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The Effects of Soil Cover on Soil Respiration and Microbial Population in the Mopane (*Colophospermum mopane*) Woodland of North Western Botswana

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ABSTRACT

Soil cover influences soil biological and chemical processes in various ways. The effects of soil cover (bare soil without litter, litter cover, Mopane tree canopy and grass cover) on soil respiration and microbial population at four sites in the Mopane woodland of north western Botswana were investigated. Soil respiration rates were measured using an infrared gas analyzer. Nitrifying bacterial populations were quantified by MPN on ammonium and nitrite calcium carbonate media, oligotrophs on 1% nutrient agar, actinomycetes on starch casein agar, bacteriovorous protozoans by MPN on sodium chloride agar baited with *Enterobacter*, and fungal biomass carbon using buried slides were also determined. Soil respiration in different habitats was significantly influenced by type of cover, diurnal temperature variations and soil moisture. Soils under cover showed significantly higher soil respiration than the bare soils. Autochthonous bacterial populations in the Mopane woodland soils consisted mostly of oligotrophs which varied insignificantly between seasons. Fungi were the most dominant microorganisms in all the Mopane woodland soils, with biomass of 162.05 to 282.14 μg C/g soil in the wet season and 11.84 to 44.16 μg C/g soil in the dry season. Microscopic observation of buried slides revealed that fungi play a vital role in holding soil particles together in these soils. The results showed a strong positive relationship between changes in fungal biomass and fluctuation of soil respiration. However, no clear correlation was observed between the variation of soil respiration and other microbial populations (oligotrophic bacteria, actinomycetes, and nitrifying bacteria). Overall these results indicate that in soils under Mopane tree canopy, fungi contribute significantly to soil structure and soil respiration. Other microbial populations consist mostly of oligotrophs which show minimal seasonal variations. Soil moisture significantly influences seasonal fluctuation in soil respiration.

Keywords: biomass, fungi, habitats, Maun, nitrification, oligotrophs

INTRODUCTION

Mopane woodland soils of north western Botswana are part of the larger Kalahari Sands Region which stretches from central part of Congo Brazzaville, running through the south eastern part of the Democratic Republic of Congo, the north western part of Angola, the south western part of Zambia, the northern western part of Namibia, the northern, central, and southern parts of Botswana and the northern tip of South Africa (Steffen 2000). These soils are highly sandy (>85% sand) with a very low moisture holding capacity (Bonyongo and Mubyana 2004). The soils also receive very low annual rainfall of approximately 450 mm (Ringrose 1999) usually between the months of December and March. Most of the year these soils are exposed to extremely dry conditions with mean maximum relative humidity of 64% and minimum of 20% (Bhalota 1987). Apart from the Okavango Delta and the immediate vicinity with a very high plant diversity and density due to flood waters (Omari *et al.* 2004), Mopane woodland soils which are further from the Delta are very rarely if at all influenced by the Delta water. This gives rise to a vegetation type of low plant diversity and low density during the dry season (May to September) which is dominated by Mopane trees (*Colophospermum mopane*) and shrubs (*Ximenia caffra*) in these Mopane woodland soils (Ellery and Ellery 1997). During the rainy season few annual grasses germinate but these usually dry out shortly. Tropical forest such as the Mopane woodland though dry and with little plant diversity are of great environmental significance in the regions they occur, as they are important in the carbon cycling budget of those areas (Mellilo *et al*. 1992; Lloyd and Taylor 1994; Veenendaal *et al.* 2003).

Among the components of carbon balance from the Mopane woodland is soil respiration. Soil respiration is the release of carbon dioxide (CO_2) from soils by living soil biota and roots respiration, and to a lesser extent, chemical oxidation of carbon compounds (Holt *et al*. 1990; Lloyd and Taylor 1994). Soil respiration is the main mechanisms of carbon transfer from soil to the atmosphere (Larionova *et al.* 1998; Yiqing *et al* 2005). Soil CO_2 efflux varies with ecosystems and environmental conditions (Fang *et al*. 1998; Yiqing *et al* 2005). Understanding the relationship between soil respiration and the influencing factors is as important as quantifying the amount of CO_2 released from the soil (Fang *et al*. 1998). Although there have been studies on soil CO_2 efflux beneath forest canopy (Fang *et al*. 1998; Yiqing *et al* 2005), the results vary between regions due to variations in environmental factors. Factors such as season, precipitation, temperature, topography, soil properties and soil organic matter due to their influence on soil microbial population and soil cover, may influence soil respiration (Holt *et al.* 1990; Fang *et al*. 1998; Ryan and Law 2005).

