 1999; Bossio et al., 2005). Temporal changes in soil microbial activity can be assessed using enzyme activities (Dick, 1997; Burns and Dick, 2002; Tabatabai and Dick, 2002). Enzyme activities can represent the ability of microoganisms to perform specific biogeochemical reactions or processes.

The objective of this study was to determine to what extent the presence of shrubs modify the soil microbial community composition, structure, and activity in the

rhizosphere and how this may change over seasons. We hypothesize that the hydraulic redistribution would maintain a moist environment around roots which would sustain at some level the activity of the communities especially during the long period of drought. Consequently, effects of dry periods on community composition and structure will be buffered against water stress.

2.2. Materials and methods

2.2.1. Experimental design

Soil samples were collected during both the dry season, March 2005, and the wet season, August 2005 in two ecological regions characterized by two different shrub species: *P. reticulatum* (annual precipitation at this site 700-1000 mm/yr) and *G. senegalensis* (annual precipitation of 400-600 mm/yr). The mean air temperatures are high, 32ºC. In each experimental site, soils were sampled randomly under six plants at four locations: in the vicinity of old roots (O) with diameter > 2 mm; in the vicinity of new roots (N) with diameter < 1 mm; in the bulk soil between roots beneath shrub (B); and two meters away from the shrubs or non-rhizosphere soil (NR). All NR and rhizosphere soils were sampled at 0-10 cm depth. The soil samples from two plants and associated NR soils were composited, homogenized, crushed to pass a 2-mm sieve, and then maintained at field moisture and stored at 4°C until analyzed. This resulted in three field replicates. All analyses were completed within one week of sampling. Soils from old and new roots were pooled for all analyses except for the DGGE analysis.

2.2.2. Laboratory Analyses

2.2.2.1. Phospholipids fatty acids analysis

Phospholipids fatty acids analysis (PLFA) was used to characterize soil microbial community composition using a modified method described by Bligh and Dyer (1959). The extraction of PLFA was done in three steps using 3 g of triplicate subsamples with a one-phase chloroform-methanol-phosphate buffer solvent. In the second step, PLFA were separated from neutral lipids and glycolipids using silicic acid columns (Supelco, Bellefonte, PA, USA). The polar lipid fraction was transesterified with mild alkali to recover the PLFA as methyl esters in 300 µl of hexane (Guckert et al., 1985). The FAME tridecanoic acid (13:0, Supelco, Inc.) was added in various concentrations as an internal standard. PLFA were analyzed by gas chromatography (GC) (Agilent Ultra 2 column; temperature ramping 120 to 260°C at a rate of 5ºC per min) using helium as the carrier gas and peaks were detected by flame ionization detector (Frostegård and Bääth, 1996).

Individual fatty acid methyl esters (FAME) 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.). Each individual fatty acid was expressed as a percentage of the total amount of fatty acids (mol %) found in a given sample. PLFA data were presented as the percentage of the total

PLFA detected within a sample for multivariate analysis and PLFA with less than 0.5% of the total relative abundance were not included in the data set.

Standard nomenclature rules were followed when referring to different fatty acids (Frostegard et al., 1993) in the form of A:BωC, where A designates the total number of carbons, B the number of double bonds, and C the distance of the closest unsaturation from the aliphatic end of the molecule. The suffixes "-c" for cis and "-t" for trans refer to geometric isomers. The prefixes "i-," "a-," and "me-" refer to iso-, anteisomethyl branching, and mid-chain methyl branching, respectively, with cyclopropyl rings indicated by "cy" (Kates, 1986). PLFA biomass was estimated by adding the amount of all fatty acids detected and was expressed in nanomoles of PLFA per g of dry weight of soil (nmol g^{-1} soil) (White et al., 1979; Frostegård et al., 1991; Bossio et al., 1998). For each microbial group and total PLFA ($PLFA_{tot}$), the total amount of PLFA identified was calculated to represent their contribution to the total microbial biomass.

A total of 23 PLFA markers out of 27 identified which represent 91% of the total PLFA were used for the multivariate analyses for *G. senegalensis*. For *P. reticulatum*, we used a total of 23 PLFA out of 29 identified (94%) for multivariate analysis. PLFA biomarkers used in this study to characterize the different groups of organisms are represented in Table 2.1. The ratio of signature fungal to bacterial fatty acids (FUN/BACT) which has often been used as an indicator of the change in the soil microbial community structure was included in the data analysis (Bardgett et al., 1998; Olsson, 1999; Zelles, 1999; Fierer et al., 2002). Stress indicators PLFA were

computed because microbes respond to various stresses by modifying cell membrane fluidity through increasing the branching and degree of saturation (monounsaturated versus polyunsaturated) of membrane lipids. The ratio cy 19:0/18:1ω7c has been suggested as an indicator of water stress primarily (Guckert et al., 1986; Lundquist et al., 1999a) and the ratio SAT/MONO mainly is characteristic of nutrient deprivation **(**Bossio and Scow, 1998; Larkin, 2003**)**.

2.2.2.2. Enzyme activities

All soil enzyme analyses were performed on well-mixed soil from which all visible plant material was removed. The activities of acid phosphatase, β-glucosidase, chitinase and urease were measured. Those enzymes are related to biogeochemical cycles of phosphorus, carbon and nitrogen.

The β-glucosidase and chitinase activities were measured using a modified method originally described by Hayano (1973) and Ndour et al. (2001). Briefly, 100 mg of fresh soil were incubated for 2 h at 37°C, with 100µl of 5mM *para*-nitrophenyl β-d-glucopyranoside substrate for β-glucosidase and *para*-nitrophenyl *N*-acetyl glucosamide (5 mM, Sigma) as the substrate for chitinase. Citrate phosphate (MacIlvain, 1921) at pH 5.8 was used to buffer the mixture. The *para*-nitrophenol (ρNP) released was measured 15 min after stopping the reaction at 400 nm for both enzymes. The results were expressed as µg ρNP released g^{-1} h^{-1}. Phosphatase activity was determined with the same principle using the method described by Tabatabai and Bremner (1969) where the ρNP released was quantified at 420 nm. The method by

Kandeler and Gerber (1988) was used to determine the urease activity where 1 g of soil was incubated with urea as a substrate. The ammonium released was measured colorimetrically with a spectrophotometer set at 660 nm.

For all enzymes, substrate was added to blank samples after incubation and read directly. A blank mixture with soil and buffer, but no substrate, was incubated in the same conditions as above and analyzed to account for the background levels of products or color developments due to reagents or soils. Two analytical replicates and one control were analyzed for each soil sample.

2.2.2.3. MBC and mineral N

Microbial biomass N was determined by chloroform–fumigation extraction (CFE) using a modification of the method of Amato and Ladd (1988). Briefly, 10 g of the moist soil was fumigated with chloroform (ethanol free) and then incubated for 10 days. Fumigated samples and unfumigated control samples were extracted with 38 mL of 2 M KCl solution for 60 min on a rotary shaker. After filtration, 2 mL of the filtrate were mixed with 0.5 mL of 0.4M sodium citrate solution. Ninhydrin-reactive N was determined by colorimetry at a wavelength of 750 nm (Schinner et al., 1996) (Evolution II, Alliance-Instrument, France). The gain in ninhydrin-reactive N after fumigation was multiply by the conversion factor (21) to estimate MBC (Amato and Ladd, 1988). Results were expressed as μg C g^{-1} of dry soil.

Soil inorganic N was quantified in the same extract (unfumigated control) colorimetrically in KCl extracts (2 M KCl) using Bremner's method (Bremner, 1965).

Ammonium was quantified using the reaction of Berthelot modified with the indophenol blue at a wavelength of 660 nm. Hydrazine sulfate is used to reduce the nitrate to nitrite in presence of copper sulfate. Nitrite is then mixed with the sulfanilamide to form a diazo complex and the absorbance was read at a wavelength of 525 nm. Inorganic nitrogen was assessed as the sum of ammonium and nitrate and the results were expressed as μg N g^{-1} of dry soil.

2.2.2.4. Denaturing Gradient Gel Electrophoresis (DGGE)

Changes in soil microbial community structure were followed by DGGE of PCR products from 16S rRNA gene for bacteria and 28S rRNA gene for fungi. The method by Porteous et al. (1997) was used to extract soil DNA from triplicate of 0.5 g of soil by adding 0.2 g glass beads (Sigma, 0.1 mm) and 1 mL lysis buffer (0.25 M NaCl, 0.1 M EDTA; pH 8) using a Bead-beater$_{TM}$ (Biospec products).

Bacterial 16S DNA was amplified using universal bacterial DGGE primers 338f-GC clamp and 518r primers (Muyzer et al., 1993). Amplification was performed by 5 min of denaturation at 94°C and then 30 cycles of 45 s each at 94°C, 45 s at 52°C and 1 min at 72°C, followed by a final extension at 72°C for 10 min using a PTC-100 thermal cycler. The primers 403f-GC (U1) and 662r (U2) described by Sandhu et al. (1995) were used to amplify the 28S DNA of the fungal population. The following cycle was used to amplified fungi 28S DNA: 5 min of denaturation at 94°C and then 35 cycles of 30 s each at 94°C; 1 min at 52°C and 2 min at 72°C; followed by a final extension at 72°C for 10 min using the same thermal cycler. The PCR taq “Ready- to-

go" (Amersham Biosciences, USA) was used to amplify both the 16S and the 28S DNA. PCR products were confirmed in a 1.0% agarose gel and stained with ethidium bromide solution (0.25 μg mL^{-1}) which makes amplified products fluorescent under UV. PCR products were then quantified using a spectrophotometer to measure the optical density.

Equivalent quantities of PCR products were resolved in an 8% polyacrylamide gel (37.5:1 acrylamide:bis-acrylamide) in 0.5× TAE (20mM Tris-HCl, 10 mM acetate, 0.5 mM EDTA) and denaturants (100% denaturant contains 7 M urea and 40% deionized formamide). A gradient of denaturants ranging from 40% to 70% was used to compare bacterial communities whereas for the fungal, the gradient used was wider and ranged from 35% to 65%. Electrophoresis was performed on an Ingeny apparatus (Ingeny phorU, Netherlands) at a constant voltage of 75 for 16 hours. After electrophoresis, gels were stained with ethidium bromide and visualized under UV light.

Given that the amplified fragments from different organisms are similar in length, melting behavior is related to the sequence composition. DNA will melt less easily in regions with many guanine and cytosine nucleotide base pairs which are held together with three hydrogen bonds, than in regions with high concentrations of adenine and thymidine bases which are held together by only two bonds. Once a fragment reaches a concentration of denaturant sufficient for denaturing to begin, the double strands begin to separate and this causes the fragment to cease migrating. The GC clamp avoids a complete separation of the bands. The fingerprint is then

comprised of different fragments migrating to different points in the gel. The software Bio-profil Biogene program (Vilber Lourmat) was used to analyze the data. This software allows detection and then identification of bands. The detected bands were used to construct a matrix indicating presence or absence of bands in each sample.

2.2.3. Statistical analysis

Rhizosphere and seasonal effects on microbial grouping PLFA were determined by linear regression (SAS Institute Inc., 1996). Spatial and temporal shifts in the composition of PLFA profiles were analyzed by principal components analysis (PCA) using the PC-ORD package (MjM Software Design, Gleneden Beach, OR) (McCune and Grace, 2002). Scores of samples on the first two axes were then analyzed by ANOVA. Before the analysis, PLFA data were converted to mol % of peak totals. For the DGGE analysis, matrices of presence and absence of bands were subject to ordination analyses to identify groups of similar samples, or structure in the dataset (Fromin et al., 2002). To assess whether community enzyme activities differed spatially and temporally, samples were analyzed using SAS software. In addition, correlations between PLFA and enzyme activities were investigated using the Mantel test (McCune and Grace, 2002).

The ability of an ecosystem to withstand extreme disturbance may depend in part on the diversity of the system. Diversity is a function of two main components: species richness or abundance and species evenness or equitability (Whittaker, 1972).

Species richness, (S), is the number of species counted in the sample, which monotonically increases with the true number of species in the community (Magurran, 1988).

For PLFA analysis, S was the number of the PLFA detected in a sample and for DGGE analysis, S was the number of 16S DNA or 28S DNA detected bands within a sample. Evenness (E) refers to the equitability of abundance. Shannon's diversity index (H') (Shannon and Weaver, 1949), represents the uncertainty in predicting the population number of an individual chosen at random (Ludwig and Reynolds, 1988), this index increases which increasing diversity. Simpson's diversity index (D) (Simpson, 1949) is the probability that two individuals chosen at random will belong to the same population (Ludwig and Reynolds, 1988).

2.3. Results

2.3.1. PCA analysis of DGGE profile from bacterial and fungal communities

Analysis of DGGE bands from 16S DNA bacteria profile (Fig. 2.1) using PCA (Fig. 2.2A) explained a total of 33% for the first two axes. Samples from the rhizosphere soil clustered and separated from the non-rhizosphere one (ANOVA $p < 0.01$). There was also a separation based primarily of the origin of the sample, whereas there was minimal separation due to season. The comparison of the bacterial community associated with the new roots and old roots for both species (Fig. 2.1) showed that the number of bands were higher and more intense (qualitative visual observations) in soil associated with new roots than soil associated with old roots

during the wet season. During the dry season, intensity of bands was similar and there was a small weak difference for the number of bands between those two communities.

For the fungal community, PCA analysis of bands explained 33% of the data. Samples from the dry season separated from other samples. This was the opposite of bacterial samples where there was a weak seasonal effect. The PCR-DGGE profiling resulted in a poor separation of fungal communities across rhizosphere location between new roots and old roots (DGGE profile not shown) compared to the bacterial communities that did show separation for this comparison (Fig. 2.2B). During the dry season, fungal community in the rhizosphere soil was similar to the one in the bulk soil. The opposite was observed during the wet season where there was a separation between rhizosphere and bulk soil samples. Additionally, the PCA of the fungal community resulted in a strong separation of samples based on their association with shrub species and soil type during the wet season.

2.3.2. PLFA analysis of the soil microbial communities

2.3.2.1. Bacterial (Gram+ and Gram-), fungal, and actinomycetal and $PLFA_{tot}$

The amount of bacterial, fungal and actinomycetal PLFA in the rhizosphere of both *G. senegalensis* and *P. reticulatum* was significantly higher regardless of the season (Fig. 2.3). However, during the wet season, bulk soil had the same amount of PLFA as did rhizosphere soil; this result was different for the dry season where PLFA from the bulk soil was significantly lower than PLFA from the rhizosphere soil.

During the dry season there was an average increase of 80% for the fungal PLFA between the rhizosphere and bulk soil. Conversely, in the wet season there was only a 20% difference between these two locations. The same level of increase was observed for the Gram+ bacteria (65%) between these locations in the dry season.

Microbial groups had the same amount of PLFA for non-rhizosphere and bulk soil which the exception of the Gram- and fungal groups for soil associated with *P. reticulatum* during the dry season (Fig. 2.3). $PLFA_{tot}$ was significantly lower for the bulk soil than the rhizosphere soil and was not different at all from the non-rhizosphere soil (Fig. 2.4). $PLFA_{tot}$ was higher in the bulk soil than in the rhizosphere soil during the dry season.

2.3.2.2. Stress Ratio Indicators

The stress indicator cy 19:0 to 18:1ω7c was high for the non-rhizosphere soil in the dry and the wet season for soil associated with both shrubs (Fig. 2.5). The same trend was observed for the ratio SAT/MONO PLFA. However, during the dry season, bulk soil and rhizosphere soil tended to show the same level of stress for *G. senegalensis* (Fig. 2.5). In comparison, during the wet season the stress was significantly higher for the the non-rhizosphere soil than the bulk soil for both species. The same trend was observed for the ratio cy 19:0/18:1ω7c for soil associated with *P. reticulatum* (Fig. 2.5). For the SAT/MONO ratio, there was a higher stress in the bulk soil than in the rhizosphere soil.

The FUN/BACT ratio was higher in rhizosphere soil compared to non-rhizosphere soil; it was also higher during the wet season as opposed to the dry season for both species. However, during the wet season the FUN/BACT ratio of the bulk soil equaled that of the rhizosphere soil and, in the dry season this ratio was the same for bulk and non-rhizosphere soils.

2.3.2.3. Principal component analysis of PLFA data

The total variance explained by the first two axes is 65% with the first axis explaining 47% of the community structure (Fig. 2.6A). The strongest factor in structuring the communities was the location of the soil sample; non-rhizosphere vs. rhizosphere soil ($p<0.001$). PCA analysis showed that the communities from the bulk soil during the wet season were similar to that of the rhizosphere soil. Samples from the non-rhizosphere soil clustered, but within this cluster they separated primarily following the season and then by the shrub species.

Fungal biomarker 18:2ω6c had the highest correlation with axis 1 ($r = 0.89$) followed by the actinomycetal biomarker 10Me 18:0 which had a correlation of $r = 0.86$. Microbial biomass carbon, mineral N and the moisture were also correlated with the rhizosphere. This is the opposite of the stress indicator cy 19:0/18:1ω7c which showed a correlation with the bulk and non-rhizosphere soils. Fungi and Gram+ bacteria had the highest correlation with the rhizosphere samples during the wet season (Fig. 2.6A).

2.3.3. Enzyme activities, MBC and mineral N

Acid phosphatase, β-glucosidase and chitinase activities were higher for the rhizosphere soil than the bulk soil (Table 2.2); the bulk soil had significantly higher activities than did the non-rhizosphere soil. This is true for both seasons for those enzymes except the chitinase activity which during the dry season was the same whether it was a bulk soil or a non-rhizosphere soil. For urease, activity was significantly higher in rhizosphere soil than in bulk and non-rhizosphere soil only during the dry season. During the wet season, this activity was the same in the bulk and rhizosphere soil but significantly different from the non-rhizosphere soil.

Microbial biomass carbon was significantly higher in rhizosphere soil than in non-rhizosphere soil and during the wet season than during the dry season for both types of soil (Table 2.2). Mineral N remained at the same value for the rhizosphere and the bulk soils; however the value was higher during the wet season than during the dry season.

Principal component analysis of enzyme results showed that the first axis explained most of the variance with 91% out of a total of 98% for the combination of the first two axes (Fig. 2.6B). Microbial communities were clearly separated depending on the location of the soil sample ($p<0.01$); microbial communities from the rhizosphere soil formed a cluster separated from the bulk soil and the non-rhizosphere soil. With respect to the location, samples from the wet season separated from the dry season and separation was also based on the shrub species. Although all enzymes were highly correlated with wet rhizosphere soil ($r > 0.90$), β-glucosidase

showed the highest correlation (r = 0.98) followed by acid phosphatase (r = 0.97) (Fig. 2.6B). With respect to the shrub rhizosphere, communities from the wet season separated from the dry season and clustered depending on the shrubs species.

2.3.4. Correlation of microbial PLFA, enzyme activities, MBC and mineral N

Analysis of enzyme activities together with PLFA markers showed overall a strong association of all enzymes with the rhizosphere communities (Fig. 2.7). The shift in microbial PLFA along axis 1 were strongly correlated with the soil moisture (r = 0.82). β-glucosidase activity showed the highest correlation (r = 0.63) compared to other microbial properties (Fig. 2.7).

2.3.5. Comparison of PCA from PLFA, Enzyme and DGGE data

Enzyme activities were best in separating the communities according to location with 98% of the variation explained by the first two axes in this present study. PLFA analysis gave also very good results (65%). This was the reverse of DGGE PCA where approximatively 30% of the variance was explained for either the bacterial or the fungal community. Although species richness was lower for PLFA analysis compared to DGGE profile of bacteria and fungi, the Simpson's diversity index was similar (Table 2.3).

2.4. Discussion

2.4.1. Composition and structure of the microbial communities

DGGE profiling of the bacterial community was successful in separating the rhizosphere communities from the non-rhizosphere one. More bacterial bands were found in rhizosphere samples than in non-rhizosphere samples. The rhizosphere contains root exudates that are leaked or secreted compounds, sloughed root cells, and mucilage. This complex mixture of organic compounds provides a source of carbon, nitrogen, and other nutrients which create a rich environment to support more diverse microbial communities. These results are consistant with other studies where higher population densities of bacteria were found in the rhizosphere using either culturable methods (Curl and Truelove, 1986; Norton and Firestone, 1991; Maloney et al., 1997; Semenov et al., 1999; Marschner et al., 2002) or unculturable methods, DGGE (Normander and Prosser, 2000; Yang and Crowley, 2000; Duineveld et al., 2001; Smalla et al., 2001; Wieland et al., 2001, Marschner et al., 2004).

The weak difference between the wet and dry season can be explained by the fact that DGGE resulted from amplified DNA from all organisms, either dead or alive. Smalla et al. (2001) reported a seasonal difference in the composition of the bacteria community. But in their study, they were comparing the microbial communities over many years. In this study, the dry season was contrasted with the wet season within a six-month period.

The bacterial community composition differed between new and old roots during the wet season. Old roots were suberised and may be less likely to exudate soluble organic compound which could result in changes in microbial community composition due to decrease and change in root exudates composition with root age (Neumann et al., 2000; Rumberger and Marschner, 2003; Cardon and Gage, 2006). This is consistent with other studies where changes in microbial community composition with root age have been shown (Marschner et al., 2001; Marschner et al., 2002; Rumberger and Marschner, 2003). Marschner et al. (2005) showed that the bacterial community was different between young roots and mature roots in rhizosphere of Banksia species trees.

For the fungal community, the seasonal effect was more effective in separating the communities than the rhizosphere effect. The weakness in separating the fungal community primarily based on the rhizosphere influence has been reported in previous studies but using PLFA methods (Buyer et al., 2002). The authors found a clear rhizosphere effect for the bacterial community but a weak one for the fungal community. During the wet season, plants usually have more nutrients available and would be expected to liberate more exudates which should stimulate microorganisms. Fungi may be elevated in rhizosphere due to two groups; 1) sugar fungi that may be stimulated because of rhizosphere depositions and or 2) fungi that are adapted to hydrolyze roots residue that is high in lignin. Greater exudation by roots would support higher microbial activity and interaction which makes more C available for fungi. This may explain the clear separation during the wet season between the

rhizosphere soil and the non-rhizosphere soil. Fungal and bacterial communities have been shown to increase in the rhizosphere soil due to roots exudates (Curl and Truelove, 1986; Maloney et al., 1997).

The strong separation of rhizosphere soil from the non-rhizosphere soil observed during the wet season for both DGGE and PLFA analysis may be due to the specificity of root exudates to shrub species and to the soil type. Indeed, root exudates from shrub species as well as soil type have shown different effects on soil microbial communities (Normander and Prosser, 2000; Duineveld et al., 2001; Smalla et al., 2001; Wieland et al., 2001; Buyer et al., 2002).

In this study, there were different soil types specific to each shrub species. Neither the soil type nor the shrub species effects have been studied separately. It would be difficult to tell which of the two factors had the stronger influence. In some studies, plants had similar microbial community structure in different soils (Grayston et al., 1998; Miethling et al., 2000). However, in many cases the rhizosphere communities of different plant species growing in the same soil are distinct (Ibekwe and Kennedy, 1998; Smalla et al., 2001; Marschner et al., 2001). The degree to which plants control the rhizosphere communities is not clear, as there are also studies in which plant species growing in the same soil had similar rhizosphere microbial communities, indicating that the influence of the soil may be greater than that of the plant (Buyer et al., 1999; Latour et al., 1999; Blackwood and Paul, 2003)

2.4.2. PLFA, Microbial biomass and Enzyme activities of the soil microbial communities

The higher amount of PLFA regardless of the microbial functional group in the rhizosphere of both shrubs was likely due to the root exudates and higher moisture that create an environment which promotes microbial activity. Compared to the bulk soil, a number of studies, mainly on crop systems, have shown that the rhizosphere soil has higher population densities of bacteria and fungi (Curl and Truelove 1986; Maloney et al., 1997; Norton and Firestone, 1991; Semenov et al., 1999; Marschner et al., 2002). However, in this PLFA study, the amount of PLFA in the rhizosphere soil was higher than PLFA from the bulk soil only in the dry season; during the wet season there was no difference. During the wet season the soil is moister and more exudates are available, this may affect the communities in the bulk soil (soil between roots). This is in accordance with studies that have shown PLFA to be unable to clearly separate bulk and rhizosphere soil in controlled conditions (Steer and Harris, 2000; Butler et al., 2003).

In this present study, rhizosphere microbial communities during the dry season were quite similar to the rhizosphere and bulk communities during the wet season. *P. reticulatum* and *G. senegalensis* have been shown to redistribute water from the roots to the subsurface (Kizito et al., 2006); this could impact the rhizosphere communities during the dry season by creating a more moist and favorable environment. Studies by Wan et al. (2000) and Brooks et al. (2002) showed that hydraulically redistributed water enhanced seedling survival, maintained overstory transpiration during summer

drought and had a significant impact on plant drought tolerance and total water utilization. Semi-arid areas and environments of periodic droughts are locations where hydraulic redistribution is likely to have a significant impact on individual tree and ecosystem water budgets (Richards and Caldwell, 1987). Kizito (2006) measured the highest peak of water redistribution during the dry season (0.1 mm day^{-1} for *P. reticulatum* and to 0.2 mm day^{-1} for *G. senegalensis*). This may explain the higher PLFA biomass in the rhizosphere during the dry season. At some point the rhizosphere soil would be moister than soil some distance from the root due to the efflux of water from the root.

Kizito (2006) also showed that shrubs buffer diurnal temperature by a 5ºC difference. Plant communities have been shown to influence associated soil microbial communities by altering the temperature and water content of the soil (Young, 1995; Stark and Firestone, 1996; Eviner, 2001; Myers et al., 2001). Furthermore, studies by Dossa (2007) showed a higher difference in nutrient availability between the rhizosphere soil and the non-rhizosphere soil. All of these factors may have contributed to the higher activity and biomass of the microbial communities in the rhizosphere soil during the dry season.

The FUN/BACT ratio was not significantly different between bulk and rhizosphere soil during the wet season and the same pattern was found for $PLFA_{Tot}$. This ratio has been suggested as an indicator of soil microbial community stability (Bardgett and McAlister, 1999; Zelles, 1999). Soil microbial communities of undisturbed terrestrial ecosystems tend to be dominated by fungal microbial biomass

(Bardgett and McAlister, 1999). In this study, this may be due to the fact that during the wet season water is not restricted to the bulk soil. This may cause a leaking of exudates from rhizosphere soil to bulk soil (between roots) creating an environment similar to the rhizosphere one.

The stress indicators cy 19:0/18:1ω7c and SAT/MONO were higher in the bulk soil than in the rhizosphere soil during the dry season. These ratios are also lower for the wet season than for the dry season. Soil water stress likely resulted in the modification of microbial membranes during the dry season. Microbial communities were affected by roots exudates associated with cell age, nutrient deprivation, desiccation (Zelles et al., 1992; Kieft et al., 1994, Zelles et al., 1995; Bossio and Scow, 1998). The hydraulic redistribution may have contributed to a reduction in water stress in the rhizosphere soil during the dry season.

Comparing the functional communities showed that fungal and Gram+ communities had the highest increase from non-rhizosphere soil to rhizosphere soil. This is in accordance with study by Priha et al. (2001) who found that fungal markers 18:2ω6,9c and branched fatty acids, which have commonly been found in Gram+ bacteria (O'Leary and Wilkinson, 1988) were dominant in birch rhizosphere (*Betula pendula* Roth). They attributed this difference to a higher dominance of *Bacillus* species in birch rhizosphere. Butler et al. (2003) also showed a greater amount of the PLFA marker, 15:0i, generally attributed to Gram+ bacteria, in the rhizosphere than non-rhizosphere soil.

Analysis of rhizosphere DNA indicated that Gram+ bacteria may be more dominant in the rhizosphere than previously believed (Smalla et al., 2001). Our results are in opposite of many studies that showed that Gram-negative were dominated in rhizosphere soil (Federle, 1986; Zelles, 1999). Gram- bacteria generally grow rapidly and may use a variety of different carbon sources. Curl and Truelove (1986) using dilution plate counts or substrate utilization assays found that the bacterial populations increased more than did fungal populations in the rhizosphere soil compared to bulk soil. Their results are, however, limited to fungi that can grow on the media, which excludes a number of important taxonomic groups (Smith et al., 1999).

2.4.3. Enzyme activities

All enzyme activities increased to a greater degree in the rhizosphere soil than in the bulk soil. Enzymes separated the communities as did PLFA. There was a strong correlation of community composition with the overall enzyme activities. However individually, with the exception of the urease, these activities had a different trend than for the PLFA biomass. During the dry season, the lack of water probably limited fungal growth and may explain why there were no differences between the non-rhizosphere and the bulk soil for the chitinase activity which is largely associated with the presence of fungi (Sinsabaugh et al., 1993; Sinsabaugh and Moorhead, 1994; Bandick and Dick, 1999). For urease activity, the similar pattern with the PLFA biomass may be due to the fact that ammonium is tightly linked to microbial assimilation (Sylvia et al., 2005).

Acid phosphatase and β-glucosidase have been shown to be sensitive to the nutrient status of the soil and plant availability of soluble carbon and phosphorus. Because nutrients in the rhizosphere of these shrubs are higher than in the bulk and non-rhizosphere soils (Dossa, 2007), one could expect an increase of these activities in the rhizosphere. Furthermore, the community composition between the rhizosphere soil and the bulk soil is different; hence we may infer that the difference in activities is linked to the composition of the microbial biomass in these soils. Studies by Marschner et al. (2005) linked bacterial composition with β-glucosidase activity in rhizosphere tree of Banksia species. Overall however, there was a good correlation between enzyme activities and PLFA ($r = 0.7$, $p<0.01$). This is consistent with studies by Fernandes (2006) who found significant correlation between enzyme activities and PLFA analysis using standardized Mantel statistics.

2.4.4. Diversity indexes

The higher percentage of variance explained by analyzing enzyme activities using PCA may be explained by the low number of variables used compared to either PLFA or DGGE profile. This study measured the activity of only four enzymes. This is very low compared to PLFA analysis where $S = 18$ and for the DGGE profiling where $S = 43.2$ for the bacterial community and $S = 38.9$ for the fungal community. Although the diversity index of the DGGE profiling was not much higher than that of PLFA, the DGGE profiling explained 33% of the variability while PLFA explained 65% of the data. This may be due to the fact that DGGE profiling was unable to

separate samples from the dry season to samples from the wet season for the bacterial community; and samples from the rhizosphere to non-rhizosphere samples for the fungal community. Moreover, DGGE was targeting bacterial or fungal groups in separate analysis whereas PLFA was profiling the whole communities, which provides a more realistic characterization of microbial communities. Using DNA does not allow a separation between living and dead organisms whereas the use of PLFA is an index of viable microbial functional groups.

2.5. Conclusions

Shrub influence in the soil microbial communities was very strong. The presence of shrubs resulted in a more active and structured communities of more diverse composition. Moreover, during the dry season shrubs maintained a moister environment which likely was important in stimulating microbial communities. This maintained higher enzyme activities in the rhizosphere soil during the dry season that was quite similar to the one in the wet season as shown by the DGGE analysis of the bacterial community. PLFA and enzyme activities were more sensitive than DGGE in reflecting the differences in activity and composition of the communities across the various soil sampling locations. Both sets of data were significantly intercorrelated and clearly showed strong rhizosphere and seasonal effects.

Fungal and Gram+ bacterial markers had higher increase in rhizosphere over non-rhizosphere soil. β-glucosidase and acid phosphatase activities were highest in the rhizosphere soil. Shrub rhizospheres clearly sustained larger, more diverse

communities than did non-rhizosphere soil – presumably by providing elevated levels of C inputs. Additionally, the maintenance of microbial communities by shrub rhizospheres between wet and dry seasons suggests hydraulic redistribution of water were important in supporting microorganisms during the dry season.

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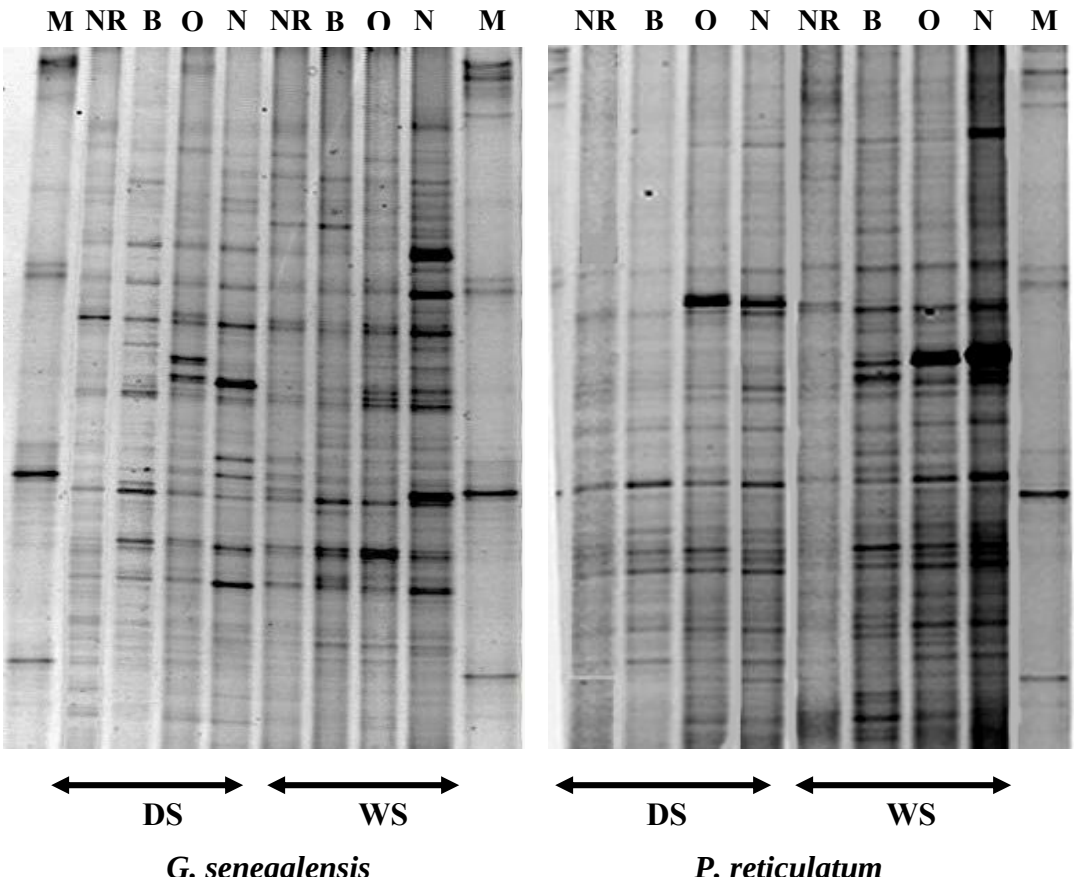

Figure 2.1. DGGE profile of 16S DNA PCR product extracted from soil using 338f/518r primers. Samples are from the wet (WS) and dry season (DS) in two sites with *G. senegalensis* and *P. reticulatum* shrubs. N = new roots, O = old roots, B = bulk soil, NR = non-rhizosphere soil, M = marker.

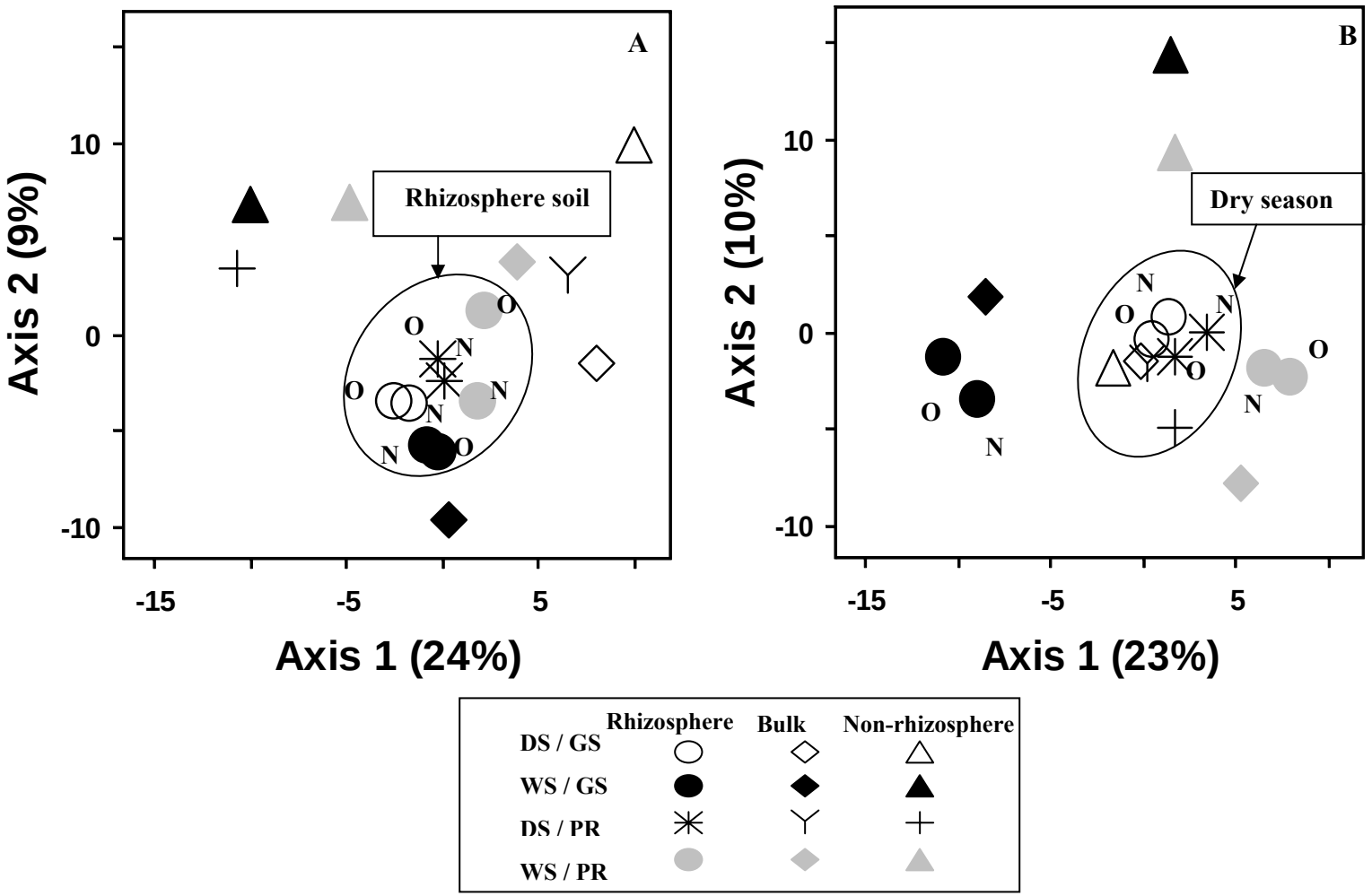

Figure 2.2. PCA plot for bacteria (A) and fungi (B) from DGGE profile of DNA extracted from soil associated with *G. senegalensis* (GS) and *P. reticulatum* (PR) sampled the dry (DS) and wet season (WS); old roots (O) ; new roots(N).

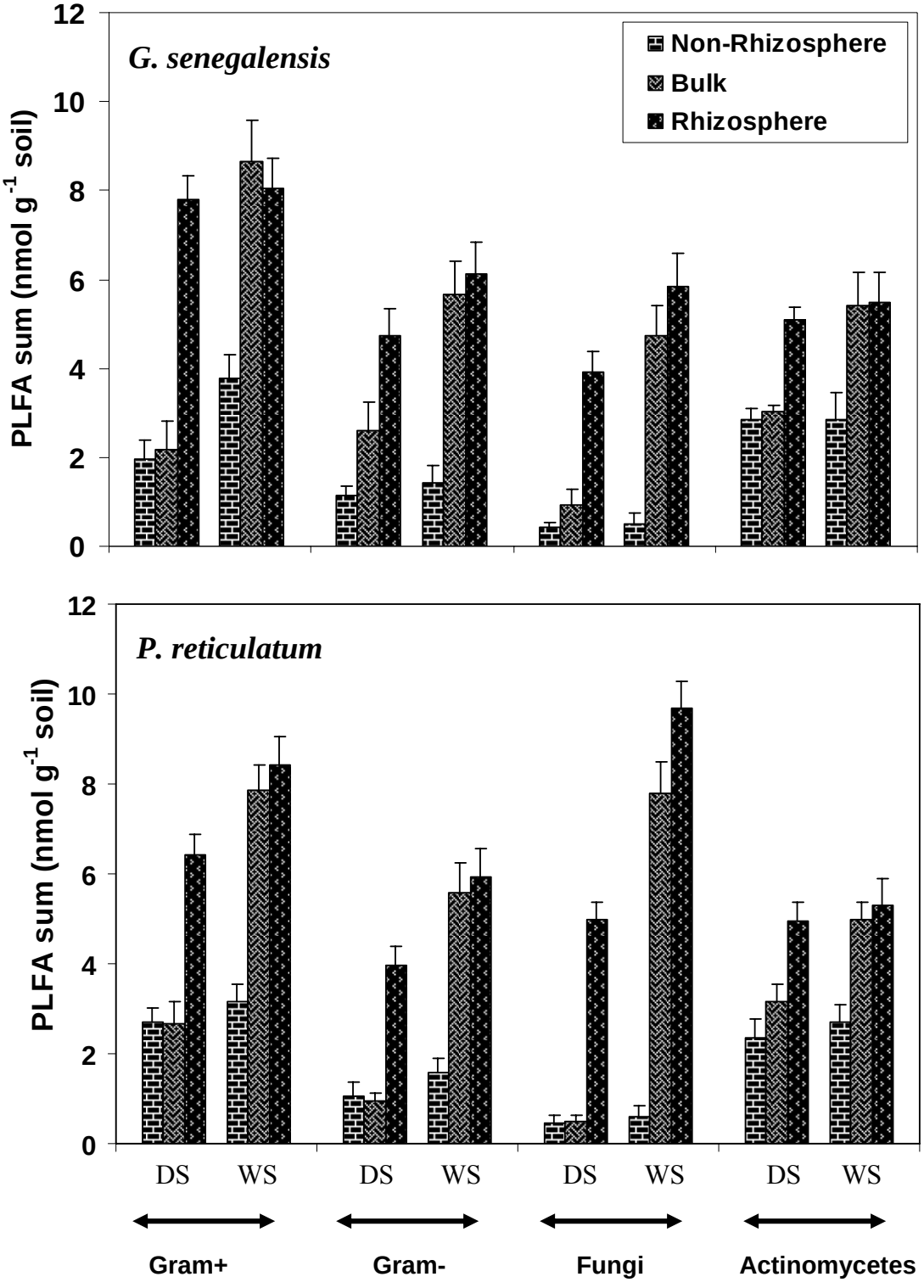

Figure 2.3. Amount of PLFA for Gram+, Gram-, fungi and actinomycetes groups extracted from soil sampled during the dry season (DS) and wet season (WS). Barres are standard of deviation.

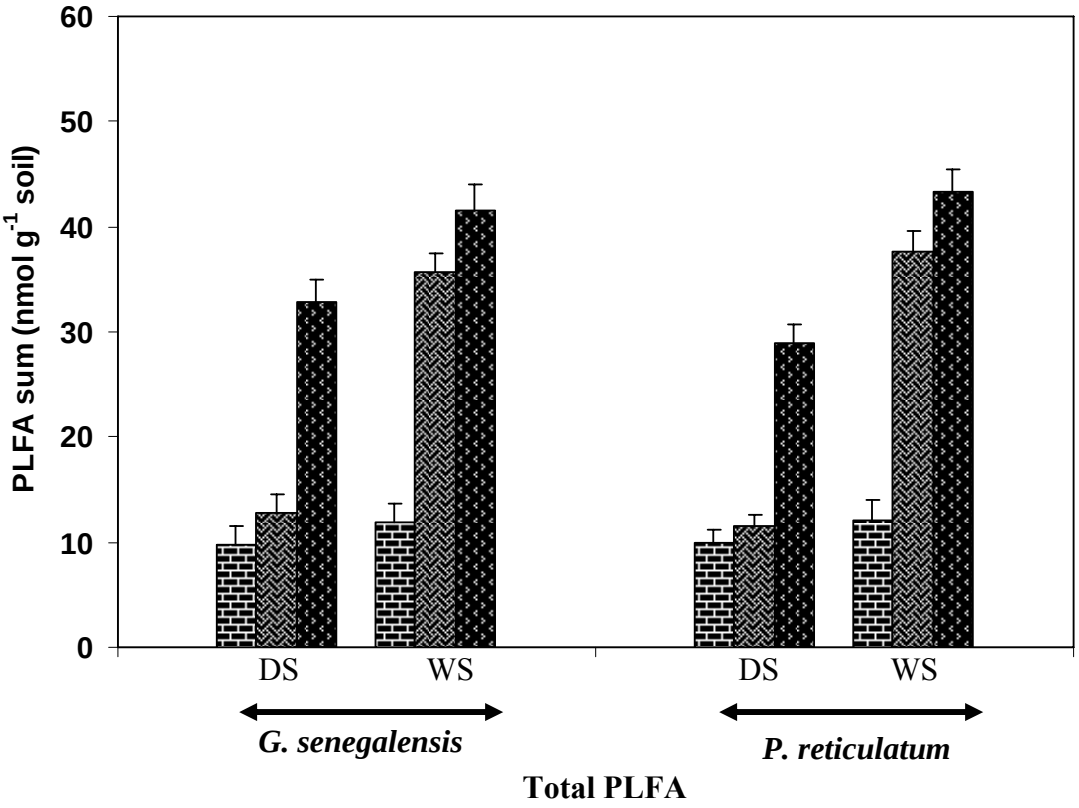

Figure 2.4. $PLFA_{tot}$ extracted from soil associated with *G. senegalensis* and *P. reticulatum*. Soil samples are from the dry season (DS) and wet season (WS). Barres are standard of deviation.

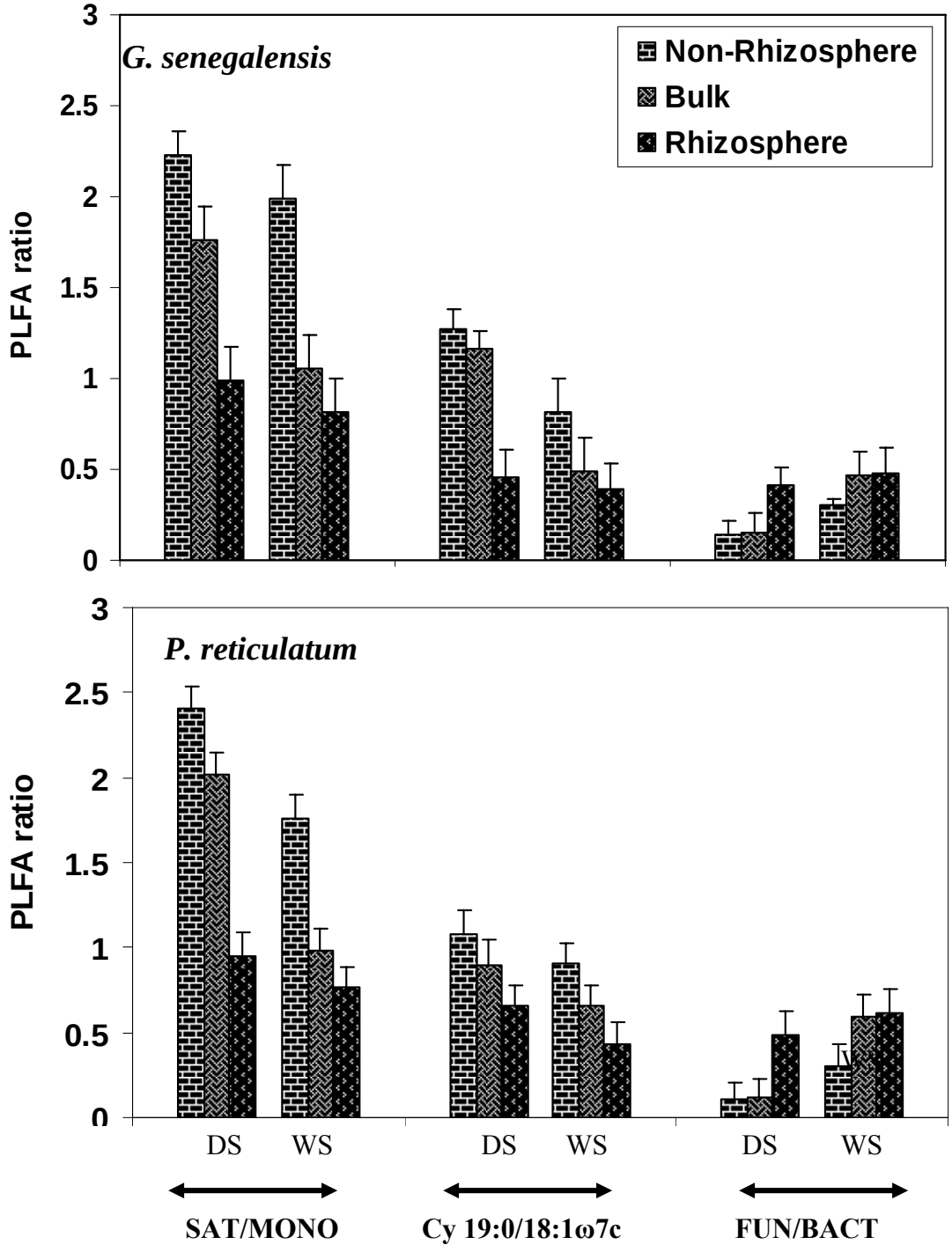

Figure 2.5. Stress indicators cy 19:0/18:1ω7c, SAT/MONO and FUN/BACT ratio extracted from soil sampled during the dry season (DS) and wet season (WS). Barres are standard of deviation.