Despite the abundance of soil respiration data, global coverage is poor with arid and tropical regions having received the least attention (Yiqing *et al* 2005). Until this study no measurements of CO_2 efflux had been undertaken in the tropical Mopane woodland soils of north western Botswana. This study was set up to determine spatial variation of CO_2 efflux on the forest floor of the Mopane woodland in Maun during different seasons. The variation was determined in four different habitats (spatial variation);

Received: 22 April, 2008. Accepted: 12 August, 2008.

Original Research Paper

soils without litter cover (bare soils), soils with cover i.e., soils with dead litter cover (litter cover), soils with grass cover (grass cover) and soils directly under Mopane trees canopy (tree canopy). The study also aimed at relating the variability to soil moisture content and soil temperature to soil respiration. Furthermore, the study attempted to determine the variability of some selected microbial populations (nitrifying, oligotrophic bacteria, fungi, actinomycetes and protozoa) between the different habitats and possible relationship to soil respiration. The study's specific objectives were to assess (i) the influence of seasonal variations i.e., soil moisture content, soil temperature and effect of time of day on soil respiration, (ii) the variability of soil respiration between different habitats and (iii) the effect of different habitats on selected microbial populations.

MATERIALS AND METHODS

Site description and sampling

The study was conducted in the Mopane woodland about 10 km east of Maun, Botswana (23° 33′ E, 19° 54′ S), 960 m above sea level (Espenshade *et al.* 1996). The area is also the surroundings of the carbon flux tower in the region (23° 33′ 09″ E, 19° 54′ 62″ S). Four sites were randomly selected around the tower area at 10, 30, 50 and 70 m away from the tower. The sites were chosen because they had the best combinations of the four habitats closest to the tower with existing environmental data. At each site four habitats: (i) bare soil without litter cover, ii) bare soil with dead litter cover, iii) soil under grass cover and iv) soil under Mopane trees canopy were chosen in a randomized block design layout. Each habitat had three replicates.

Sampling was carried out in August and October 2004, and January and May 2005. Based on the rainfall patterns in the study area, October to January were treated as the wet season and the May to August as the dry season. Soil samples were collected from the A_1 horizon at each sampling spot using a disturbed auger. Each 500 g soil sample collected was put in a separate sterile plastic bag. The soil samples were placed in a cooler box and transported to the laboratory for analysis. Once in the laboratory, the samples were partitioned according to use. The soil samples for microbiological analysis were analysed immediately upon arrival at the laboratory. Whenever it was not possible to perform microbiological analysis immediately upon arrival at the laboratory, samples were refrigerated at 4°C and analysed within 2 days. The soil samples to be used for physical analysis were air dried, sieved through a 2 mm sieve and then stored at room temperature.

Soil respiration determinations

Soil respiration was measured using a portable 12V, battery driven soil respiration system consisting of an infrared gas analyzer (model EGM–3. PP Systems) with a data-logger and integral pump, an environmental sensor for soil temperature (temperature probe) and a soil respiration chamber (PP Systems–Infrared Gas Analyser (EGM–3). The chamber enclosed a surface area of 7×10^{-3} m^2 and was equipped with a small low speed fan for mixing the air in the chamber. To avoid disturbance during repeated measurements and to ensure consistency at different locations, 48 polyvinyl chloride (PVC) collars with sharpened edges (150 mm tall, wall thickness 3 mm) were installed to exactly fit the chamber's outer diameter.

The collars were hammered 100 mm into the forest floor 3 days before the first measurement at each habitat. For measurement, the chamber was placed tightly onto the PVC collar on the forest floor surface. During the measurement, air was drawn from the chamber through tubing into the analyzer at intervals of 120 sec. Forest floor CO_2 was regarded as being proportional to the rate of change of CO_2 concentration in the chamber (Blanke 1996). Fourty-eight measurements were taken between 06.30 hrs and 11 hrs and another 48 between 13 and 18.30 hrs. Each time the soil respiration was measured, soil temperature in the A_1 horizon (5 cm) was also measured using the temperature probe of the EGM–3. PP System.