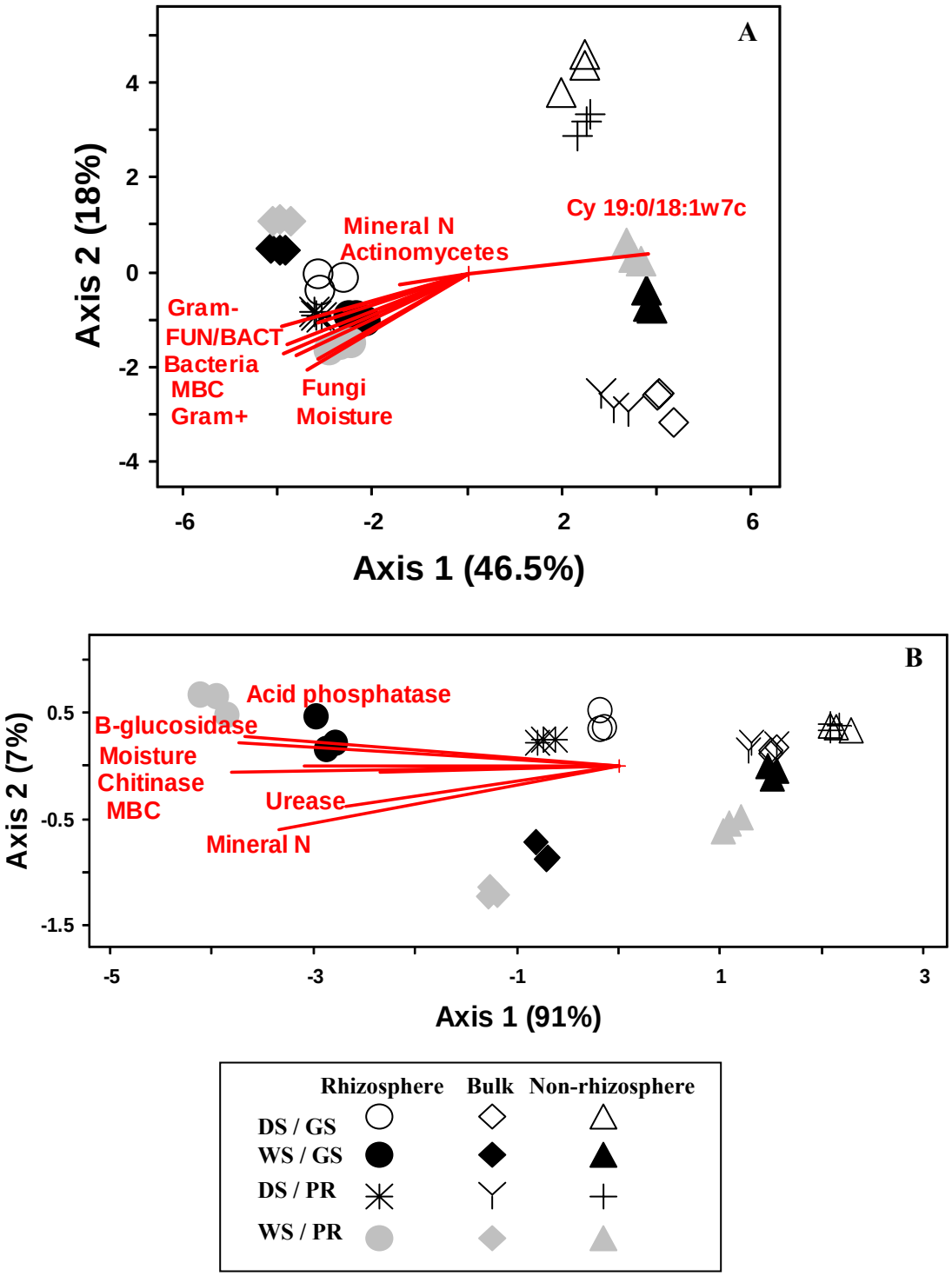

Figure 2.6. PCA analysis based on 23 PLFA markers (A) and four enzyme activities (B) from soil associated with *G. senegalensis* (GS) and *P. reticulatum* (PR) during the dry season (DS) and wet season (WS). Vectors represent microbial grouping PLFA, enzymes and some soil properties.

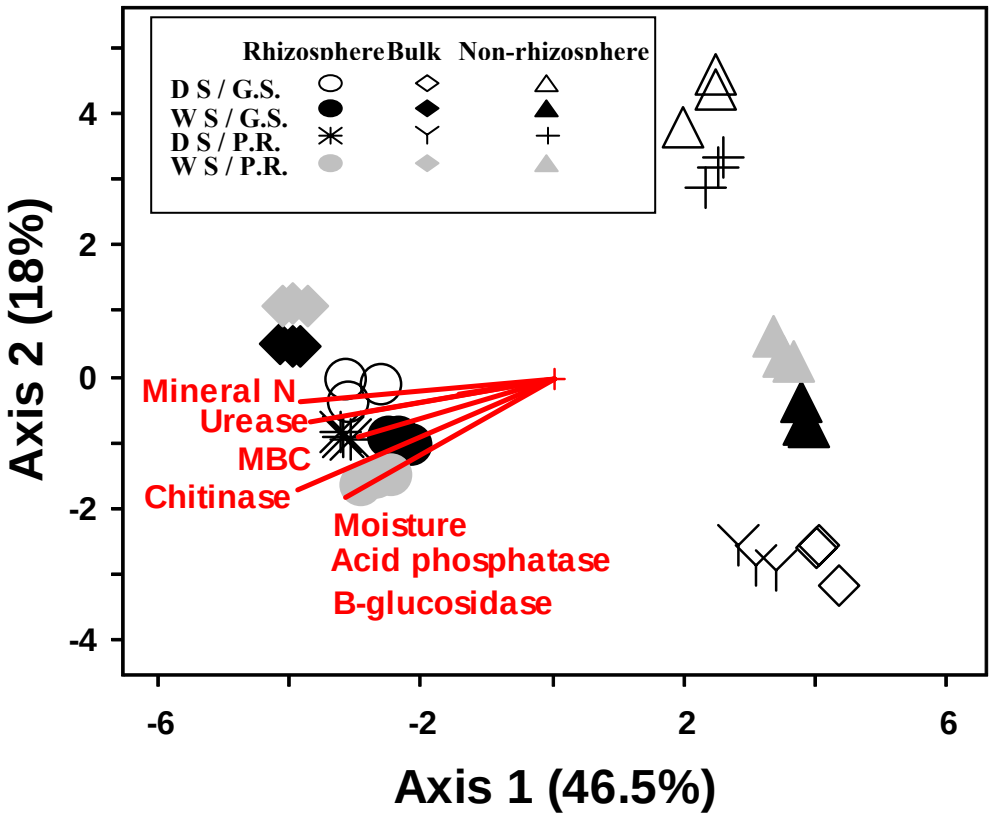

Figure 2.7. PCA based on 23 PLFA markers quantified from soil associated with *G. senegalensis* (GS) and *P. reticulatum* (GS) during the dry season (DS) and wet season (WS). Enzyme activities are represented by vectors.

Table 2.1. PLFA used as biomarkers for different groups of microorganisms.

Microbial Groups	PLFA markers	References
Fungi	18:2ω6c 18:1ω9c	Frostegård and Bååth, 1996; Olsson, 1999
Gram-positive bacteria (Gram+)	15:0i; 15:0a; 16:0i; 17:0i; 17:0a	O'Leary and Wilkinson, 1988
Gram-negative bacteria (Gram-)	18:1ω7c; cy 17:0; cy 19:0	Wilkinson, 1988
Bacteria	Gram+, Gram-	
Actinomycetes (Actino)	10Me 16:0; 10Me 17:0; 10Me 18:0	Kroppenstedt, 1992
Saturated PLFA (SAT)	15:0; 16:0; 17:0; 18:0	
Monounsaturated PLFA (MONO)	16:1ω9c; 16:1ω7c; 16:1ω5c; 17:1ω9c; 18:1ω9c; 18:1ω7c; 18:1ω5c	

Table 2.2. Enzyme activities, MBC and mineral N of soil associated with *G. senegalensis* and *P. reticulatum* sampled during the wet and dry season.

Treatment	Acid phosphatase		β-glucosidase		Chitinase		Urease		MBC		Mineral N	
	---------- μg pNP $h^{-1}g^{-1}$ ----------						μg $N\text{-}NH_4^+$ $h^{-1}g^{-1}$		μg C g^{-1}		μg N g^{-1}	
Guiera senegalensis												
Dry season												
Non-rhizosphere	193	(27)*	142	(14)	65	(7)	1.0	(0.0)	7.3	(0.9)	6.7	(0.4)
Bulk	333	(29)	218	(15)	72	(8)	2.4	(0.1)	25.6	(1.1)	8.1	(1.6)
Rhizosphere	717	(22)	324	(20)	250	(13)	4.7	(0.3)	36.1	(1.4)	13.6	(1.9)
Wet season												
Non-rhizosphere	271	(26)	198	(20)	83	(9)	3.1	(0.1)	12.3	(1.0)	8.5	(1.1)
Bulk	556	(28)	457	(23)	210	(23)	8.0	(0.4)	34.0	(1.3)	16.2	(1.6)
Rhizosphere	1145	(99)	590	(30)	502	(21)	8.7	(0.1)	46.1	(1.2)	17.5	(1.2)
Piliostigma reticulatum												
Dry season												
Non-rhizosphere	196	(26)	148	(14)	74	(7)	1.0	(0.0)	6.5	(0.8)	8.1	(0.5)
Bulk soil	356	(34)	254	(25)	79	(7)	2.2	(0.1)	27.0	(1.3)	10.1	(1.9)
Rhizosphere	794	(36)	345	(23)	302	(20)	6.2	(0.1)	40.1	(1.1)	15.8	(1.7)
Wet season												
Non-rhizosphere	273	(20)	208	(17)	96	(9)	5.3	(0.2)	16.5	(1.0)	9.1	(1.2)
Bulk soil	566	(28)	496	(19)	245	(9)	9.7	(0.2)	38.8	(1.8)	18.7	(1.8)
Rhizosphere	1359	(98)	650	(27)	660	(17)	9.8	(0.2)	49.1	(1.9)	19.3	(1.8)

* Values in parenthesis are standard of deviation.
All values are expressed per g of dry soil (g^{-1} soil).

Table 2.3. Diversity indexes computed using microbial data from different microbial methods of analysis.

	Richness (S)	Evenness (E)	Shannon's diversity index (H')	Simpson's diversity index (D)
DGGE bacteria	43.20	1.00	3.74	0.97
DGGE fungi	38.90	0.99	3.51	0.95
PLFA	18.10	0.93	2.68	0.92

CHAPTER 3

Microbiology and Macrofaunal Activity Relative to Rates of Indigenous Shrub Residue Decomposition in Farmers' Fields of Senegal

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Abstract

A major limitation for crop productivity in Sahelian degraded soils is the lack of organic inputs. *Piliostigma reticulatum* and *Guiera senegalensis* are prominent, unrecognized and unmanaged vegetative components of the Sahelian ecosystem that have the potential to provide significant biomass C for soil. These shrubs co-exist in row-crop fields, but are coppiced and burned every spring. Therefore, the objective of this study was to develop fundamental information about non-thermal management of shrub residues in relation to microbial dynamics. The experiment was a 2 X 3 X 2 factorial design with two soil treatments (0-5 cm depth beneath and outside the influence of the shrub), three residue amendments (leaf, leaf+stem and stem), and 2 litterbag mesh size treatments (<0.7 mm to exclude macrofauna and >2 mm to permit entry by macrofauna). Litterbags were destructively sampled at 15, 30, 60, 120 and 210 days after the first rain of June 30, 2005. At each sampling, litter mass loss was measured and soil was assessed for microbial carbon biomass (MBC), inorganic N and enzyme activities. When macrofauna were not restricted in decomposing litter, there were greater rates of mass loss, MBC, enzyme activities, and mineral N. Rates of decomposition and microbial response were higher with soils beneath canopy than outside the canopy influence. β-glucosidase had a high correlation with mass loss and MBC. Chitinase showed a strong correlation with mass loss. The results provide evidence that non-thermal management has potential for practical use of coppiced shrub residues.

3.1. Introduction

Studies have shown that regular input of organic matter is needed to maximize yields and fertilizer efficiency in the Sahel of Africa (Badiane et al., 2000a). Keeping plant residues in the field is a critical component of soil management, not only for nutrient value, but also for soil protection from wind and water erosion. Organic matter inputs are central for optimizing nutrient uptake and water efficiency to maximize crop productivity in the Sahel (Sanchez et al., 1997; Badiane et al., 2000b; Masse et al., 2004). Adding only fertilizer results in a slow decline in yields over time (Sanchez et al., 1997; Merckx et al., 2001), and animal manure or household refuse do not provide the quantities needed at landscape scales to significantly improve soils (Badiane et al., 2000b). Realistically, because the Sahel has low net primary productivity (low and variable rainfall), farmers are at risk averse and there are few biological options or human incentives for sustainable biomass conservation.

In the semi-arid Sahel of Africa, there are two shrubs that predominate in farmers' fields, *Piliostigma reticulatum* and *Guiera senegalensis.* They are coppiced and burned just prior to the rainy season which is an ineffective use of this biomass. Burning residues reduces the amount of carbon (C) and nitrogen (N) returned to soils, does not build organic matter, and resulting charcoal would be largely unavailable for biological activity. To adopt non-themral management required the dependence of biological mediated decomposition to avoid build up and interference of shrub residues with cropping operations.

Although abiotic process can cause decomposition, microbial communities tend to dominate this process in their quest for nutrients using extracellular enzymes (Sinsabaugh and Moorhead, 1994). In drier regions, macrofauna and in particular termites dominate litter decomposition (Lepage, 1982; Josens, 1983; Mando and Miedema, 1997; Manlay et al., 2004; Diallo, 2005) by shredding litter into smaller pieces and thereby increasing the surface area that enables greater degradation by the soil microbial communities.

Termites enhance the decomposition of surface applied organic materials by stimulating enzyme activities, resulting in rapid mineralization of nutrients, which then can be used by growing plants (Beare et al., 1992; Tian et al., 1995). Enzymes have been suggested as potential indicators of soil quality (Dick, 1997; Bandick and Dick, 1999; Badiane et al., 2001) and can be closely related to microbial activity and biochemical reactions and nutrient cycling in the soils (Burns, 1982; Burns and Dick, 2002; Tabatabai and Dick, 2002; Nannipieri et al., 2003).

Substrate chemistry can influence the decay rate in various ways. Shifts in microbial population or changes in decomposition rates can be tightly linked with modification of litter chemistry. Higher nitrogen content speeds the decomposition process, whereas high content of lignin and polyphenolics may delay and inhibit the process (Palm and Sanchez, 1991; Vanlauwe et al., 1996; Bernhart-Reversat, 1999).

Use of litterbags remains popular for decomposition studies involving comparisons of placement and management effects on mass loss and N from organic materials (Diack et al., 2000; Kwabiah et al., 2001; Shane et al., 2002; Njunie et al.,

2004; Manlay et al., 2004; Diallo, 2005). There is very little information on rates of decomposition of residues from *P. reticulatum* and *G. senegalensis* and associated microbial responses (Diack et al., 2000).

The objectives of this study were to evaluate: 1) the role of macrofauna, 2) the influence of the presence of shrubs; and 3) the residue type on the decomposition rate, and the microbial structure and activity.

3.2. Materials and methods

3.2.1. Experimental Sites

This experiment was carried out in farmers' fields, in semi-arid Senegal. The work was done on two ecological regions: the first region is characterized by the presence *G. senegalensis* in a loamy sand soil with an annual rainfall regime of 400-600 mm; the second area, with an annual precipitation of 700-1000 mm is dominated by *P. reticulatum* in a sandy loam soil. The carbon content of these soils varied from 0.3% to 0.8%. The temperatures are high through the year averaging 32°C in April-June and 22.8°C in December-February.

3.2.2. Experimental Design

Leaves and stems were harvested in April for each shrub, cut in pieces of approximatly 3 cm and then air-dried in an area protected from sunlight. After one month, 10 g of residue was placed into 12 x 15 cm bags of two different mesh sizes: 2

mm mesh size to allow in microfauna and macrofauna; and 0.7 mm to exclude macrofauna. The bags were then sealed as litterbags.

The experiment was a 2 X 3 X 2 factorial design with 2 soil treatments (0-5 cm depth beneath and outside the influence of the shrub), 3 residue amendments (leaf, leaf+stem and stem), and 2 mesh litterbag size treatments (<0.7 mm to exclude macrofauna and >2 mm) which was done with one of the two shrub species in each of two ecological regions. In mid-June the bags were buried randomly in two locations beneath and outside shrub canopy influence within the top 0-5 cm of the soil in a field previously under peanut cropping. There were six replicates at each site.

3.2.3. Sample Collection and Mass Loss Measurement

Litterbags were destructively sampled at 15, 30, 60, 120, 210 days after the first rain which occurred 16 days after bags were buried (30 June). At each sampling date, litterbags and soil under litterbags were collected (0.3 cm below bag) and brought to the laboratory. Litterbags were first washed under low pressure water in a sieve with 200 µm mesh size until the water became clear. Residue was then poured in to another sieve with 200 µm and washed under running water. The cleaned residue was air-dried at 30ºC for a week and then weighed. Mass remaining was computed at each sampling date. Linear regressions were performed with the natural logarithm of percent remaining dry weight to calculate k (the annual fractional loss rate or decay rate), using an exponential decay model (Olson, 1963): $\ln(X/X_0) = -kt$; where X = final weight, X = initial weight and t = time.

Two soil samples from the same treatment were composited, homogenized and passed through a 2-mm sieve and kept at 4ºC until analysis. This resulted in three field replications. All microbiological and biochemical analyses: microbial biomass carbon, mineral N, enzyme activities (β-glucosidase, acid phosphatase, urease and chitinase) were done within one week of each sampling period.

3.2.4. Laboratory Analyses

A fumigation extraction method by Amato and Ladd (1988) was used to determine the microbial biomass. This method is based on the quantification of α-amino N of microorganisms. Two sets of 10 g of non-fumigated and fumigated soil were extracted with 75 mL of KCl (2 M) in a shaker for one hour at 25°C. Ninhydrin-reactive N was determined from 0.5 mL of extract from each of the fumigated and unfumigated soils. Aliquots were mixed with 1.5 mL of 2 M KCl and 2.0 mL of ninhydrin reagent. Ninhydrin-reactive N was quantified colorimetrically at 750 nm, by flow injection analysis (Evolution II, Alliance-Instrument, France). MBC was estimated by multiplying by 21 the gain in ninhydrin-reactive N after fumigation (Amato and Ladd, 1988). Results were expressed as μg C g^{-1} of dry soil.

Soil inorganic N was determined colorimetrically in KCl extracts (2 M KCl) by flow injection analysis according to the method of Bremner (1965). Ammonium was quantified using the reaction of Berthelot modified with the indophenol blue. Magnesium and others metals (iron, aluminum) were complexed using a mixture of EDTA and a tartrate of sodium and potassium. The ammonium was quantified at a

wavelength of 660 nm. Nitrate is reduced to nitrite using hydrazine sulfate in presence of copper sulfate. Nitrite is then mixed with the sulfanilamide to form a diazo complex with N-1 naphtilethylene diamine dichlorohydrate. The absorbance was read at a wavelength of 525 nm. The results were expressed as μg N (NH_4^+ or NO_3^-) g^{-1} of dry soil. Inorganic N was assessed as the sum of ammonium and nitrate.

The activities of enzymes β-glucosidase and chitinase were measured because of their importance in the C cycle along with a N cycling enzyme assay (urease) and acid phosphatase because of its importance in the phosphorus cycle. Soils were assayed for alkaline phosphatase activity, but since the soil was acid, activity was very low, and thus only the acid phosphatase activity was assessed in this study.

A modified method originally described by Hayano (1973) and Ndour et al. (2001) was used to measure the activity of β-glucosidase and chitinase. An aliquot of 100μl of 5mM *para*-nitrophenyl β-d-glucopyranoside substrate for β-glucosidase and 100μl ρ-nitrophenyl *N*-acetyl glucosamide (5 mM, Sigma) as the substrate for chitinase were added to 100 mg of fresh soil. The mixture is buffered by the citrate phosphate (MacIlvain, 1921) at pH 5.8 and then incubated at 37ºC. After 2 h of incubation, the reaction was stopped; the ρ-nitrophenol (ρNP) released was measured 15 min later at 400 nm and expressed as μg ρNP released g^{-1} h^{-1}. A similar principle was used to measure the acid phosphatase activity using a modified method described by Tabatabai and Bremner (1969); the ρNP released was quantified at 420 nm. For urease activity, urea was added as a substrate to a mixture containing 1 g of soil. After

incubation, the ammonium released was quantified with a spectrophotometer set at 660 nm.

Control of enzyme activities was done by adding substrate to blank samples after incubation. The background product of the soil was discounted by incubating a blank mixture with no substrate. For each soil sample, there were two replicates and one control.

3.2.5. Statistical Analysis

The effect of litterbag location, litterbag mesh size, residue amendment, and time of incubation in relation to mass loss were investigated by analyzing the data using multiples comparisons procedures (SAS, Institute Inc., 1996). This permited separating of effect means using the least significant difference test. Bonferroni correction which is a multiple-comparison correction that lower the alpha value, was used in order to account for the number of comparisons being performed. Correlation was further done among the properties using SAS.

Multivariate analysis using principal components analysis was performed with the combination of all data using the PC-ORD package (MjM Software Design, Gleneden Beach, OR) (McCune and Grace, 2002). The analysis offers a more visual presentation of the results with samples having similar characteristics clustered. Scores of samples in axis 1 and axis 2 were further analyzed using permutational multivariate analysis of variance (PerMANOVA) (Anderson, 2001) in order to assess statistically the significance of any treatment. PerMANOVA is a nonparametric procedure that

tests the simultaneous response of one or more variables to one or more factors in an ANOVA experimental design on the basis of any distance measure, using permutation methods.

3.3. Results

G. senegalensis and *P. reticulatum* have the same C:N ratio which varied between 20-27 for leaves and the mixture of leaves + stems (Table 3.1). However, lignin content was higher for *G. senegalensis* than for *P. reticulatum* (18.1 vs. 13.6). Although the shrubs had the same polyphenol content, the ratio of polyphenol + lignin over nitrogen is higher for *G. senegalensis* than *P. reticulatum* (Table 3.1).

3.3.1. Mass Loss

Mass loss was highest when macrofauna were allowed access to the residue ($p<0.001$) with a value of 90% percent for *P. reticulatum* and at least 60% for *G. senegalensis* after 210 days (Fig. 3.1 and 3.2). Excluding the macrofauna resulted in a reduction of the decomposition by 30% for *P. reticulatum* and 20% for *G. senegalensis*. The decay constant (-k) was higher in general with stems residue as opposite to leaves residue (Table 3.2). For *G. senegalensis*, litterbags that were placed under the canopy influence had a significantly higher mass loss (Table 3.3. $p< 0.01$) at the end of the incubation period than did litterbags placed outside the canopy influence regardless of the mesh size. Unlike *G. senegalensis*, this result is only found for litterbags with small mesh size (<0.7 mm) for *P. reticulatum* residue (Fig. 3.2). When

the macrofauna were not restricted outside the canopy influence, there were a higher rate and a higher mass loss ($p<0.01$) for *P. reticulatum* (Fig. 3.1 and 3.2).

The highest rate of mass loss for *G. senegalensis* occurred after day 30 with litterbags having leaf residue when placed beneath the canopy. This rate of mass loss was greater at day 15 than at day 60 when there was a steep decrease in decomposition rate. The same trend was observed for *P. reticulatum* with the small mesh size, but the highest rate occurred at day 30 for litterbags with leaf residue and at day 60 for stem residue regardless of the location. Overall, for both shrubs the highest decomposition rate was observed in the litterbags of coarse mesh size.

3.3.2. Microbial biomass, Soil Inorganic N, Enzyme Activities and Moisture Content

3.3.2.1. Microbial biomass

The amount of microbial biomass was different with respect to the shrub species with *G. senegalensis* having a higher biomass at day 15 and *P. reticulatum* at day 30. There was no significant difference in MBC at day 15 with regard to the location of soil samples for *G. senegalensis*. However, at days 30 and 60, MBC was higher beneath the canopy than outside the canopy influence (Fig. 3.3). The same trend is observed at day 30 for *P. reticulatum* but only for litterbags where macrofauna were excluded. Conversely, at day 15, when macrofauna were not restricted, samples located beneath canopy had a higher biomass than those outside canopy. A significant macrofauna effect was observed for all samples in the early stages of decomposition

which caused higher biomass for soil associated with litterbags where macrofauna were not restricted

3.3.2.2. Soil inorganic N

Like the MBC trend, the N trend was also different depending of the species involved (Fig. 3.4). For *G. senegalensis*, the mineral N was higher at day 15 regardless of the presence or absence of macrofauna. This is followed by a decrease from 35 µg N g^{-1} soil to 4 µg N g^{-1} soil for all locations and litterbags mesh size up to day 120 (Fig. 3.4). After day 30, the amount of N was significantly higher for soil beneath canopy than outside canopy for *G. senegalensis* as well as *P. reticulatum*. It was only at day 210 where there was an increase in mineral N.

Unlike *G. senegalensis*, *P. reticulatum* had the highest mineral N at day 30 with a higher value of 12 µg N g^{-1} soil. This was followed by a decrease up to day 210. Mineral N was high in presence of macrofauna for *G. senegalensis* at day 210 ($p<0.01$). For *P. reticulatum* at day 210 there no macrofauna effect; at this time, there were almost no residue, thus it was difficult to compare the amount of N with respect to macrofauna at day 210. Soil beneath canopy, previously amended with stem residue, had the highest amount of mineral N.

The effect of macrofauna was also observed with the quantification of the nitrate which was significantly higher (Fig. 3.5, $p<0.001$) at day15 for soil beneath canopy associated with macrofauna than soil where macrofauna were excluded for *G. senegalensis*. After day 15, there was a decrease of nitrate followed by an increase at

day 210 with a higher value ($p<0.01$) for soil beneath the than outside the canopy. For *P. reticulatum*, the values were overall higher for soil beneath canopy than outside canopy and there was a decrease at days 60 and 120 followed by an increase of mineral N at day 210 (Fig. 3.5, Table 3.3).

Ammonium quantification resulted in a different trend with a significant location effect for *G. senegalensis* and *P. reticulatum* (Fig. 3.6, Table 3.3). Soil beneath the canopy had the higher ammonium than outside canopy for *G. senegalensis* ($p<0.01$). Ammonium content decreased at day 30 before increasing at day 60 and then decreased again at day 210. For *P. reticulatum*, there was an overall increase at 30 day and a steep decrease up to day 210.

3.3.2.3. Enzyme activities

The activities of acid phosphatase and β-glucosidase were highest at day 30 of incubation for all samples (Fig. 3.7 and 3.8). A strong location effect was observed at days 15 and 30 for soil from both shrubs with a higher activity for soil beneath the canopy. Additionally, there was a significant macrofauna effect resulting in a higher activity with sample associated with macrofauna at days 15 and 30. Overall at day 30, the amendment with the mixture leaf + stem had the highest enzyme activities when macrofauna were allowed.

Chitinase activity had a steep decrease with time after day 15 (Fig 3.9) with the exception of samples associated with macrofauna and amended with *P. reticulatum* residue which had the highest activity at day 30. This activity was influenced by the

location of the sample and the presence or absence of macrofauna (Table 3.3). For urease, the highest activity was observed at day 15 for *G. senegalensis* and at day 30 for *P. reticulatum* (Fig. 3.10). This activity decreased thereafter with time. When macrofauna are not restricted, urease activity tended to be higher ($p<0.01$). In addition, for *P. reticulatum*, samples beneath canopy had higher value than samples outside canopy influence ($p<0.001$).

3.3.2.4. Moisture Content

The moisture content was highest at day 30 with a different pattern for the different shrubs species (Fig. 3.11). Soil moisture associated with *G. senegalensis* was 7% whereas *P. reticulatum* had a moisture content of 10%. For both species the moisture was high only up to day 60.

3.3.3. Principal Components Analysis (PCA)

Multivariate analysis of all samples for *G. senegalensis* showed a strong clustering by incubation periods ($p<0.01$). The first axis of the PCA accounted for nearly 60% of variation in the data, while the second axis accounted for 26% (Fig. 3.12). Samples from day 15 clustered separately from other sampling dates and at this time there was further separation between beneath and outside the canopy influence. Mineral N as well as ammonium and nitrate were strongly correlated with samples from day 15 ($p<0.01$), whereas the moisture, mass loss rate and β-glucosidase were correlated with samples from day 30 (Fig. 3.12, $p<0.01$).

PCA for *P. reticulatum* explained a variance of 80% with the first axis accounting for 64% (Fig. 3.13). Samples are primarily separated according to the time of sampling with days 15 and 30 forming a cluster ($p<0.001$). Samples from days 60 and 120 clustered but away from day 210 samples. The location effect resulted in a clearer separation of samples beneath and outside the canopy influence for days 60, 120, and 210 ($p<0.01$). The ammonium was highly correlated with samples from day 60 beneath the canopy, while all the remaining parameters correlated with samples at days 15 and 30 (Fig. 3.13). Decomposition rate appeared to be closer to the MBC at day 30.

3.3.4. Correlation of Mass Loss, Enzyme Activities, MBC and Inorganic N with Main Factors

A correlation of all parameters using SAS within each incubation period showed for *G. senegalensis*, at day 15, ammonium, β-glucosidase, mineral N, nitrification and moisture were strongly correlated with location of the samples, beneath or outside the canopy influence ($p<0.01$). The rate of mass loss was significantly correlated with the absence or presence of macrofauna ($r = 0.87$, $p<0.0001$), β-glucosidase ($r = 0.47$, $p<0.001$) and chitinase ($r = 0.35$, $p<0.01$). Ammonium and acid phosphatase were primarily correlated with the location and the type of residue amended, while MBC was correlated with the nitrate content ($r = 0.51$ $p<0.01$). At day 30, the rate of mass loss depended more on the location of the sample in addition to the termite's effect, moisture, chitinase, urease, mineral N, nitrate, β-

glucosidase ($p<0.01$). MBC was strongly correlated with the location of the samples, the mineral N and the moisture ($p<0.001$).

For *P. reticulatum* at day 15, the rate was primarily correlated with mesh size ($r = 0.7$, $p<0.0001$) and ammonium content ($r = 0.39$, $p<0.01$) as well as residue amended. All enzyme activities were strongly correlated with the location of the samples ($p<0.01$) and in addition, β-glucosidase strongly correlated with MBC ($r = 0.34$, $p<0.01$). The rate of decomposition was strongly correlated with the presence of macrofauna ($r = 0.85$, $p<0.0001$) and the chitinase activity ($r = 0.6$, $p<0.0001$). MBC showed also a correlation with the presence of macrofauna as well as the activity of chitinase ($p<0.01$).

3.4. Discussion

3.4.1. Macrofauna Activity

The macrofauna access to residue had a much larger impact on decomposition than did the shrub canopy or residue treatments. When macrofauna were present, mass loss reached 70 to 100% by day 120 for *P. reticulatum* and day 210 for *G. senegalensis*. Conversely, in the absence of macrofauna rates of decomposition seemed to plateau at <60% mass losses; indicating further decomposition was not proceeding, pointing to the crucial role of the macrofauna in maintaining a high decomposition rate of the organic material in all systems.

This was most likely due to termites which, in the drier region, are the predominant soil fauna and mostly consist of the subterranean type that does not create

mounds on the soil surface (Lepage, 1982; Josens, 1983; Lavelle et al., 2000). Termites consume more than 90% of dry wood in semi-arid tropical areas and directly mineralize up to 20-40% of the net primary production in wetter savannas (Mando and Miedema, 1997; Rouland, 2000; Lavelle et al., 2000). Our results are consistent with other similar litterbag size exclusion studies in semi-arid tropics where macrofauna access to residue increased rate of litter decomposition (Tian et al., 1992; Diack et al., 2000; Manlay et al., 2004). Furthermore study by Mando and Miedema (1997) showed in a field experiment of semi-arid Burkina-Faso that crops were enhanced by the field colonization of termites. Termites enhanced the decomposition of surface-applied organic materials that stimulated nutrient release for crop uptake.

In this study, macrofauna access resulting in an increase of MBC was shown only at days 15 and 30 for *G. senegalensis* and *P. reticulatum*. Day 30 had the highest mass loss rate and was also the time where there was elevated mineral N. The accelerated decomposition when macrofauna were allowed into litterbags is likely due to termites. Evidence for this was visual observation of termites and sheetings constructed inside and beneath litterbags. The response at this time was a combination of new litter being added to soil and the beginning of rainy season. The soil moisture of soil at *P. reticulatum* site was (10%) and (7%) at *G. senegalensis* site.

Termites accelerate residue decomposition in soil by breaking down available litter into smaller pieces and thereby increasing the surface area of the substrate that can be acted upon by soil microbial communities, mainly fungi. Through this process, along with termite feces which concentrate microorganisms and partially or

completely decomposed plant material, termites release enzymes and make nutrients available for microorganisms.

Mineral N levels increased between days 120 and 210 for treatments beneath the canopy of both shrubs. This may be due to several reasons. One reason may be because there is a slightly higher total N level beneath shrubs than outside (Dossa, 2007). However, the total N levels only increased in small amount (0.2 g N kg^{-1}) beneath canopy of both shrubs. A second reason may be due to slightly higher level of soil moisture beneath the shrubs which could be due to hydraulic redistribution. Lastly a study by Berg and McClaugherty (1989) showed that lignin decomposition caused a late release of N during decomposition. Consequently, in our study it may well be that from days 120 and 210 only the recalcitrant lignin was remaining, and combining this with more favorable conditions beneath the shrubs, may have resulted in a release of N from lignin. In a nutrient study with *P. reticulatum* leaves, Iyamuremye et al. (2000) also found that net mineralization ocurred after 77 days of incubation in soil from beneath the canopy.

In this study, enzyme activities, particularly chitinase, was enhanced by the presence of termites. Chitinase (N-acetyl-glucosamidase) is involved in the degradation of chitin, the major structural component of fungi and insects. Since fungal biomass is orders of magnitude greater than insect biomass in soils, chitinase is an indirect indicator of fungal biomass (Bandick and Dick, 1999) because as fungal biomass and turnover increases, there is a corresponding increase in chitinase by the

microbial communities. In addition, fungi are the major litter decomposers, thus one would expect chitinase to correlate with the rate of decomposition.

Chitinase also is involved in N utilization processes by microorganisms. Chitinase activity has been shown to be inhibited by inorganic N availability and it is thus often used as an indicator of N demand by microbes (Chrost, 1991; Sinsabaugh et al., 1993; Sinsabaugh and Moorhead, 1994) and has been shown to be correlated with N mineralization (Tabatabai and Dick, 2002). In this study, a net increase in mineral N occurred at day 210 and at this time the chitinase activity and MBC were very low.

3.4.2. Shrub Canopy Effect

Litter placed below the shrub canopy generally had greater rates of decomposition and had higher levels of microbial properties than litter located outside the canopy. The strong effect of shrub canopy on the decomposition process is tightly linked to the higher nutrient value of soil beneath the canopy than soil outside the influence of the shrub (Dossa, 2007). This is due to the direct impact of shrubs through inputs of organic matter from litter, root turnover, and root exudates This follows the concept of "fertility/hydrologic islands" beneath woody species, which influence biogeochemistry/decomposition of uncultivated desert environments such as the NSF Jornada LTER site (Schlesinger et al., 1996; Schlesinger and Pilmanis, 1998; Schade and Hobbie, 2005; Rango et al., 2006).

Moreover, *G. senegalensis* and *P. reticulatum* have been shown to keep a moister environment in their vicinity (Kizito et al., 2006). With higher nutrients

available in a moist environment, decomposition is more likely to be enhanced and this would increase microbial biomass and activity. Sinsabaugh and Moorhead (1994) and Bilgo et al. (2007) showed that the decomposition processes was the result of the collective activity of microbial communities driven by the acquisition of nutrients released by extracellular degradation of detritus.

However, the mass loss did not follow the same trend for *P. reticulatum* with coarse mesh size. In this case, litter outside the canopy showed a faster decomposition after day 30 than with litter beneath the canopy. The highest rate of mass loss occurred at day 30 when the moisture is at its highest level, one may assume the preference of termite for a drier environment in the bare soil than beneath the canopy influence (Lepage, 1982; Josens, 1983; Rouland, 2000; Lavelle et al., 2000; Fall et al., 2001).

3.4.3. Litter Chemistry Amendment

The effect of residue-type amendments on the microbiological properties was not as strong as the other factor effects. However, the effect on the mass loss is due to the fact that microorganisms first decomposed the easily decomposable materials.

Substrate chemistry can influence the decay rate in many ways. Residues with low values of the C:N ratio decompose more quickly than residues with high C:N values. In this study, leaves decomposed faster than the treatment of either stem or a mixture of stem and leaf. The leaf material of both species has a much lower C:N ratio compared to stem (Dossa, 2007). Although high N concentration speeds

decomposition, high contents of polyphenolics can inhibit this process (Palm and Sanchez, 1991; Bernhart-Reversat, 1999; Vanlauwe et al., 1996).

The role of lignin in the decomposition process is debated. Previous studies showed that lignin/N exerts control over the rate of decomposition (Mellilo and Aber, 1982; Palm and Sanchez, 1991; Vanlauwe et al., 1996; Preston et al, 2000; Prescott et al., 2004). Recently, many studies have shown that lignin is not (as previously thought) hard to decompose (Amelung et al., 1999; Lobe et al., 2002; Dignac et al., 2005; Preston et al., 2006; Rasse et al., 2006). The whole issue came up as different methods were used to study lignin. In former times, methods that were used extracted not lignin only, but lignin, tannins and cutins; therefore, the lignin content in a sample was always overestimated (Preston et al., 2006; Lutzow et al., 2006). Also, in this study, there was a high correlation between the soil ammonium content and the rate of mass loss with respect to the residue type. This is consistent with studies by Jensen et al. (2005) that identified N content as a good predictor of mineral N.

3.4.4. Time of Incubation

The higher mass loss rate/day at day 30 strongly correlated with the moisture level and showed the importance of water in the decomposition process. The diminution of the rate is also due to the fact that at the beginning, easily decomposable material, e.g. sugars and starch, are decomposed (Kuhad et al., 1997). However, as the simple compounds are depleted, these "sugar" microorganisms will decline because they lack the hydrolytic enzymes to break down these complex compounds.

Subsequently, hydrolytic enzymes capable of attacking larger, complex compounds are produced to first hydrolyze cellulose. With the depletion of cellulose, only lignin remains which stimulates production of ligninases (Heal et al., 1997; Silver and Miya, 2001; Sall et al., 2003). As decomposition proceeds the loss of readily available C and nutrient sources causes decomposition to slow down which is what we found in this study. However, what is called lignin in these studies might be a complex including lignin defined as acid unhydrolysable residue by Preston et al. (2006) who showed that lignin could be decomposed faster than could cellulose.

In this study there was a decrease of N in the early stage of decomposition. This is consistent with studies showing that in the first phase of decomposition, element such as N, which are readily utilized by the microorganisms, were immobilized in microbial biomass (Berg and McClaugherty, 1989; Coûteaux et al., 1995; Songwe et al., 1995). This decrease may depend not only on the disappearance of soluble carbohydrates but also on the presence of polyphenols (Marstrop, 1996) which could inhibit microbial growth (Boufalis and Pellissier, 1994) as well as limit the decomposition of organic materials (Bernhart-Reversat, 1999).

Nutrient availability status, particularly by the consistent depletion of inorganic N, may cause shifts in enzyme activities and microbial biomass as shown in this study. The depletion of nitrate may limit microorganism growth with further consequences on the enzyme activities. Sall et al. (2003) showed that soil microbial biomass and enzyme activities increased in the litter-amended soils during the first 15 days of incubation and decreased thereafter.

β-glucosidase was more sensitive among all enzymes to the rate of mass loss and nutrient depletion. Paz Jimenez et al. (2002); Turner et al. (2002); and Dux et al. (2006) showed that β-glucosidase activity was strongly affected by plant leaf-residue types and mass loss. Polyphenols may inhibit β-glucosidase activity (Benoit and Starkey, 1968; Swain, 1979) while lignin may impede the growth of cellulolytic bacteria and fungi (Roper and Gupta, 1995). β-glucosidase has been found to be sensitive in discriminating soil management effects in periods as short as two years (Bandick and Dick, 1999). The strong correlation of MBC with inorganic N reflected the strict dependence of microbes on nutrients.

3.5. Conclusions

Macrofauna access which was most likely termites significantly impacted the decomposition process with resulted in greater decomposition rate, MBC, β-glucosidase and chitinase activities. Shrub canopy influence on the decomposition process was also significant with a higher increase of the decomposition rate, and higher microbial biomass and activity beneath than outside the canopy influence. Although the decomposition rate was higher for leaf residue, the impact of residue type on the soil microbial communities was either weak or inexistent. There appear to be real possibilities to develop improved systems to manage shrub residue non-thermally, sequester C, and offset soil degradation.

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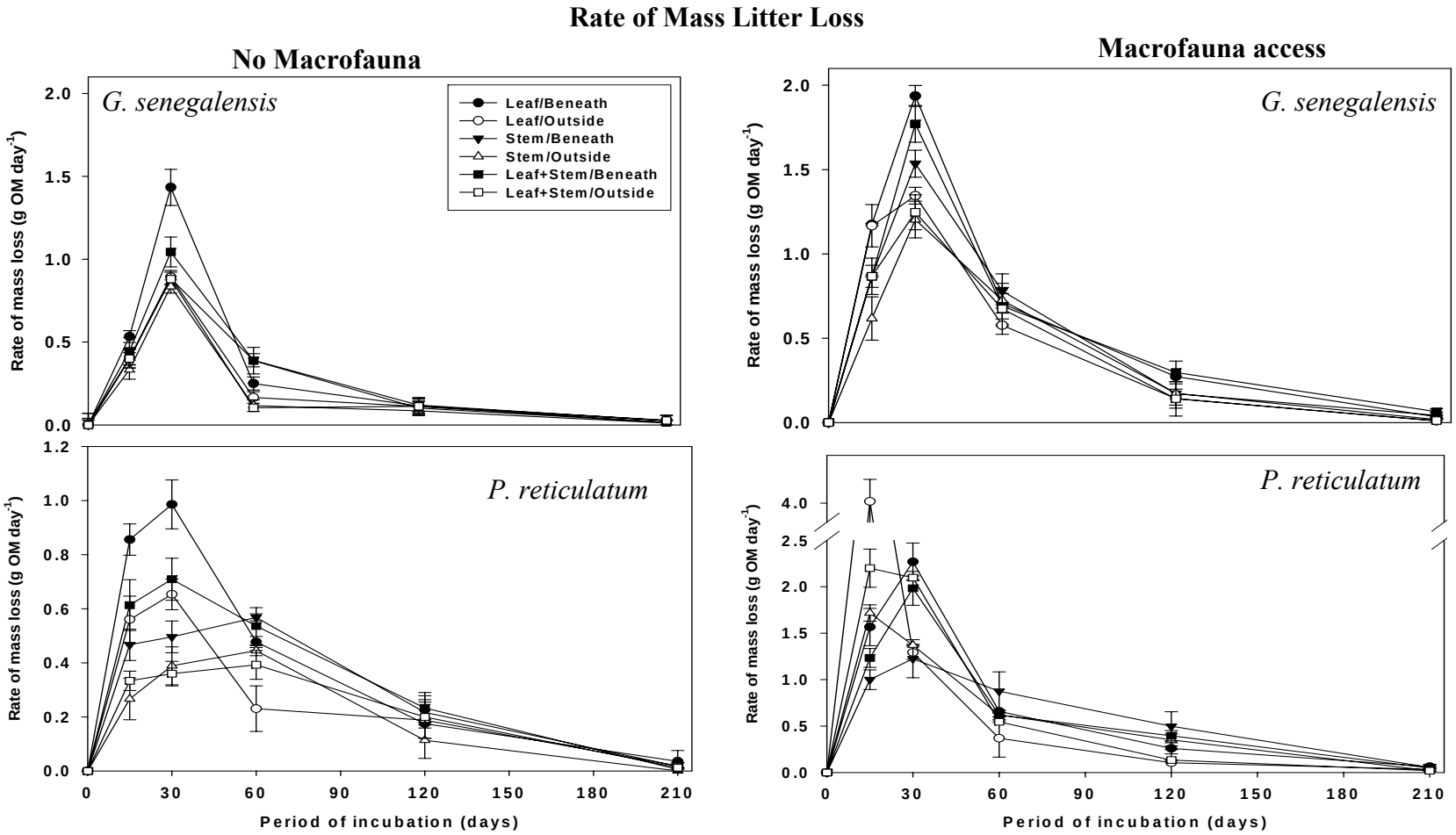

Figure 3.1. Rate of mass loss from litterbags with less than 0.7 mm diameter (graphs on the left side) and litterbags with 2 mm diameter (graphs on the right side). Barres are standard of deviation.