Microbial soil analyses

Fungal biomass carbon determination using the Rossy-Cholodney slide technique

Fungal biomass carbon was determined *in situ* using the Rossy–Cholodney slides technique (Rossy *et al.* 1936; Parkinson *et al.* 1971; Frankland 1975; Parkinson and Paul 1984; Atlas and Bartha 1993). The technique assumes that the glass slide surface is non selective and acts like the surface of mineral particles in soil. Thus organisms that adhere to the slide can be considered representative of that soil community in general (Atlas and Bartha 1993).

Two slides (stuck together back to back) were buried vertically in the field soils at each habitat. After 7 days, the slides were removed by agitating them from side to side and slowly pulling them out of the soil. Each slide was heat fixed and stained with Methylene blue (1 g 90% Methylene blue dissolved in 0.5 g NaCl solution in water) for 1 min. The stain was then washed off under a gentle running tap water and allowed to air-dry. The slides were examined under a compound microscope (Zeiss, Germany GSZ) and fungal hyphal lengths were measured (using a calibrated eyepiece micrometer) at 400X magnification. Fungal hyphae lengths in 15 fields of view per slide were measured and recorded. Mycelial hyphae was then expressed in µg C/g soil biomass carbon using the following assumptions: Fungal hyphae mean diameter is 3-4 µm, density 1.0 $\mu g/\mu m^3$, dry weight 20%, carbon content 50% on dry weight basis and 1m mycelium contains approximately 1 µg carbon (Rossy *et al.* 1936; Parkinson *et al.* 1971; Frankland 1975; Parkinson and Paul 1984).

Microbial diversity

Microbial populations were determined using plate count technique on solid agar media and MPN. Serial dilutions of soil in sterile tap water were prepared up to 10^{-5} and then used to plate on different solid agar media. Total bacteria populations were determined by spread plating the serial soil dilutions on Trypticase soy broth (BIOMEREUX Y42830) amended with 15 g/1 agar (High Media M290). Oligotrophic bacteria were enumerated by plating 0.1 ml dilutions of the 10^{-3}-10^{-4} on to nutrient poor solid media consisting of soil extract agar amended with 1% nutrient broth (Wollum 1982).

Actinomycetes populations were estimated by spread plating serial soil dilutions on starch casein agar (Williams and Wellington 1982) and then incubating at 25°C for 14 days, to obtain ashy-like colonies typical of actinomycetes. The colonies were counted using a colony counter and recorded for each habitat. The most probable number (MPN) of biophagic protozoan was determined using the baited plate technique as outlined by (Gupta and Germida 1988). Soil dilutions (10^{-2} to 10^{-5} were plated on to the 24 multi-well MPN plates containing 0.8% NaCl solid 15% agar and over laid with 0.5 ml of concentrated cell suspension of *Enterobacter aerogenes* as prey. The plates were incubated at room temperature and microscopic observations of protozoa were made from 8 to 14 days. To verify the method, control samples were made using soils obtained from the Biological Sciences Nature Reserve in Gaborone with known populations of soil protozoa. The MPN of predatory protozoa were determined with reference to the table of Cochran (1950) for use with 10-fold dilutions and 5 replicates per dilution.

MPN of nitrifying bacteria were estimated on ammonium-calcium carbonate medium and nitrite-calcium carbonate media for ammonium oxidizers and nitrite oxidizers respectively. Soil aliquots (0.330 ml) of soil dilutions (10^{-4} to 10^{-1}) were plated out on to MPN plates containing the media as outlined in the procedure by Schmidt and Belser (1984). The plates were incubated in the dark at room temperature for 4 weeks and then tested for the presence of nitrite using Griess Ilosvay reagent and for nitrite oxidizers using Zn–Cu–MnO_2 mixture (Schmidt and Belser 1984). The MPN of nitrifying bacteria was also calculated using Cochran's (1950) table for a 10-fold dilution series for 5 replicates per dilution.

Table 1 Soil physical-chemical characteristics of the different Mopane habitats.