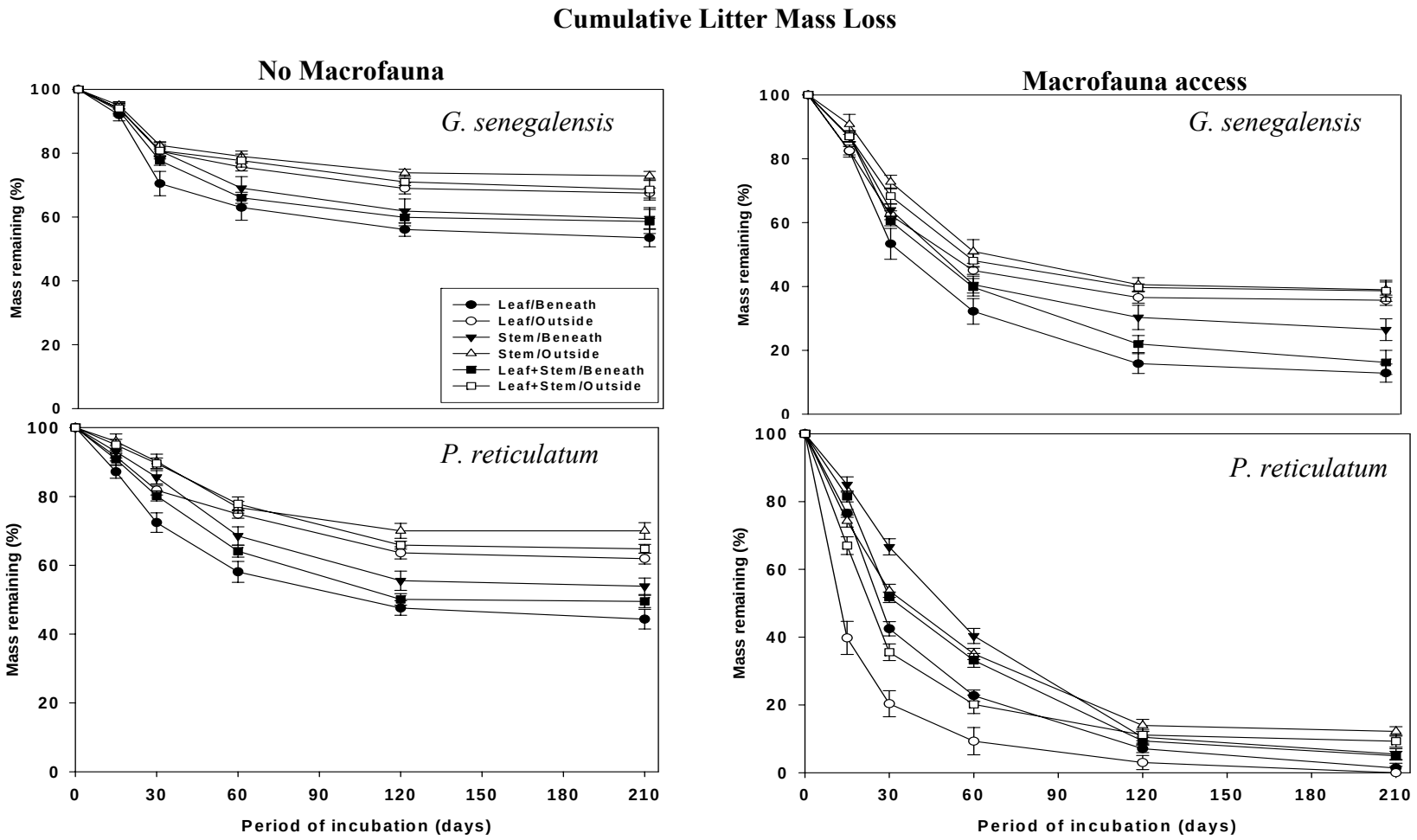

Figure 3.2. Cumulative mass loss of litterbags with less than 0.7 mm diameter (graphs on the left side) and litterbags with 2 mm diameter (graphs on the right side). Barres are standard of deviation.

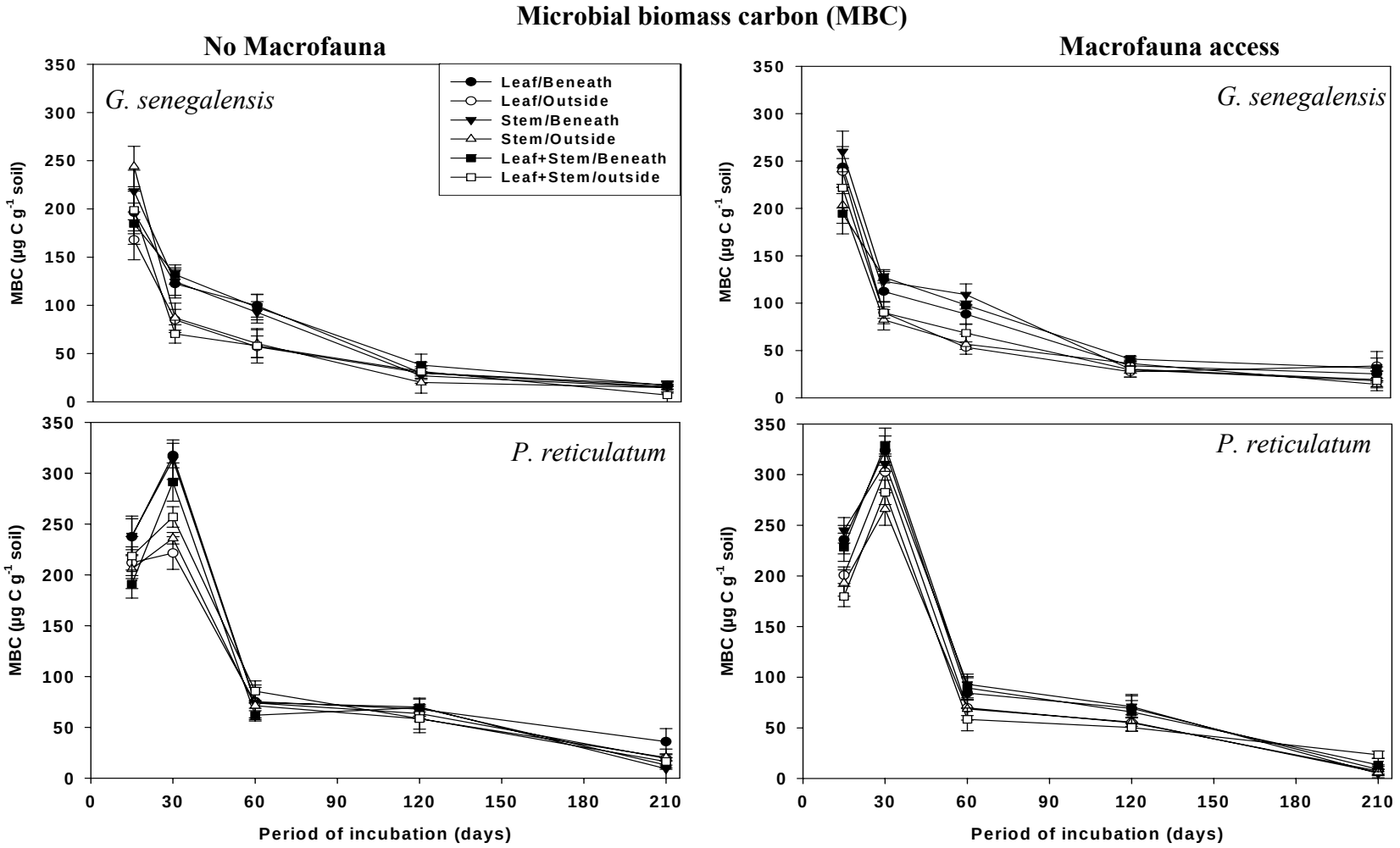

Figure 3.3. MBC of soil sampled under litterbags with less than 0.7 mm diameter (graphs on the left side) and under litterbags with 2 mm diameter (graphs on the right side). Barres are standard of deviation.

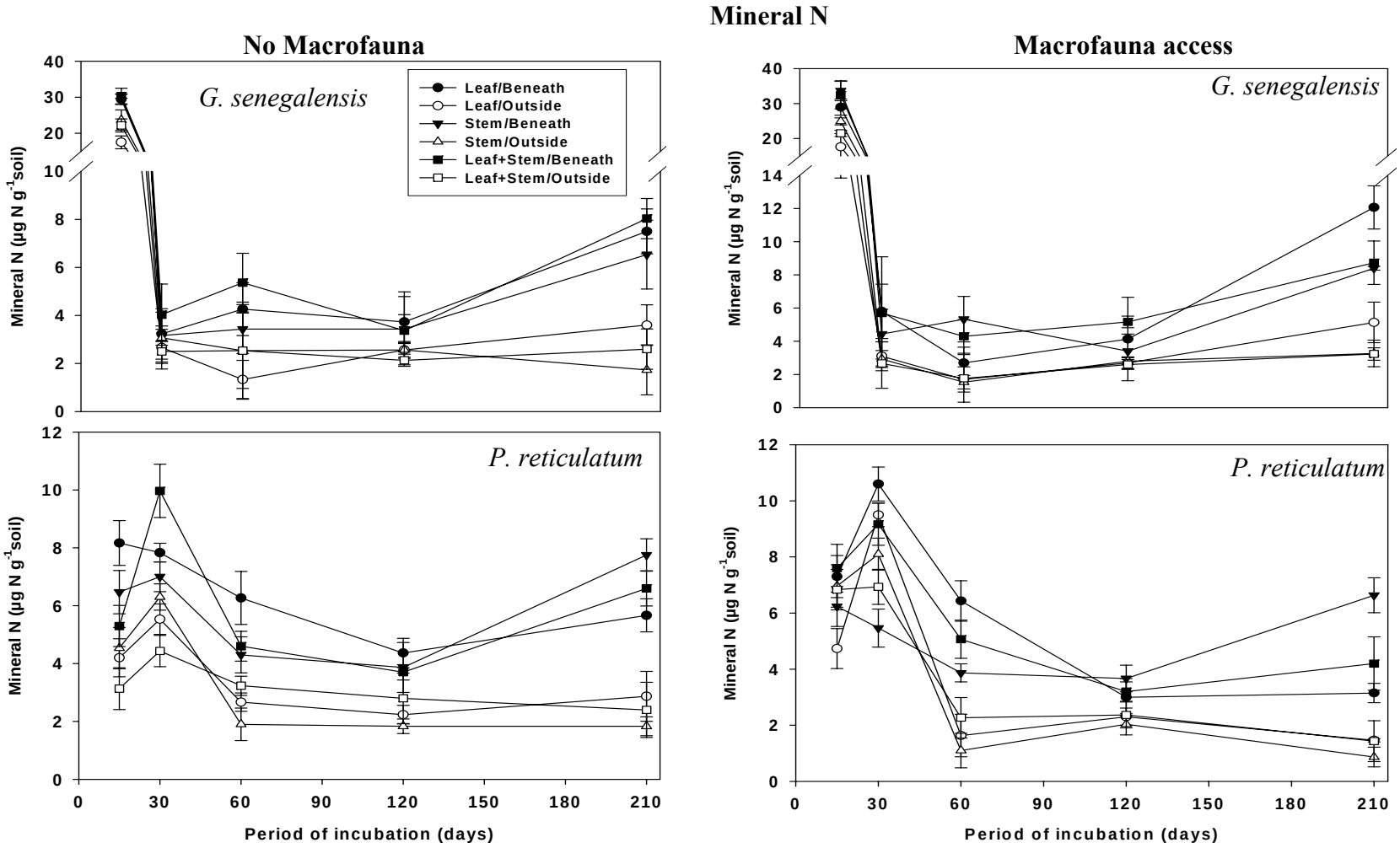

Figure 3.4. Mineral N of soil sampled under litterbags with less than 0.7 mm diameter (graphs on the left side) and under litterbags with 2 mm diameter (graphs on the right side). Barres are standard of deviation.

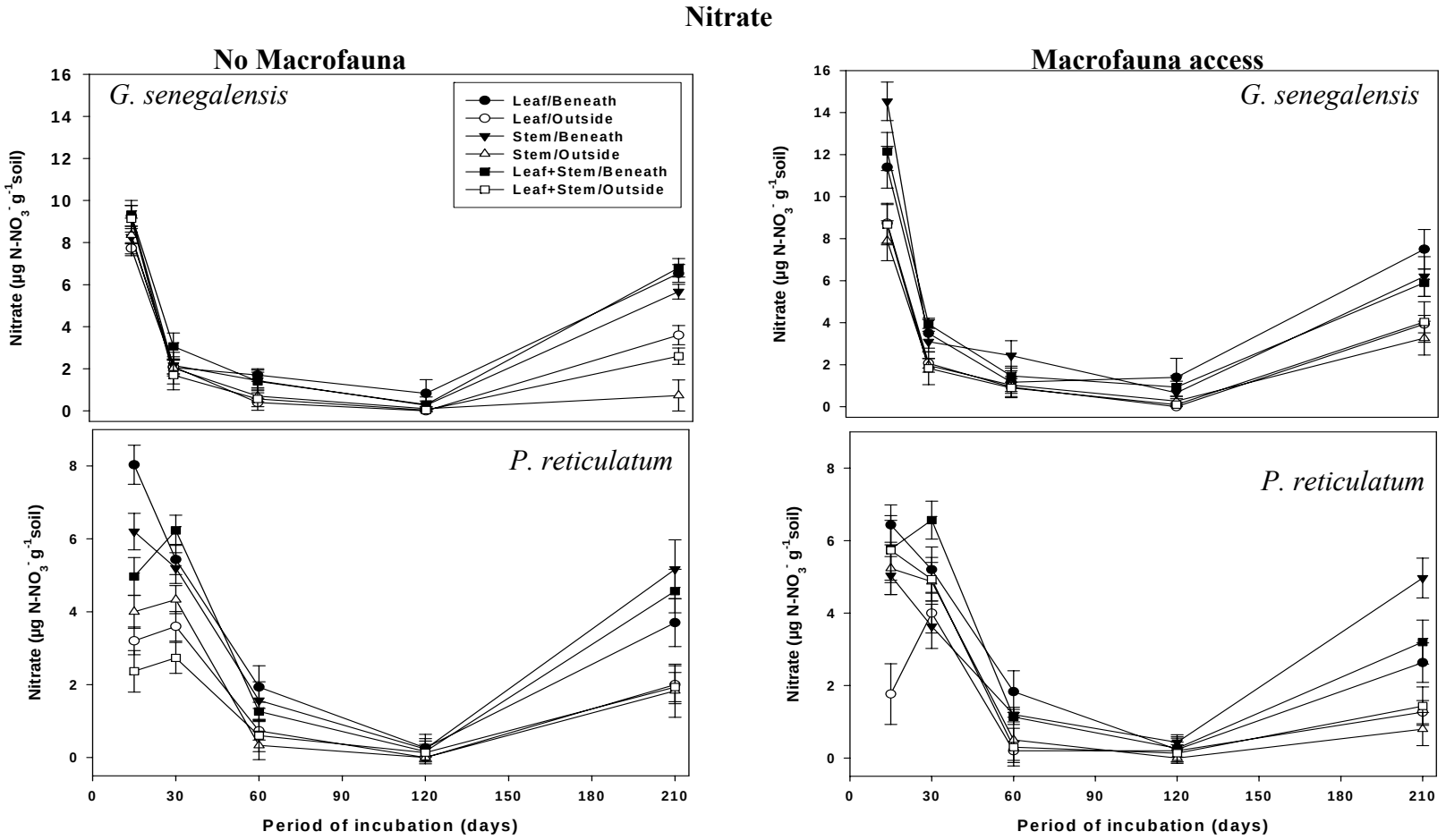

Figure 3.5. Nitrate content of soil sampled under litterbags with less than 0.7 mm diameter (graphs on the left side) and under litterbags with 2 mm diameter (graphs on the right side). Barres are standard of deviation.

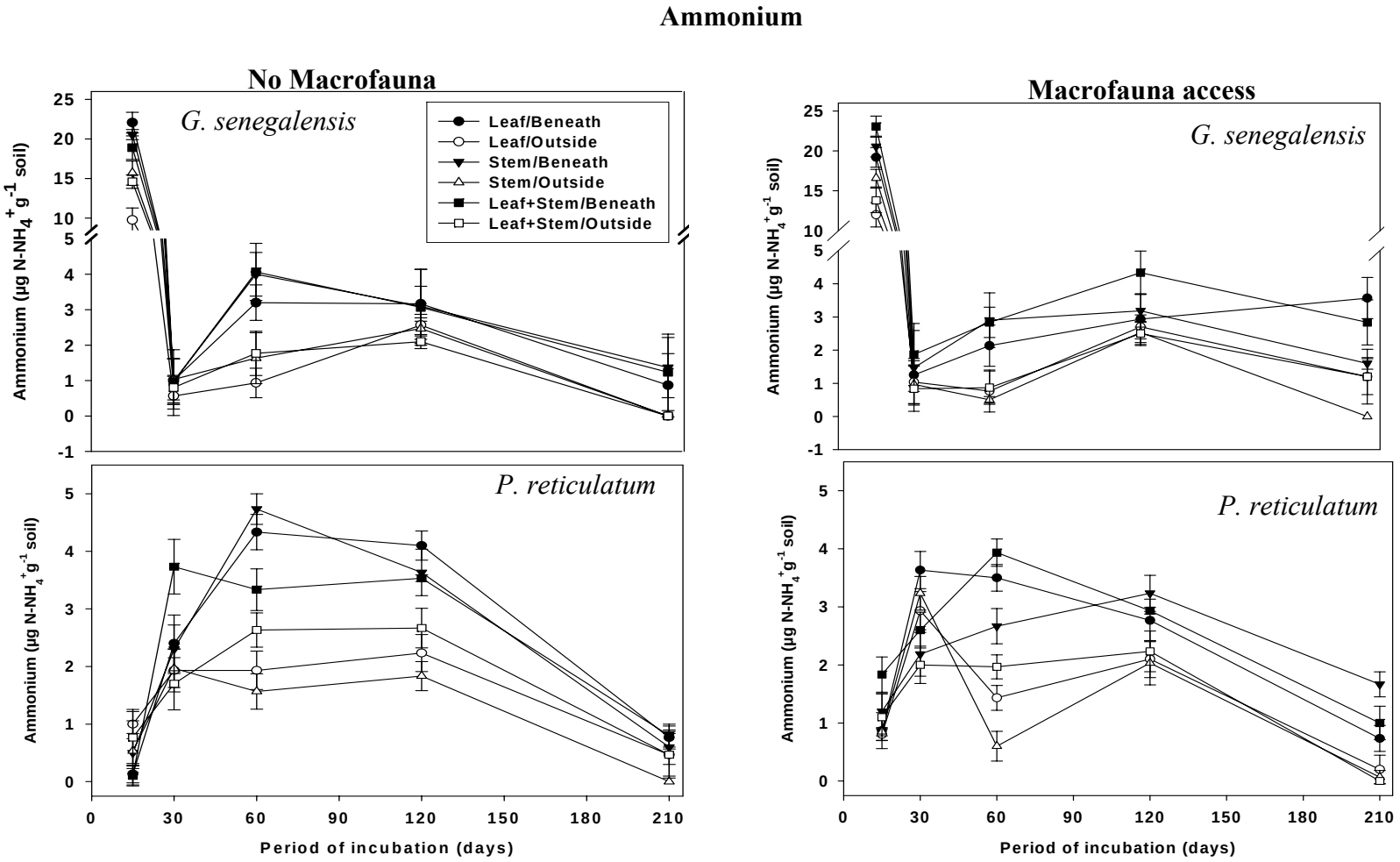

Figure 3.6. Ammonium content of soil sampled under litterbags with less than 0.7 mm diameter (graphs on the left side) and under litterbags with 2 mm diameter (graphs on the right side). Barres are standard of deviation.

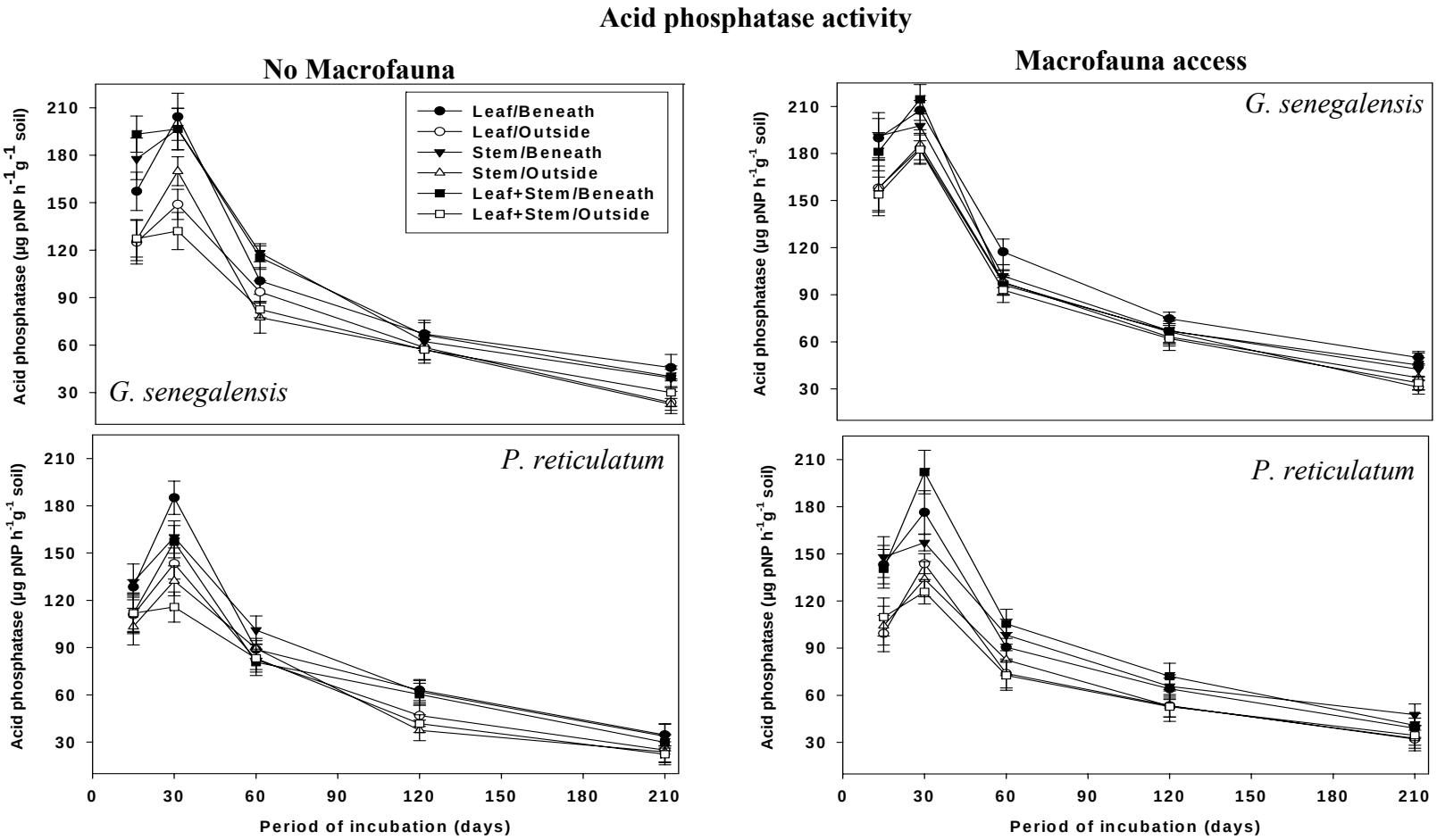

Figure 3.7. Acid phosphatase activity of soil sampled under litterbags with less than 0.7 mm diameter (graphs on the left side) and under litterbags with 2 mm diameter (graphs on the right side). Barres are standard of deviation.

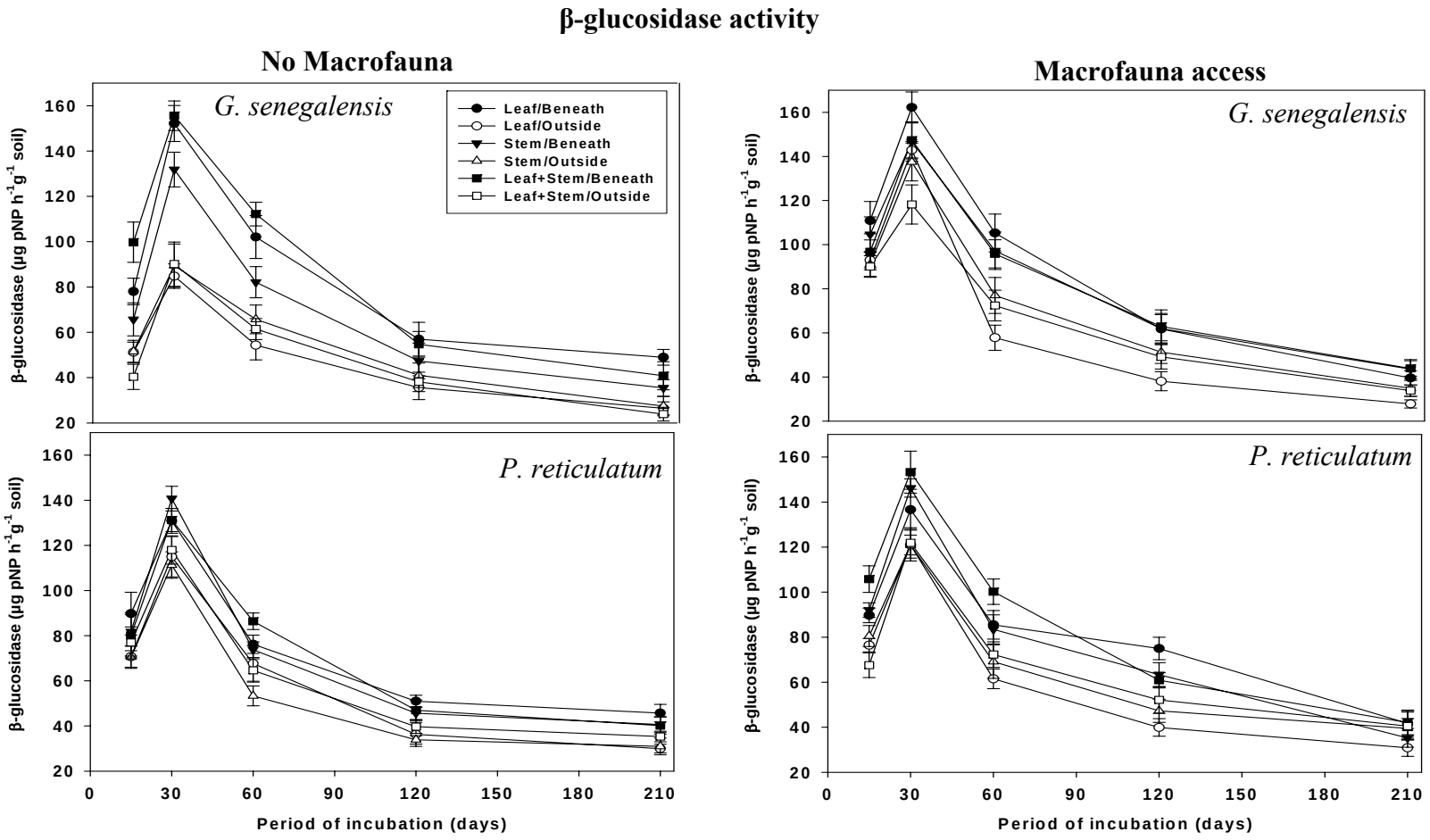

Figure 3.8. β-glucosidase activity of soil sampled under litterbags with less than 0.7 mm diameter (graphs on the left side) and under litterbags with 2 mm diameter (graphs on the right side). Barres are standard of deviation.

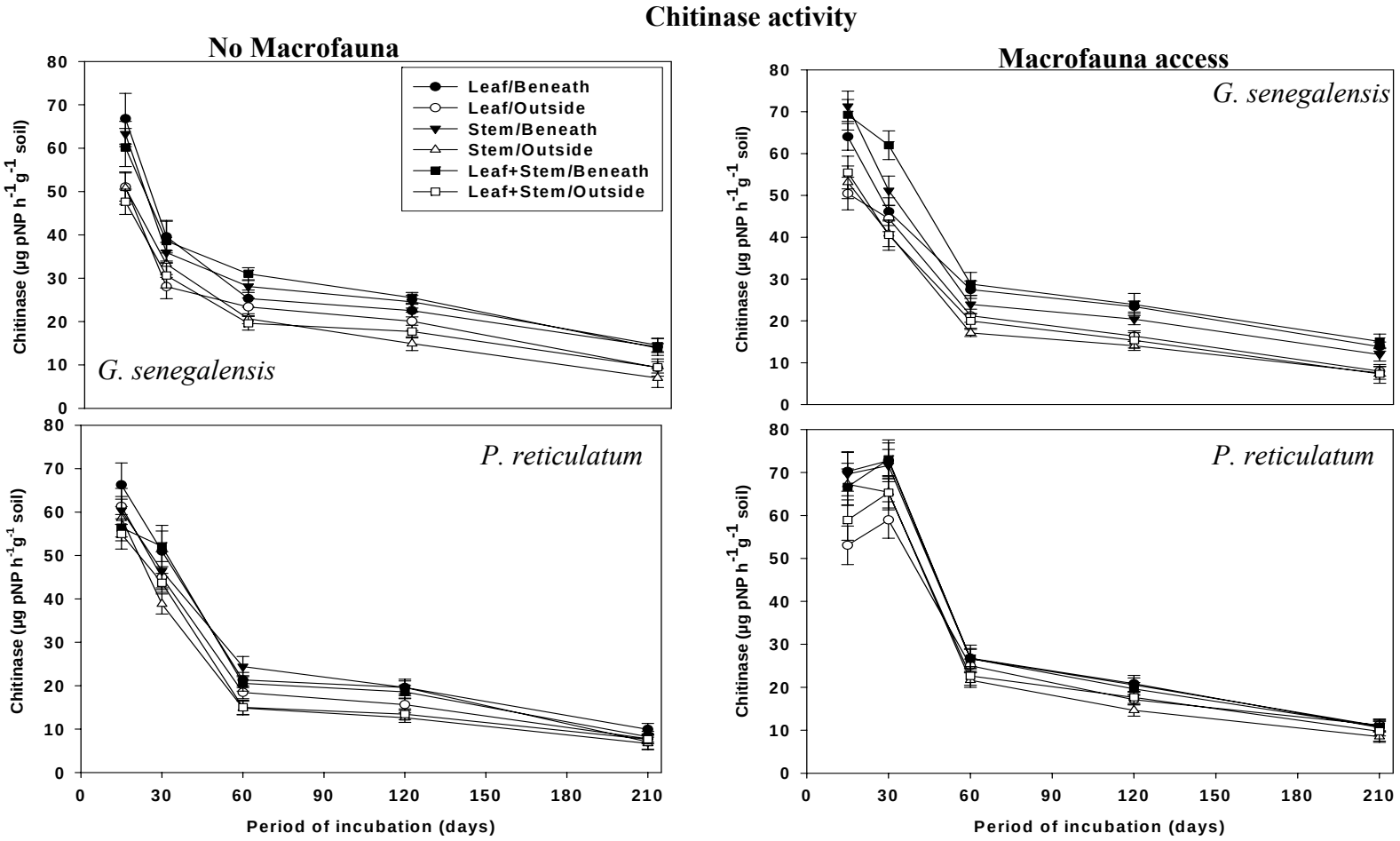

Figure 3.9. Chitinase activity of soil sampled under litterbags with less than 0.7 mm diameter (graphs on the left side) and under litterbags with 2 mm diameter (graphs on the right side). Barres are standard of deviation.

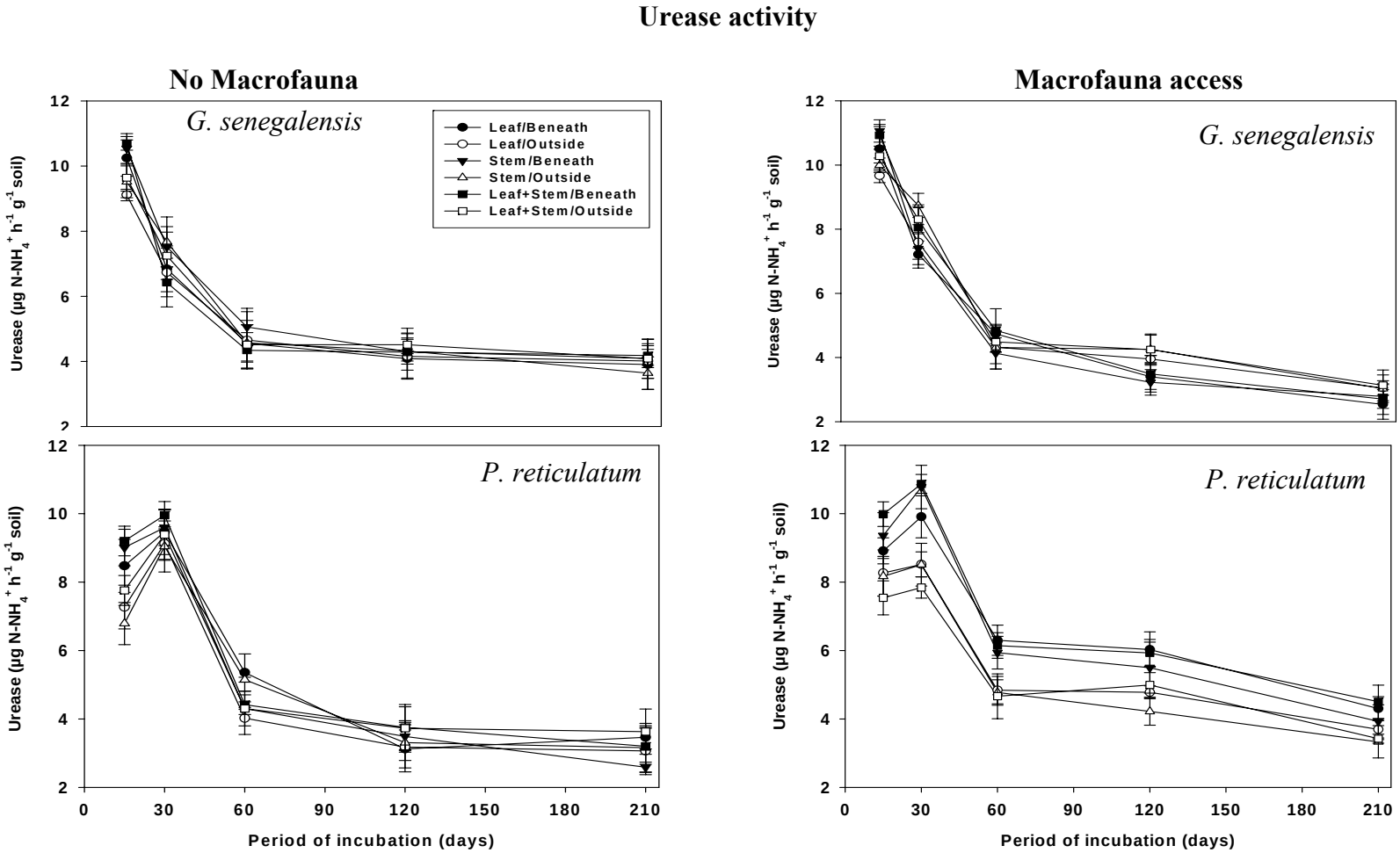

Figure 3.10. Urease activity of soil sampled under litterbags with less than 0.7 mm diameter (graphs on the left side) and under litterbags with 2 mm diameter (graphs on the right side). Barres are standard of deviation.

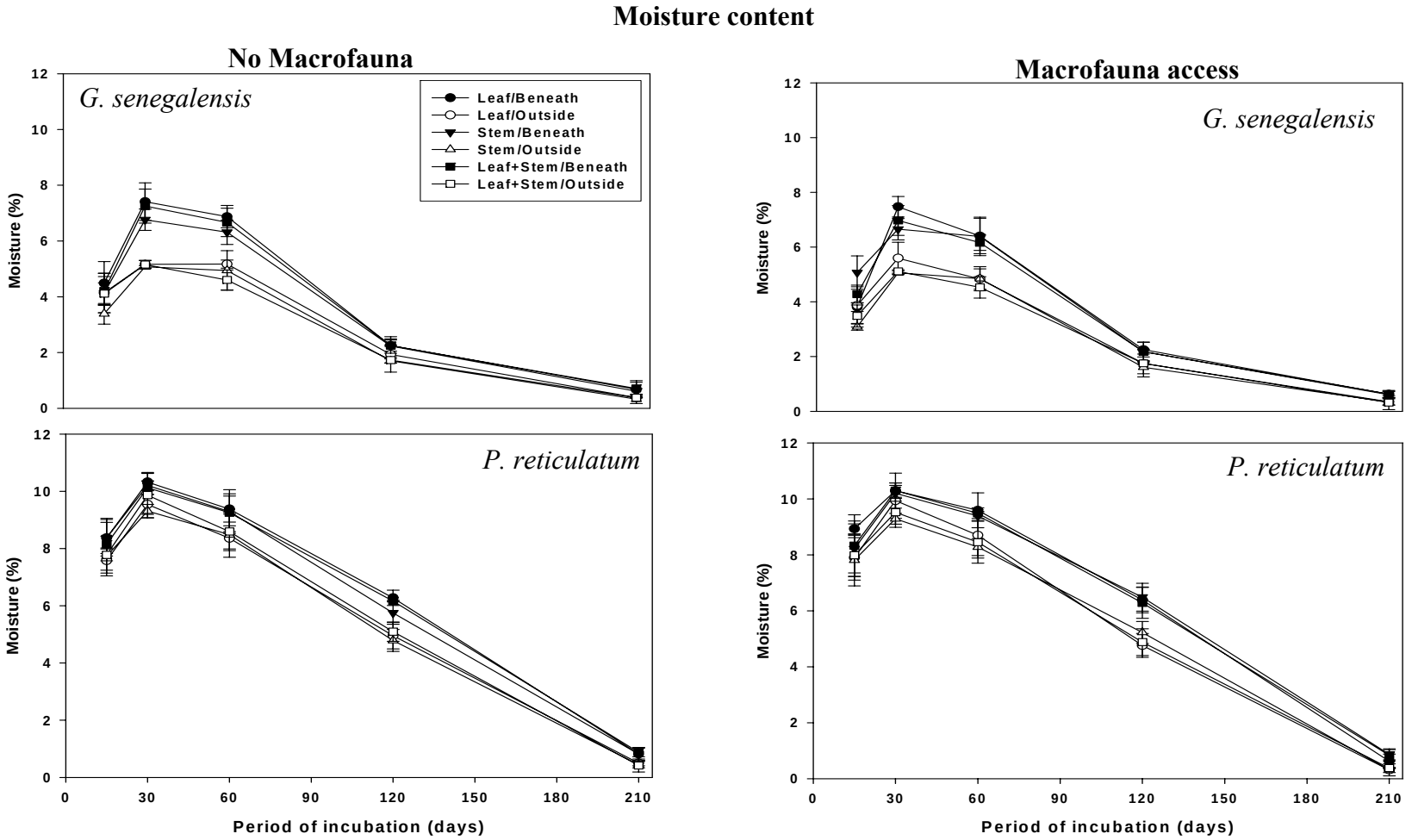

Figure 3.11. Moisture content of soil sampled under litterbags with less than 0.7 mm diameter (graphs on the left side) and under litterbags with 2 mm diameter (graphs on the right side). Barres are standard of deviation.

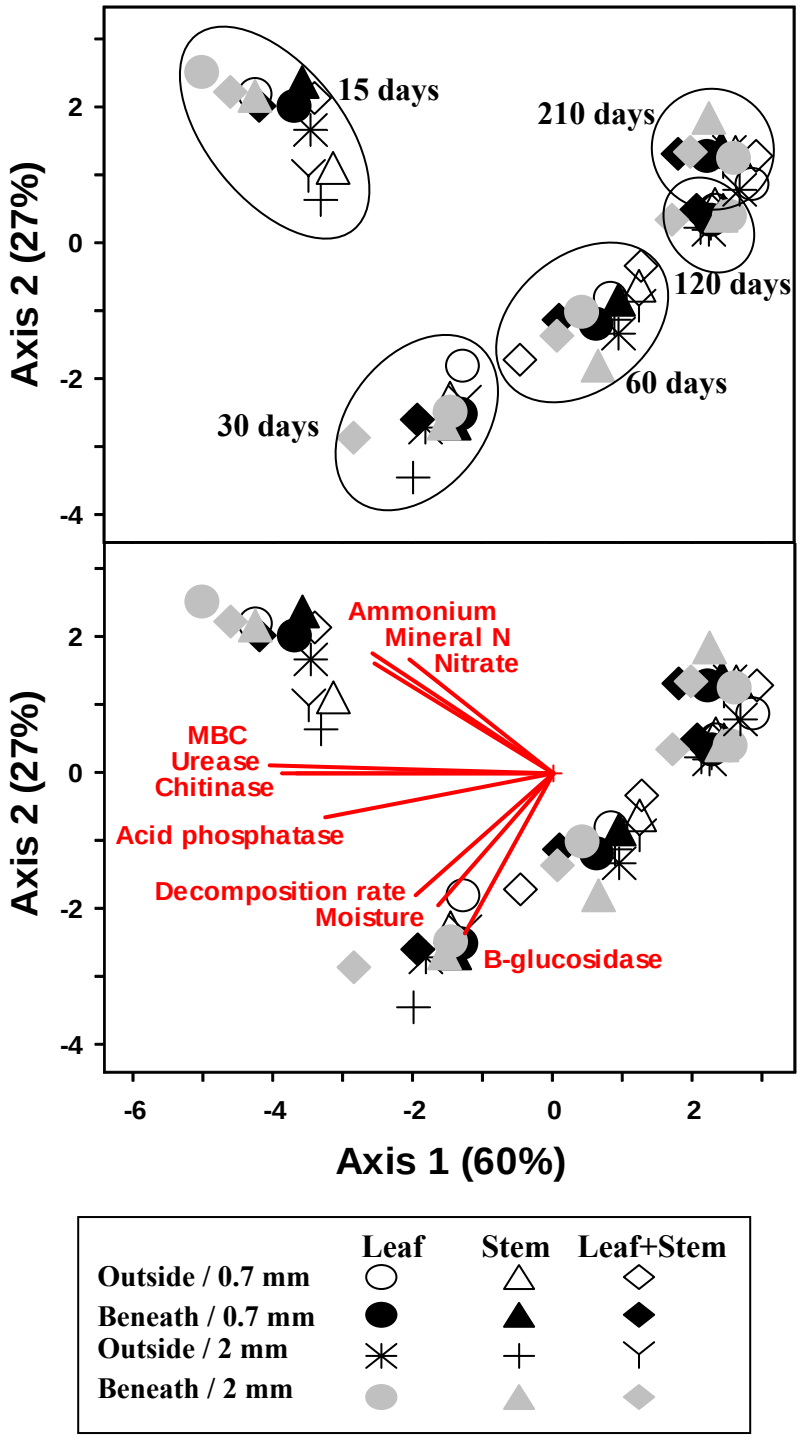

Figure 3.12. PCA representation of soil sampled under *G. senegalensis* residue enclosed in litterbags with less than 0.7 mm diameter and 2 mm diameter beneath and outside the canopy influence. Vectors represent correlations of the properties with the microbial communities.

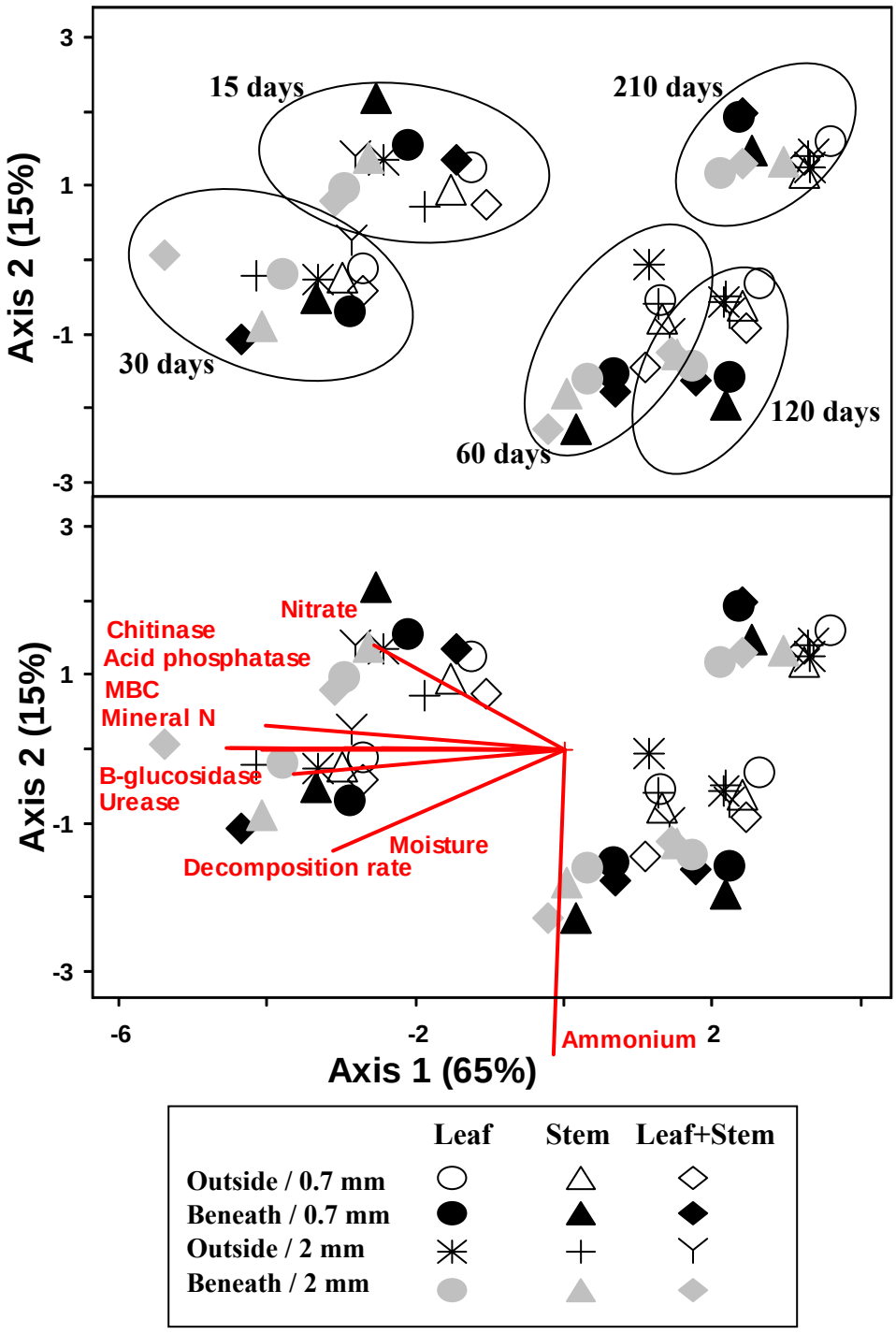

Figure 3.13. PCA representation of soil sampled under *P. reticulatum* residue enclosed in litterbags with less than 0.7 mm diameter and 2 mm diameter beneath and outside the canopy influence. Vectors represent correlations of the properties with the microbial communities.

Table 3.1. Initial chemistry of shrub residues, *G. senegalensis* and *P. reticulatum* (n = 4).

	C	N	Lignin (LG)	Cellulose	Hemicellulose	Polyphenols (PP)	C:N	LG:N	PP:N	(PP+LG): N
	----------%----------									
					G. senegalensis					
Leaf	35.4	1.6	10.3	21.6	12.8	6.4	21	6.3	3.9	10.2
Leaf + Stem	33.3	1.3	18.1	45.2	13.3	7.9	26	14.2	3.1	20.3
					P. reticulatum					
Leaf	35.2	1.8	13.1	19.8	13	5.3	20	7.4	3.0	10.4
Leaf + Stem	33.7	1.2	13.6	44.4	13.2	7.3	27	10.8	5.8	16.7

Table 3.2: Decay constant (-k) of litter from *G. senegalensis* and *P. reticulatum* (n = 6)

Litter treatment	Beneath canopy		Outside canopy	
	No macrofauna	Macrofauna access	No macrofauna	Macrofauna access
	----------Decay constant (-k year^{-1}) ----------			
	G. senegalensis			
Leaf	0.24	0.45	0.17	0.31
Stem	0.22	0.40	0.14	0.35
Leaf + Stem	0.23	0.46	0.16	0.32
	P. reticulatum			
Leaf	0.28	0.48	0.19	0.25
Stem	0.27	0.53	0.18	0.41
Leaf + Stem	0.28	0.49	0.21	0.37

Table 3.3. Test of significance of different factors used to monitor soil or residue from *G. senegalensis* and *P. reticulatum*.