Soil habitat	pH Water	pH $CaCl_2$	Textural class[¥]	% organic matter	% total N
Bare	5.59	5.45	Sandy	0.586	<0.001
Litter cover	6.24	6.12	Sandy	0.828	<0.001
Grass cover	6.19	5.97	Sandy	0.759	<0.001
Tree canopy	6.40	6.31	Sandy	0.690	<0.001

[¥] % Sand higher than 95%;
pH water- active acidity;
pH $CaCl_2$-potential acidity

Determination of soil characteristics

Soil pH was determined in a 1:2 soil: distilled water suspension (active acidity) and 1:2 soil: 0.01 M $CaCl_2$ (potential acidity) and then measured using a Corning scale pH meter electrode (model 215). Soil texture (%sand, silt and clay) was determined by the hydrometer method (*Bouyoucos* method). The soil textural class was then determined by using the soil textural triangle (Anderson and Ingram 1993). Soil moisture content was determined gravimetrically from weight loss after 10g of each soil sample was oven dried at 105°C overnight (Anderson and Ingram 1993). Soil organic matter content was determined using the Walkley-Black method (Walkley 1947).

Statistical analysis

Analysis of variance was performed using the SPSS 10.0 package. *Post hoc* analyses were performed using the Tukey Test. In the analysis, group separation was based on habitat (bare soils without litter cover, soils with litter cover, grass cover and soils directly under Mopane tree canopy), season, time of the day and the parameter studied.

RESULTS

Effect of environmental factors on soil respiration

The Mopane woodland soils studied were found to be highly sandy with 95% sand and 1.4 % silt in all the habitats from which samples were collected. These Mopane woodland soils were found to be slightly acidic with pH values ranging from 5.59 to 6.40. The pH values did not vary significantly between habitats and between seasons (**Table 1**). The differences in active and potential acidity were also minimal. Generally the soils had very low organic matter of less than 1% in all the habitats. Total nitrogen levels in these soils were also very low and were always below 0.001% in all the habitats. None of the soil physical characteristics varied with season. Statistical analyses of the data obtained showed that not all factors studied significantly influenced soil respiration (**Table 2**). Only soil moisture content, temperature, habitat, and fungal biomass were observed to influence soil respiration.

Soil respiration at different habitats differed with season, with higher rates recorded in the wet season than the dry season (**Table 3**). There were significant differences ($P< 0.05$) in soil moisture content between the seasons, with the wet season having significantly higher soil moisture content than the dry season. Although there were significant differences in soil moisture content between the two seasons, no significant difference were observed between habitats within the same season. In this study, soil respiration also differed with season (**Table 3**). Generally higher rates of mean daily respiration (0.24 to 0.65 $gCO_2/m^2/hr$) were observed during the wet season with high soil moisture content (2.01% to 2.69%), while relatively low soil respiration rates (0.05 to 0.12 $gCO_2/m^2/hr$) were recorded during the dry season when soil moisture was very low (0.68 % to 0.88%) (**Table 3**). Within each season the bare habitat (bare soils without litter cover) showed significantly lower soil respiration than habitats with cover (bare soils with dead litter cover, soils with grass cover and soils directly under Mopane trees canopy). Time of the day did not seem to significantly influence soil respiration within each habitat as there were no significant differences in soil respiration rates between the mornings and afternoons (**Table 3**). Among the different habitats both in the mornings and afternoons, soil respiration rates did not differ significantly between the three habitats with cover. However, soil respiration rates in the bare soil without cover was significantly lower ($P < 0.05$) than habitats with cover.

Although there were no significant differences in soil temperatures between the wet and dry season, there were soil temperature variations with time of the day. Afternoon soil temperatures were generally higher than the morning temperatures (**Table 4**). Soil temperature across the seasons did not differ significantly; therefore it had no observable influence on seasonal variation in soil respiration. Soil temperatures also did not significantly differ between the habitats within each season although they were observed to be

Table 2 Probability levels for statistical significance for the effect of the different parameters on soil respiration in the Mopane woodland.

Source of variation	Probability
Dry season	
Habitat vs mean daily SR	*
Moisture vs mean daily SR	**
Mean temp vs mean daily SR	NS
Fungal bio C vs mean daily SR	NS
Morning temp vs morning SR	**
Afternoon temp vs afternoon SR	**
Wet season	
Habitat vs mean daily SR	**
Moisture vs mean daily SR	NS
Mean temp vs mean daily SR	**
Fungal bio C vs mean daily SR	NS
Morning temp vs morning SR	**
Afternoon temp vs afternoon SR	**
Both wet and dry season	
Habitat vs mean daily SR	**
Moisture vs mean daily SR	NS
Mean temp vs mean daily SR	NS
Fungal bio C vs mean daily SR	**
Season vs mean daily SR	**
Moisture vs mean daily SR	**
Mean temp vs mean daily SR	NS

NS = Non significant
* $p < 0.05$
** $p < 0.01$

Table 3 Mean soil respiration in the different habitats during the two seasons.