	Location					Amendment					Termites				
	15d	30d	60d	120d	210d	15d	30d	60d	120d	210d	15d	30d	60d	120d	210d
G. senegalensis															
Rate of mass loss	**	**	NS	**	NS	**	**	NS	NS	NS	**	**	**	**	NS
MBC	NS	**	**	NS	NS	NS	NS	NS	NS	NS	**	**	NS	NS	NS
Mineral N	**	**	**	**	**	NS	NS	NS	NS	NS	NS	*	NS	NS	**
Ammonium	**	NS	**	**	NS	**	NS	NS	NS	NS	NS	NS	NS	NS	**
Nitrate	**	**	**	**	**	NS	NS	NS	NS	NS	**	*	NS	NS	**
Moisture	**	**	**	**	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Acid Phosphatase	**	**	**	**	NS	**	NS	NS	NS	NS	NS	**	NS	NS	**
β-glucosidase	**	**	**	**	**	**	**	*	NS	NS	**	**	**	NS	NS
Chitinase	**	NS	**	**	**	NS	NS	NS	NS	NS	**	**	NS	NS	NS
Urease	NS	NS	NS	**	NS	*	NS	NS	NS	NS	NS	**	NS	NS	NS
P. reticulatum															
Rate of mass loss	**	**	**	**	NS	NS	**	**	NS	NS	**	**	**	**	NS
MBC	**	**	NS	**	NS	NS	NS	NS	NS	**	**	**	NS	NS	NS
Mineral N	**	**	**	**	**	NS	NS	NS	NS	**	NS	NS	NS	NS	**
Ammonium	NS	NS	**	**	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Nitrate	**	**	**	*	**	NS	NS	NS	NS	NS	NS	NS	NS	*	NS
Moisture	NS	**	**	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Acid Phosphatase	**	**	NS	**	NS	*	**	NS	NS	NS	**	**	NS	NS	NS
β-glucosidase	**	**	**	**	**	**	**	NS	NS	NS	**	**	**	**	NS
Chitinase	**	**	**	**	NS	NS	**	NS	NS	NS	**	**	NS	NS	NS
Urease	**	**	NS	*	NS	NS	NS	NS	NS	NS	NS	**	**	**	NS

*significant

CHAPTER 4

Succession of Soil Microbial Communities during Decomposition of Native Shrub Litter of Semi-Arid Senegal

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Abstract

The parkland system of the Sahel where farmers allow trees to grow in cropped fields has an unrecognized component, native woody shrubs. In semi-arid Senegal two shrubs dominate and coexist in farmers' fields, *Guiera senegalensis* and *Piliostigma reticulatum*, which are coppiced and burned prior to crop planting. None-the-less, the year-round presence of the rhizosphere should improve soil quality, but the interactions of these shrubs with the microbial communities are uninvestigated. Hence, the objective was to determine the influence of shrub rhizosphere soil and residue chemistry on the microbial community composition and activity during the decomposition process as a means to develop non-thermal residue management. The experimental design was a 2 X 3 factorial design with two soil treatments (0-5 cm depth beneath and outside the influence of the shrub) and three residue amendments (leaf, stem+leaf and control). The samples were incubated for 105 days in laboratory conditions with intermediate destructive samplings at days 7, 14, 45, 75, or 105 after incubation. At each sampling, soils were assessed for phospholipids fatty acid (PLFA) to profile microbial functional groups and activity of two C hydrolyzing enzymes (β-glucosidase and cellulase) were quantified. PLFA patterns of the soil microbial communities shifted due to the residue amendments and time of incubation. Shrub canopy influence 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+ bacteria and the fungal markers

18:2ω6c and 18:1ω9c were highly correlated with both cellulase and β-glucosidase activities. The actinomycete marker (10Me 16:0) was, in contrast, poorly correlated with enzyme activities. This study showed that 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.

4.1. Introduction

In Senegal and throughout neighboring Sahelian countries, farmers allow trees and shrubs to grow randomly in their fields. This is known as the parkland system. Trees in parkland systems and other agroforestry systems have been receiving increasing attention in Africa (Young, 1989; Samba, 2001). However, two native and dominant shrubs, *Piliostigma reticulatum* and *Guiera senegalensis* are largely overlooked components of parkland systems in semi-arid Sub-Sahelian Africa (Lufafa, 2006). In some areas these shrubs nearly cover the landscape but in others there is a less dense distribution (Diack et al., 2000; Lufafa, 2006). In farmers' fields throughout the Sahel shrubs regrow after the cropping season. In the spring, the shrubs are coppiced and burned to prepare for the next cropping period. Non-thermal management of these organic materials holds potential to add organic matter to soils through litter input and root exudates, which would increase soil microbial biomass C.

A second aspect is that 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 (Kieft et al., 1998), as well as high microbial biomass and activity (Gallardo and Schlesinger, 1995). These favorable conditions stimulate soil organic matter formation and nutrient cycling under shrub canopies compared to outside the influence of the canopy. 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 management systems to optimize the use of the litter for improving soil quality and crop productivity. In order to utilize non-thermal management of these residues, fundamental studies examining the shrub canopy influence on the microbial community structure and decomposition are needed.

Soil microbial communities control the partitioning of 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; fungi and bacteria are the most important litter decomposers over soil fauna. Bacterial and fungal biomass increased as decomposition proceeds (Parmelee et al., 1989; Henriksen and Breland, 1999; Malosso et al., 2004). Utilization of these C pools may be associated with different groups of microorganisms, resulting in an orderly microbial succession that is linked to the changes in residue chemistry during decomposition. However, the real situation is more complex, and each succession is unique (Frankland, 1998).

Since the decomposition processes are largely controlled by the soil microbial communities, enzyme activities can be used to determine the potential of the soil to perform key hydrolytic reactions in response to substrates or environmental factors. Phospholipid fatty acid (PLFA) analysis is a quantitative method to determine the viable microbial biomass, as phospholipids are integral components of cell membranes and are rapidly converted into neutral lipids upon microbial death (King et al., 1977). Therefore, they provide an accurate index of living biomass. The composition of PLFA from samples has been sensitive for detecting shifts in soil microbial communities during decomposition and 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). Changes in PLFA profiles are indicative of changes in the overall structure of microbial communities (Frostegård et al., 1996) and "signature" PLFA can provide information on specific groups of microorganisms present in a community (Frostegård et al., 1993).

The objectives of this research were to study the structure and activity of the microbial communities during decomposition of above ground residues for *G. senegalensis* and *P. reticulatum* with respect to 1) temporal microbial succession; 2) impacts of soils from beneath or outside the shrub canopies and 3) shrub residue chemistry.

4.2. Materials and methods

4.2.1. Site Description

The experimental location was the semiarid agro-ecological zone in the peanut (*Arachis hypogea*) production zone of Senegal, between 13° 35' and 14° 30' northern latitude and 14° 35' and 16° 45' western longitude. The region is characterized by a tropical Sudanian climate with an annual rainfall of 700 mm and potential evapotranspiration of 1,800 mm yr^{-1}. Temperatures range from averages of 22.8°C in December-January to averages of 32°C in April-June.

A Dior loamy sand was collected for *G. senegalensis* at Keur Matar-Aram, Senegal (annual precipitation of 400-600 mm) and similarly another set of sandy loam soil samples was collected for *P. reticulatum* at Nioro, Senegal (annual precipitation of 700-1000 mm). Soils are low in C and N (Table 4.1). They are classified as Lixisols (FAO classification) and are leached ferruginous tropical soils (probably Ultisols according to USDA 7^{th} Approximation). All sites were in farmers' fields that were under peanut (*Arachis hypogea*) and pearl millet (*Pennisetum thyphoides*) crop rotation where farmers coppice and burn aboveground residue every spring season prior to planting of row crops.

4.2.2. Laboratory Incubation Study

The incubation study had a completely randomized 2 x 3 factorial design for each shrub type where there were two soil treatments (soil beneath or outside the

shrub canopy) and three residue treatments (leaf, a proportional mix as found in the field of 60% stem plus 40% leaf, and control soil with no residue). The shrub residues were collected in March, 2002 (approximately 1 m in height at harvest time). 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 were homogenized and then crushed to pass 2-mm mesh screen, air-dried and stored at 22°C.

The incubation study was sarted by first briging soils to 2/3 of field capacity and then incubating at 25°C to allow equilibration for three days prior to the start of the experiment. Residues were then 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. Soil moisture was maintained gravimetrically every 2-3 days.

4.2.3. Phospholipids fatty acids analysis

Microbial community structure was determined by analysis of PLFA using a modified method described by Bligh and Dyer (1959). All chemicals used were of analytical grade. Briefly, fatty acids were extracted in three steps from 3 g of triplicate

sub-samples soil 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 in various concentrations as internal standard and PLFA were analyzed by gas chromatography (GC) (Agilent Ultra 2 column; temperature ramping 120°C to 260°C at a rate of 5ºC per min). 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.).

Standard nomenclature rules were followed when referring to different fatty acids (Frostegård et al., 1993). Fatty acid nomenclature is in the form of A:BωC, where A designates the total number of carbons, B the number of double bonds, and C the distance of the closest unsaturation from the aliphatic end of the molecule. The suffixes "-c" for cis and "-t" for trans refer to geometric isomers. The prefixes "i-," "a-," and "me-" refer to iso-, anteisomethyl branching, and mid-chain methyl branching,

respectively, with cyclopropyl rings indicated by "cy" (Kates, 1986). 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 per gram 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 value expressed in nmol g^{-1} C of PLFA per g of soil has been 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%) have been used for multivariate analysis. Specific PLFA markers have been used in this study as biomarkers for different groups of organisms (Table 4.2) and total PLFA ($PLFA_{tot}$) was summed across each sampling date and was used as an indicator of microbial biomass.

The ratio of the sums of the fungal and bacterial fatty acids signature (FUN/ BACT) was included in the data analysis; it has been used as an indicator of overall changes in the soil microbial community structure (Bardgett et al., 1998; Olsson, 1999; Zelles, 1999; Fierer et al., 2002). In addition, we calculated and analyzed two

stress indicators; the ratio saturated to monosaturated PLFA (SAT/MONO) and the ratio cy 19:0 / 18:1ω7c. The low value of the ratio SAT/MONO has been suggested an indicator of substrate availability and monounsaturated fatty acids have been used as indicators of aerobic and high substrate conditions (Bossio and Scow, 1998; Larkin, 2003). Cyclopropyl fatty acids (cy 17:0, cy 19:0) are produced from the corresponding monounsaturated fatty acids (16:1ω7c, 18:1ω7c) by many Gram- bacteria response to depletion of substrate or stress (Guckert et al., 1986; Petersen et al., 1997; Lundquist et al., 1999a), thus the ratio of cy 19:0 / 18:1ω7c was also used as a stress indicator.

4.2.4. 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). 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}.

Two analytical triplicates were used for each soil sample and enzyme. For each sample, a blank mixture with soil and buffer, but no substrate, was incubated in the same conditions as above and analyzed to discount the background of reducing sugars in the soil.

The 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 one hour at 25°C as described by Tabatabai (1994). The quantity of ρNP released was determined colormetrically on a spectrophotometer set at 420 nm and was reported as μg ρNP g^{-1} dry soil hr^{-1}.

4.2.5. 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). Before the analysis, PLFA data were 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 axis 1 and 2. PerMANOVA is a nonparametric procedure that tests the simultaneous response of one or more variables to one or more factors in an ANOVA experimental design on the basis of any distance measure, using permutation methods. In addition, correlations between PLFA relative concentrations and NMS coordinates were calculated to identify PLFA whose gradients were represented by

axis 1 and 2. Amount of PLFA have been also correlated with enzyme activities as well as some specific PLFA groups using SAS (SAS Institute Inc., 1996).

4.3. Results

G. senegalensis and *P. reticulatum* had lower C:N ratio (20 – 27) with very low N content (Table 4.3). Lignin is higher for *G. senegalensis* than for *P. reticulatum.*

4.3.1. Temporal and Residue Amendment Responses

4.3.1.1. 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 higher than at day 45 ($p < 0.01$). Soils amended with leaf had a higher amount of $PLFA_{tot}$, fungal and actinomycetal PLFA than soil amended with leaf/stem mix up to day 45 regardless of source of soil (Fig. 4.1). 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 amendment, $PLFA_{tot}$, 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).

Specific markers, functional groups and stress indicators were averaged for day15 or averaged across all sample dates (Table 4.4). The MONO, SAT and 18:2ω6c PLFA and the SAT/MONO ratio were significantly different with respect to the residue treatment at day 15. The monosaturated PLFA and stress indicator cy 19:0/18:1ω7c were also significantly different with respect to soil source. The sum of all PLFA across time of incubation resulted in greater amounts of PLFA beneath than outside the canopy and for amended soil than non-amended soil.

4.3.1.2. Piliostigma reticulatum

As an index of microbial biomass, $PLFA_{tot}$ was higher at the first sampling date at day 7 for all treatment except for the control (Fig. 4.2). 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 day15 (15 nmol g^{-1} soil). Control soil had much lower $PLFA_{tot}$ compared to amended soil. The residue effect was significant for all samples ($p< 0.03$). PLFA for the control soil were higher at day 15 than at day 7 for all microbial groups with the exception of the actinomycete groups. $PLFA_{tot}$, 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 highest amount of $PLFA_{tot}$ was for soil beneath canopy amended with leaf litter (120 nmol g^{-1} soil) or outside canopy (98 nmol g^{-1} soil) at days 7 and 15. 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 (Table 4.5). 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 10Me 16:0, 18:2ω6c and SAT and MONO PLFA were higher on soil amended with leaf compared to soil amended with leaf + stem at day 15 and for $PLFA_{tot}$.

4.3.2. PLFA analysis using Non-metric Multidimensional Scale (NMS)

4.3.2.1. Guiera senegalensis

Non-metric multidimensional scale analysis of the data showed that a percentage of 98% of the data was explained by the first two axes with the first axis explaining 82% and the second axis 13% (Fig. 4.3). 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). Axis two was highly correlated with the branched bacterial marker 16:1ω5c (r = 0.72).

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). 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 insignificant. The amended soil beneath the canopy tended to separate from soil outside 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 soil ($p < 0.01$). Within unamended soil, each sampling date was strongly different from one another ($p < 0.005$).

4.3.2.2. 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 (Fig. 4.4). The marker 18:0 2OH and the fungal markers 18:2ω6c and 18:1ω9c had the highest positive correlation with axis 1 respecticely ($r = 0.78$, $r = 0.77$ and $r = 0.76$), whereas the actinomycete marker 10Me 18:0 had the strongest negative correlation ($r = -0.86$). For the second axis, the highest correlation was with the actinomycete marker 10Me 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 $r = 0.66$).

The analysis of NMS scores of PLFA using PerMANOVA showed a strong difference between control soil and amended soil ($p<0.0001$). However, no difference was found within amended soils. There is 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 4^{th} and 5^{th} sampling ($p= 0.01$).

4.3.3. 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 = 0.64 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); 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 both enzymes r = 0.62 for β-glucosidase, and 0.81 with the cellulase activity. The marker 10Me 16:0 is the least correlated with the β-glucosidase activity (r = 0.18) and its correlation is low with cellulase (r = 0.25).

4.4. Discussion

4.4.1. Temporal Dynamics

The highest amount of $PLFA_{tot}$ after seven days of incubation for *P. reticulatum* may be due to the availability of easily decomposable materials that can support a much larger biomass. As the decomposition proceeds, recalcitrant materials remain which leads to a decrease in the population and only organisms adapted to

decompose these materials will be predominant (Boufalis and Pellissier, 1994; Bernhart-Reversat, 1999; Sall et al., 2003).

In reverse, actinomycete markers were higher at a later date, day 15. Actinomycetes have been shown to thrive under stressful conditions whereas bacteria and especially fungi have been shown to generally respond to readily available and easily decomposable material (Morris and Boerner, 1999). This may explain why the actinomycetes PLFA were higher at day 15 for *P. reticulatum*.

Unlike P. reticulatum where microbial PLFA had a large response at day 7, *G. senegalensis* had a later response at day 15. This may be because *G. senegalensis* has a higher ratio of lignin + polyphenol to nitrogen (20.3 vs. 16.7) than *P. reticulatum* 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).

Compared to other functional microbial groups, the fungal PLFA remained steady between days 7 to 15. Fungi have hyphae to more fully (spatially) explore nutrient reserves compared to place bound bacterial communities (Holland and Coleman, 1987) which gives them an advantage in maintaining biomass. Saprotrophic fungi play a central role in decomposition. Many fungi possess enzyme systems that can attack simple carbohydrates, parts of protein, cellulose and lignins. Fungal enzymes include cellulase, hemicellulase lignin peroxidase, MN peroxidase, proteases,

chitinase, and other enzymes (Carlile et al., 2001). This would allow them to sustain biomass and activity as the chemistry of the substrates change during decomposition.

4.4.2. Effects of Shrub Canopy Litter and Rhizosphere

The location of soil was important in determining microbial community profiles during decomposition. Soil taken beneath the canopy had higher PLFA levels than soil outside the influence of shrubs across residue treatments and sampling dates. For both plant species, soil beneath canopy consistently had a higher C:N ratio than soil outside canopy. Shrub canopies provide litter input and their rhizosphere have root turnover and exudates which contribute to increase C and nutrient levels which has been widely reported for various tree or shrub species (Boerner and Koslowsky, 1989; Boettcher and Kalisz, 1990; Bolton et al., 1993).

In our study, 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 (Swift et al., 1979; Heal et al., 1997) which in turn may affect decomposition of plant material (Elliott and Elliott, 1993). This is particularly true for the fungi where fungal PLFA were significantly higher in soils amended with leaf than soils amended with leaf + stem. Similar results were found in this study with enzyme activities higher beneath than outside the canopy influence. Enzyme activities were higher in leaf-amended soil at the beginning of the decomposition process. The activity was also

higher depending on the litter chemistry and results are consistent with finding by Fernandes (2006).

Organic materials added to soils contain a wide range of C compounds that vary in their rate of decomposition. The content of carbon, nitrogen, and lignin determines the rate of decomposition. Leaf litter had a lower C/N ratio (20-21) compared to the mixture of leaf + stem (26-27). This is consistent with studies that have shown fungi to be more responsive to C/N ratio and organic matter input chemistry than other microbial groups (Mafongoya et al., 1996).

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 shown by Broder and Wagner (1988) who reported fungal response to residue chemistry during successional stages of wheat straw decomposition.

SAT/MONO PLFA ratio is an indicator of microbial stress; this ratio was lower beneath canopy than outside regardless of the residue amended. The ratio cy 19:0 over 18:1ω7c, which is a stress indicator, was significantly lower in soil taken beneath canopy. The stress indicators, ratio of cy 19:0 over 18:1ω7c and MONO/SAT 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. The higher correlations of the fungal markers (18:2ω6c, 18:1ω9c) and the actinomycete marker (10Me 18:0) with axis 1 indicate that actinomycetal and fungal groups were important in the structure of the communities.

However, the actinomycete markers 10Me 16:0 and 10Me 18:0 were correlated in opposite directions with axis 1 compared to 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 then decreased thereafter. Conversely actinomycetes started low and steadily increased over 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 for both shrubs 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+ bacteria markers also had a strong correlation with cellulase and β-glucosidase activities in *G. senegalensis* amended soils. Unlike Gram- bacteria which colonize readily decomposable compounds Gram+ bacteria can thrive on more recalcitrant materials and under more stressed environments (King et al.,

2001) and, furthermore can dominate in agricultural soils that typically are under more stress than uncultivated soils (Haack et al., 1994).

In contrast to other functional groups, the actinomycete marker 10Me 16:0 had the lowest correlation with enzyme activities. None-the-less, as mentioned above, this marker was stimulated by residue treatments at certain times during decomposition. This would suggest they may have a less direct role in decomposition but are able to indirectly take advantage of the ability of fungi to degrade residues. Also, they are known for dominating during stressful periods.

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 ratio (Broder and Wagner, 1988; Lundquist et al., 1999b). As decomposition proceeds, the chemical composition of the residue changes (Horwath and Elliott, 1996), this affects directly 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 is in opposite of studies by 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 and similar to the residues into these studies, it should be noted that all of these residues came from

herbaceous plants that are known to have lower lignin content and be less recalcitrant than woody species. On the other hand, 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 (Drijber et al., 2000; Schutter and Dick, 2002; Jackson et al., 2003; Rosenberg et al., 2003; Hinojosa et al., 2005; Jandl et al., 2005).

4.5. Conclusions

The dominant factor that resulted in the largest shift in microbial communities is the effect of shrub canopies/rhizosphere over non-rhizosphere soil. Secondarily, residue amendments affected microbial communities which had 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 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+ bacteria had high correlations with enzyme activities and it was particularly stimulated by *G. senegalensis*-amended soils. Conversely, the actinomycetal marker (10Me 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 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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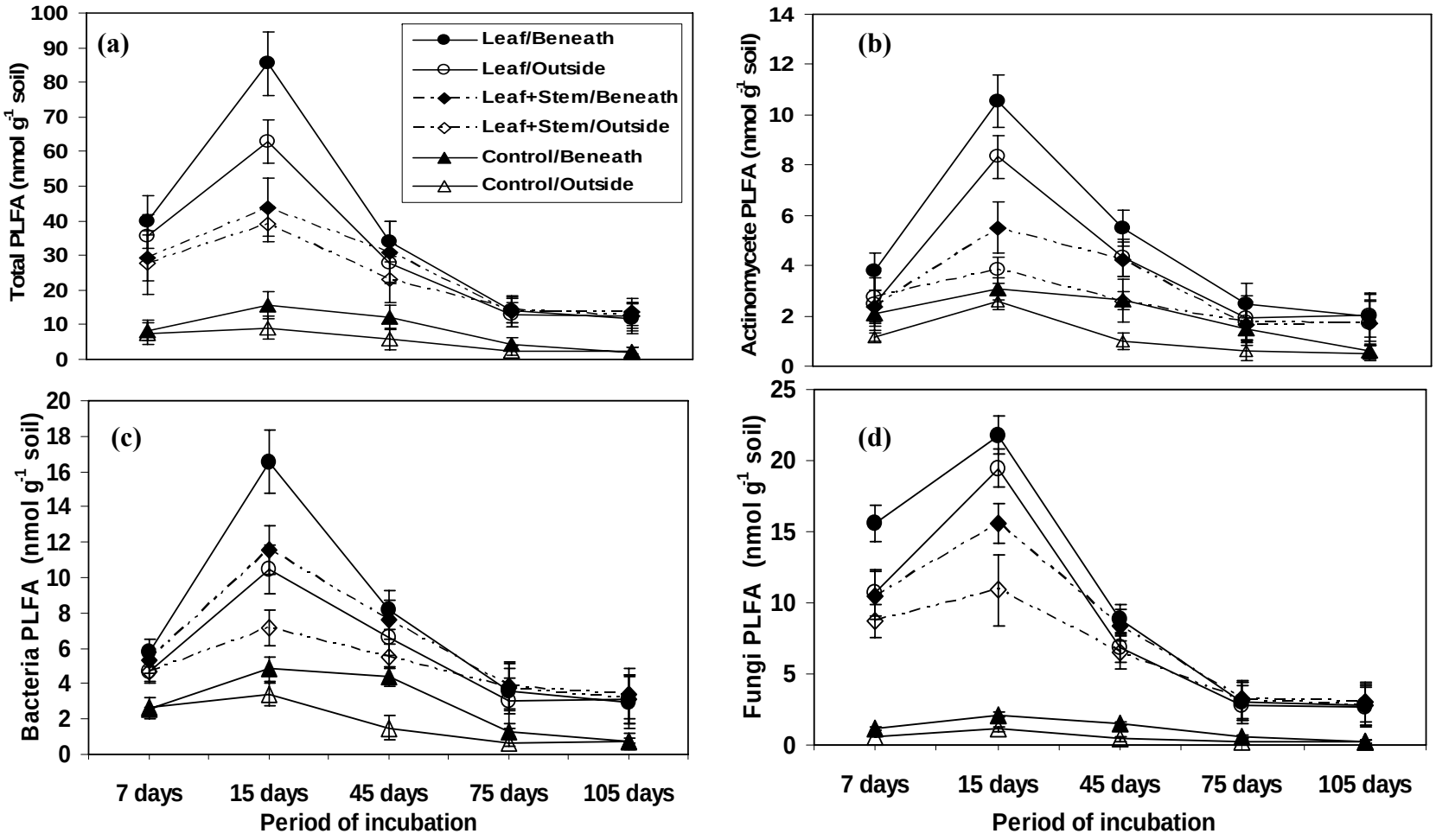

Figure 4.1.Total PLFA (a), actinomycete (b), bacteria (c) and fungi (d) PLFA of soil sampled beneath and outside the canopy of *G. senegalensis* and amended with different residues treatment. Barres are standard of deviation.

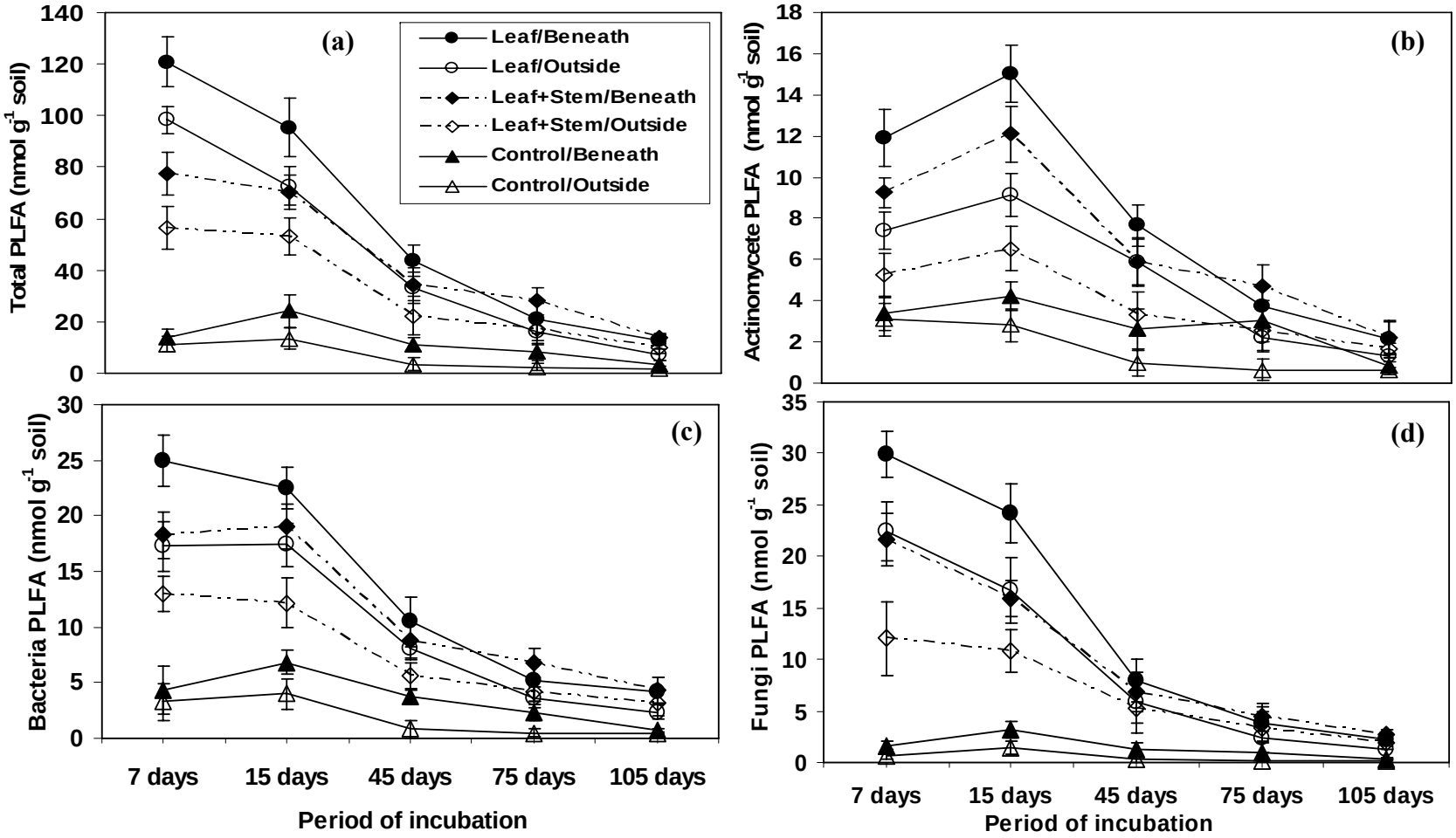

Figure 4.2.Total PLFA (a), actinomycetes (b), bacteria (c) and fungi (d) PLFA of soil sampled beneath and outside the canopy of *P. reticulatum* and amended with different residues treatment. Barres are standard of deviation.

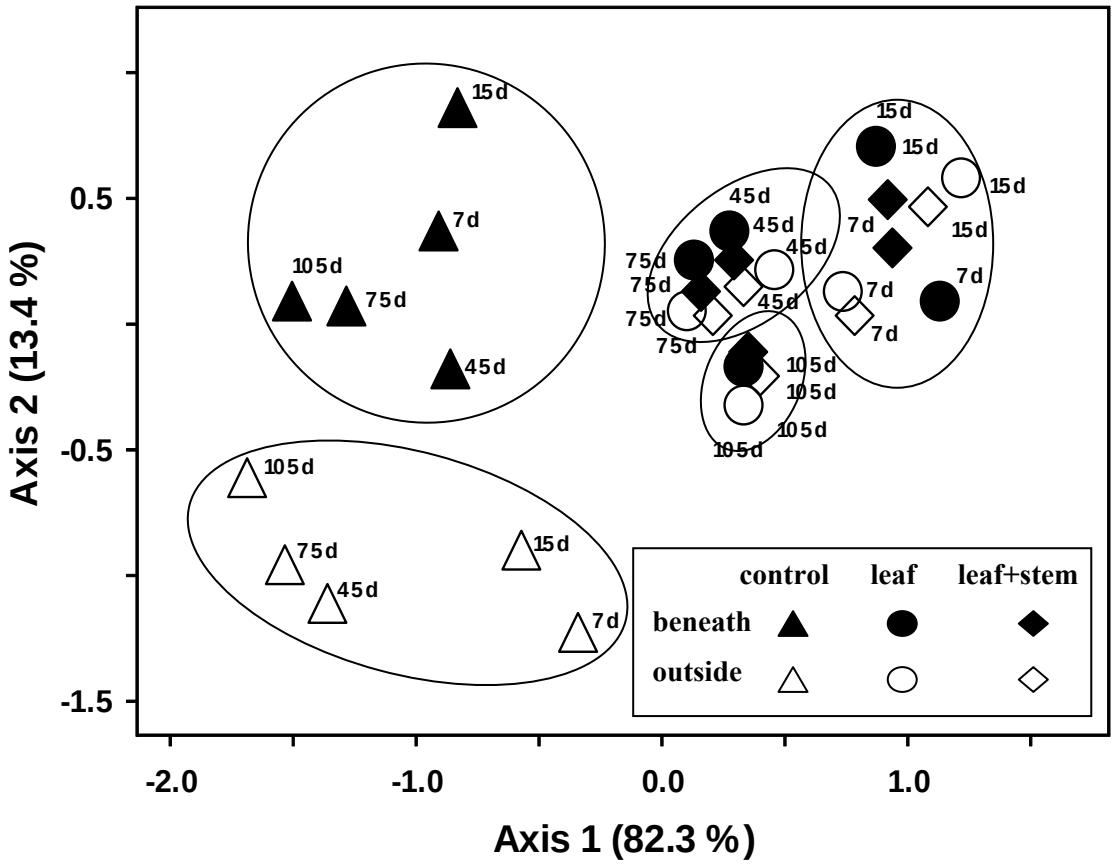

Figure 4.3. NMS representation of soil sample distances based on the mol % of 30 PLFA peaks extracted from soil associated with *G. senegalensis* amended with different residues and incubated over time (d = days of incubation).

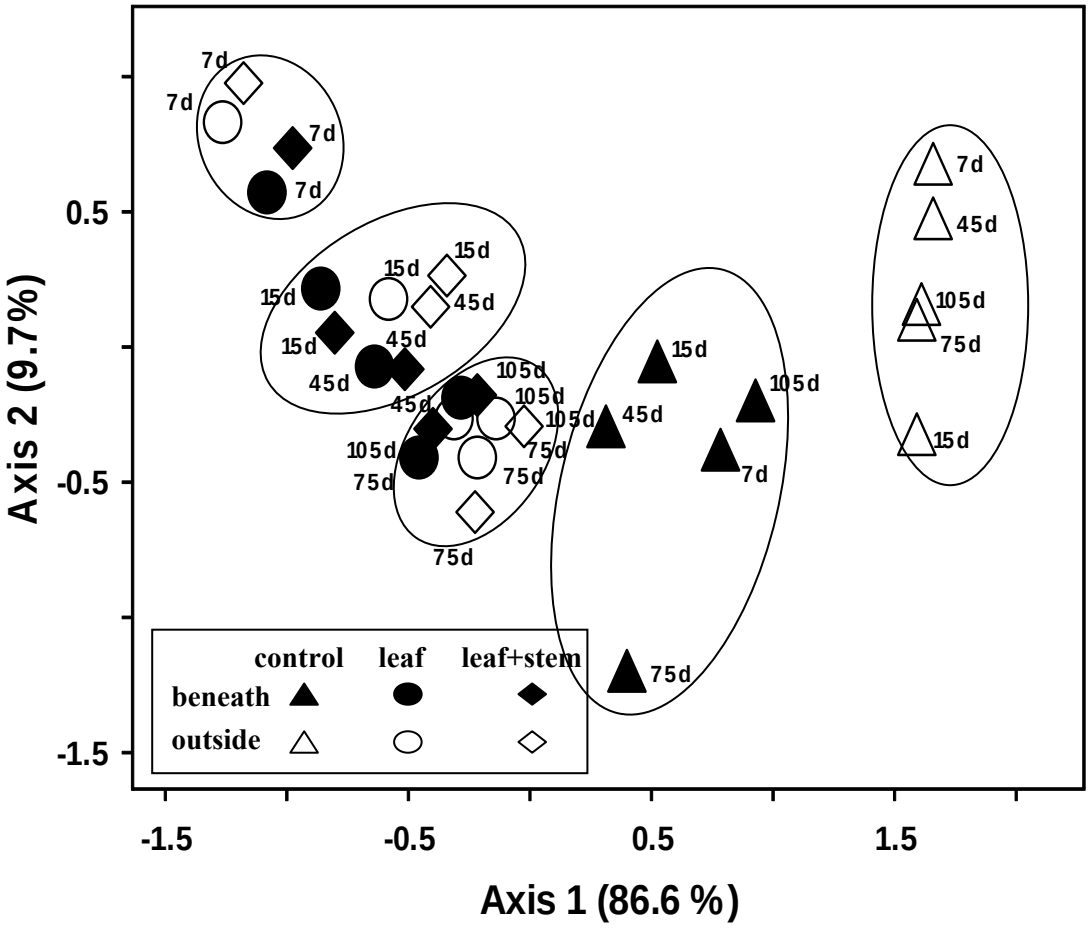

Figure 4.4. NMS representation of soil sample distances based on the mol % of 32 PLFA peaks extracted from soil associated with *P. reticulatum* amended with different residues and incubated over time (d = days of incubation).

Table 4.1. Total C and N content of soils used in the incubation study (n = 4)

Soil location	Total C	Total N	pH
	----------g kg^{-1} ----------		
	G. senegalensis		
Beneath canopy	3.35	0.20	5.2
Outside canopy	2.51	0.18	5.4
	P. reticulatum		
Beneath canopy	5.77	0.21	6.4
Outside canopy	3.23	0.19	5.8

Table 4.2. PLFA used as biomarkers for different groups of microorganisms.

Microbial Groups	PLFA markers	References
Fungi	18:2ω6c 18:1ω9c	Frostegård and Bååth, 1996; Olsson, 1999
Gram-positive bacteria (Gram+)	15:0i; 15:0a; 16:0i; 17:0i 17:0a	O'Leary and Wilkinson, 1988
Gram-negative bacteria (Gram-)	18:1ω7c; cy 17:0; cy 19:0	Wilkinson, 1988
Bacteria	Gram+, Gram-	
Actinomycetes (Actino)	10Me 16:0; 10Me 17:0; 10Me 18:0	Kroppenstedt, 1992
Saturated PLFA (SAT)	15:0; 16:0; 17:0; 18:0	
Monounsaturated PLFA (MONO)	16:1ω9c; 16:1ω7c; 16:1ω5c; 17:1ω9c; 18:1ω9c; 18:1ω7c; 18:1ω5c	

Table 4.3. Initial chemistry of shrub residues, *G. senegalensis* and *P. reticulatum* (n = 4).

	C	N	Lignin (LG)	Cellulose	Hemicellulose	Polyphenols (PP)	C:N	LG:N	PP:N	(PP+LG): N
	----------%----------									
					G. senegalensis					
Leaf	35.4	1.6	10.3	21.6	12.8	6.4	21	6.3	3.9	10.2
Leaf + Stem	33.3	1.3	18.1	45.2	13.3	7.9	26	14.2	3.1	20.3
					P. reticulatum					
Leaf	35.2	1.8	13.1	19.8	13	5.3	20	7.4	3.0	10.4
Leaf + Stem	33.7	1.2	13.6	44.4	13.2	7.3	27	10.8	5.8	16.7

Table 4.4. PLFA (nmol g^{-1} soil) for soil taken beneath and outside canopy of *G. senegalensis* (n = 3).

		Day 15				PLFA averaged across all sampling dates			
		Beneath canopy		Outside canopy		Beneath canopy		Outside canopy	
Residue	Taxonomic groups								
Control	10Me 16:0	1.6	(0.2)	1.4	(0.2)	5.4	(0.5)	4.3	(0.3)
	18:2ω6,9c	0.9	(0.3)	0.5	(0.0)	1.8	(0.3)	0.5	(0.0)
	FUN/BACT	0.4	(0.0)	0.3	(0.0)	1.8	(0.1)	1.5	(0.1)
	SAT	4.1	(0.5)	2.8	(0.8)	13	(1.5)	10	(3.0)
	MONO	4.3	(0.9)	1.2	(0.1)	11	(1.1)	5.8	(0.4)
	SAT/MONO	1.0	(0.1)	2.3	(0.2)	1.2	(0.1)	1.7	(0.2)
	Cy 19:0/18:1ω7c	0.7	(0.1)	1.7	(0.1)	8.5	(1.7)	9.7	(1.1)
Leaf	10Me 16:0	2.3	(0.5)	2.0	(0.6)	7.8	(1.0)	5.5	(1.3)
	18:2ω6,9c	9.2	(1.5)	8.7	(1.0)	21	(2.1)	17	(1.1)
	FUN/BACT	1.3	(0.1)	1.9	(0.3)	6.0	(1.0)	6.2	(1.0)
	SAT	28	(2.5)	21	(1.9)	60	(3.1)	52	(2.9)
	MONO	30	(2.3)	18	(1.3)	54	(4.7)	42	(2.7)
	SAT/MONO	1.0	(0.1)	1.2	(0.1)	1.1	(0.1)	1.3	(0.1)
	Cy 19:0/18:1ω7c	0.6	(0.0)	1.6	(0.3)	5.8	(0.9)	6.0	(1.4)
Leaf+stem	10Me 16:0	2.0	(0.1)	1.3	(0.1)	7.3	(1.1)	5.2	(1.1)
	18:2ω6,9c	7.2	(0.5)	5.2	(1.0)	18	(2.0)	14	(1.1)
	FUN/BACT	1.3	(0.1)	1.5	(0.2)	5.5	(1.1)	5.7	(1.9)
	SAT	18	(1.7)	14	(2.8)	32	(2.1)	39	(3.1)
	MONO	20	(1.2)	11	(1.1)	42	(3.2)	31	(2.1)
	SAT/MONO	0.9	(0.1)	1.3	(0.1)	0.8	(0.1)	1.2	(0.1)
	Cy 19:0/18:1ω7c	0.5	(0.0)	1.5	(0.2)	6.9	(1.1)	8.2	(1.2)

*Values in parentheses are standard of deviation.

Table 4.5. PLFA (nmol g^{-1} soil) for soil taken beneath and outside canopy of *P. reticulatum* (n = 3).

		Day 15				PLFA averaged across all sampling dates			
		Beneath canopy		Outside canopy		Beneath canopy		Outside canopy	
Residue	Taxonomic groups								
Control	10Me 16:0	2.0	(0.1)*	1.9	(0.2)	7.3	(0.9)	5.3	(0.4)
	18:2ω6,9c	1.0	(0.2)	0.8	(0.0)	2.3	(0.5)	1.1	(0.5)
	FUN/BACT	0.5	(0.1)	0.4	(0.1)	2.0	(0.1)	1.6	(0.2)
	SAT	7.1	(1.3)	4.2	(0.5)	17	(2.1)	10	(1.1)
	MONO	9.4	(1.8)	4.5	(1.2)	22	(3.1)	10	(1.1)
	SAT/MONO	0.8	(0.0)	0.9	(0.1)	0.7	(0.2)	1.0	(0.0)
	Cy 19:0/18:1ω7c	1.0	(0.1)	0.7	(0.0)	3.3	(0.7)	3.2	(1.0)
Leaf	10Me 16:0	8.4	(1.1)	4.8	(0.9)	23	(2.1)	15	(2.7)
	18:2ω6,9c	15	(2.0)	8.7	(1.2)	34	(3.0)	19	(1.1)
	FUN/BACT	1.1	(0.1)	1.0	(0.0)	4.3	(0.3)	4.2	(1.0)
	SAT	26	(3.9)	19	(3.1)	77	(6.0)	64	(3.5)
	MONO	38	(4.3)	28	(4.7)	112	(7.6)	85	(4.7)
	SAT/MONO	0.6	(0.0)	0.7	(0.0)	0.7	(0.2)	0.8	(0.1)
	Cy 19:0/18:1ω7c	0.4	(0.0)	0.5	(0.0)	2.3	(0.5)	3.0	(0.3)
Leaf+Stem	10Me 16:0	6.9	(1.5)	4.2	(0.7)	21	(1.1)	13	(1.1)
	18:2ω6,9c	8.2	(1.7)	5.7	(1.1)	28	(4.0)	17	(1.0)
	FUN/BACT	0.8	(0.1)	0.9	(0.1)	4.1	(0.5)	4.1	(0.8)
	SAT	17	(3.0)	17	(1.7)	53	(5.0)	45	(2.0)
	MONO	24	(4.2)	17	(2.3)	82	(4.0)	56	(2.0)
	SAT/MONO	0.7	(0.1)	1.0	(0.1)	0.6	(0.2)	0.8	(0.1)
	Cy 19:0/18:1ω7c	0.5	(0.1)	0.6	(0.1)	3.1	(0.3)	3.2	(0.5)

*Values in parentheses are standard of deviation.

CHAPTER 5

GENERAL CONCLUSION

Despite the permanent regrowth of shrubs in the parkland system of Sub-Sahelian Africa and their persistent green presence during the nine-month dry season, few studies have investigated their potential influence on nutrient availability. Furthermore, the microbial work was done mainly with nitrogen-fixing trees. This study has the particularity of investigating the influence of two native predominant shrubs, *Guiera senegalensis* and *Piliostigma reticulatum* on the soil microbial community composition, structure and activity.

In Chapter 2, the rhizosphere influence, as well as the seasonal influence (dry vs. wet) on the soil microbial communities were characterized using denaturing gradient gel electrophoresis (DGGE) for the bacterial and fungal communities in rhizosphere soil of new and old roots, in the bulk soil (between roots) and 2 m away from the canopy influence. In addition, analyses of phospholipids fatty acid (PLFA), enzyme activities (acid phosphatase, β-glucosidase, cellulase and urease), microbial biomass carbon (MBC) and mineral N were carried out to determine the composition, biomass and activity of the communities.

- Soils beneath canopy (rhizosphere and bulk soils) had a more diverse composition and active community than soil outside the canopy influence.

- During the rainy season, rhizosphere soil and bulk soil showed similar microbial activities as well as similar composition. Conversely during the dry season the two communities were very different. In addition, the rhizosphere communities during the dry season were quite similar to the soil microbial communities beneath canopy (rhizosphere and bulk soils).
- PLFA and enzyme activities were highly correlated and were more sensitive than DGGE in reflecting the strong rhizosphere and seasonal effects on the communities across various soil sampling locations.
- Fungal and Gram+ bacterial PLFA had the highest increase in rhizosphere over non-rhizosphere soil.
- Activities of β-glucosidase and acid phosphatase were more correlated with the rhizosphere communities.

In the Chapter 3, in a field study, we investigated the influence of shrub canopy (beneath and outside the canopy influence), residue type (leaf, stem, leaf+stem), and macrofauna (with or without macrofauna) on the decomposition rate during the rainy season in farmers' field over 210 days. Enzyme activities (acid phosphatase, β-glucosidase, cellulase, urease), MBC and mineral N also were quantified over time.

- Macrofauna access strongly increased the rate of decomposition which was overall higher at day 30. MBC increased under macrofauna influence as well as chitinase activity.

- Shrub canopy influence on the decomposition process was also very strong with a higher increase beneath canopy of the decomposition rate, and a higher microbial biomass and activity than outside the canopy influence.
- Although the decomposition rate was higher for leaf residue, the impact of residue type on the soil microbial communities was either weak or nonexistent.
- For *G. senegalensis*, at day 30, the rate of mass loss depended more on the location of the soil sample in addition to the macrofauna's effect, moisture, chitinase, urease, mineral N, nitrate, β-glucosidase. MBC was strongly correlated with the location of the samples, the mineral N and the moisture.
- For *P. reticulatum*, the rate of decomposition was strongly correlated with the presence of macrofauna and the chitinase and β-glucosidase activities at day 30. β-glucosidase also strongly correlated with MBC.

In Chapter 4, the last part of this thesis, we investigated shrub canopy influence (beneath and outside the canopy influence) and residue type (leaf, stem+leaf, and no residue = control) during the decomposition process in an incubation study done in laboratory conditions. The succession of the soil microbial communities using PLFA and the activity of two enzymes, β-glucosidase and cellulase, were investigated over time during 105 days.

- Shrub canopies/rhizosphere were the dominant factors in determining microbial community composition. Soil sampled beneath canopies had higher enzyme activities and higher amounts of PLFA than did soil sampled outside the canopy influence.
- Residue treatment type has less influence than did the shrub canopy on the activity and composition of the communities.
- Fungi were more sensitive to the type of litter added than were the bacterial and actinomycetes communities. There was indirect evidence that the residue chemistry affected soil communities.
- There were strong correlations of cellulase and β-glucosidase activities with fungal PLFA; this provided evidence for the dominance and the ability of fungi to degrade shrub residues.
- Gram+ bacteria had high correlations with enzyme activities and it was particularly stimulated by *G. senegalensis*-amended soils whereas the actinomycete marker (10Me 16:0) had low correlations with enzyme activities.

Overall, shrub rhizospheres clearly sustained a larger, more diverse community than non-rhizosphere soil – presumably by providing elevated levels of C inputs. The rhizosphere communities during the dry season were similar to the rhizosphere and bulk communities during the wet season. *Piliostigma reticulatum* and *Guiera senegalensis* are stimulating microbial activity and communities even in the dry season after six or more months without rainfall. Besides C inputs through litter fall,

root turnover and exudates, this suggests that shrubs maintain moisture levels in the dry season for microorganisms by performing hydraulic redistribution of water from wet subsoils to dry surface soils at night through a passive water potential gradient. Consequently, shrubs rhizosphere and litter input are maintaining soil health and can drive biogeochemical processes year round which has not been previously recognized, although the idea of island of fertility have long been established.

These results have practical implications in that it appears the presence of shrubs, as well as macrofauna access, enhanced the decomposition and stimulate microbial activity and diversity. This is a reasonable basis to develop non-thermal residue management to replace the current destructive farming practices in order to improve soil and optimize agricultural productivity.

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