Soil habitat	% Moisture	Mean morning soil respiration ($gCO_2/m^2/hr$)[Ψ]	Mean afternoon soil respiration ($gCO_2/m^2/hr$)[Ψ]
Wet season			
Bare	2.01 ± 0.74 b	0.24 ± 0.07 b	0.49 ± 0.14 c
Litter cover	2.23 ± 1.01 b	0.62 ± 0.48 c	0.58 ± 0.12 cd
Grass cover	2.69 ± 1.46 b	0.55 ± 0.30 c	0.65 ± 0.20 d
Tree canopy	2.58 ± 1.31 b	0.64 ± 0.40 c	0.65 ± 0.11 d
Dry season			
Bare	0.75 ± 0.20 a	0.05 ± 0.03 a	0.08 ± 0.03 a
Litter cover	0.68 ± 0.15 a	0.05 ± 0.02 a	0.07 ± 0.01 a
Grass cover	0.84 ± 0.21 a	0.08 ± 0.03 ab	0.09 ± 0.04 ab
Tree canopy	0.88 ± 0.21 a	0.11 ± 0.02 b	0.12 ± 0.03 b

[Ψ] Means followed by the same letter in the same column are not significantly different from each other ($p < 0.05$).
Each value is a mean of 24 replicates

Table 4 Effect of mean soil temperature and time of the day on the mean soil respiration in the different habitats during the dry and wet seasons.

Soil habitat	Morning		Afternoon	
	Temp (°C)	Respiration ($gCO_2/m^2/hr$)$^{\Psi}$	Temp (°C)	Respiration ($gCO_2/m^2/hr$)$^{\Psi}$
Wet season				
Bare	27.4 ± 2.0	0.24 ± 0.07	40.5 ± 2.7	0.49 ± 0.14
Litter cover	26.9 ± 1.0	0.62 ± 0.48	38.1 ± 5.0	0.58 ± 0.12
Grass cover	27.2 ± 1.3	0.55 ± 0.30	38.4 ± 4.9	0.65 ± 0.20
Tree canopy	26.8 ± 1.4	0.64 ± 0.4	37.5 ± 5.5	0.65 ± 0.11
Dry season				
Bare	25.3 ± 4.4	0.05 ± 0.03	31.7 ± 3.7	0.08 ± 0.03
Litter cover	28.7 ± 4.5	0.05 ± 0.02	32.7 ± 4.3	0.09 ± 0.04
Grass cover	31.9 ± 5.8	0.08 ± 0.03	34.0 ± 4.3	0.09 ± 0.04
Tree canopy	28.3 ± 5.0	0.11 ± 0.02	26.7 ± 4.7	0.12 ± 0.03

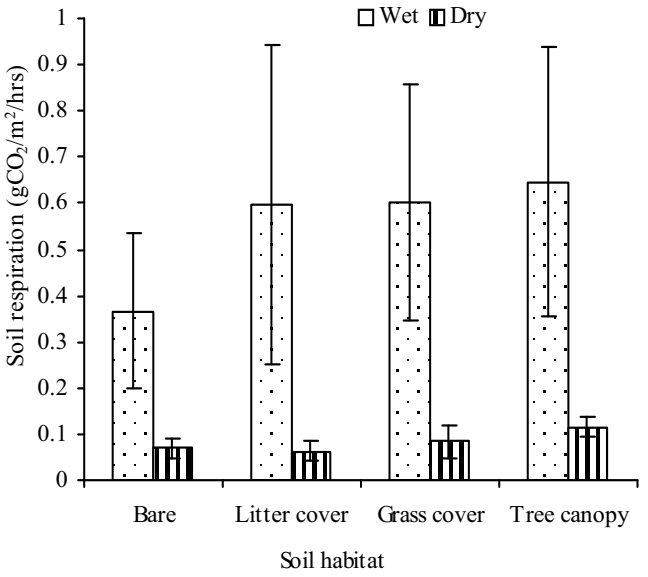

Fig. 1 Soil respiration in the different habitats in the wet and dry seasons.

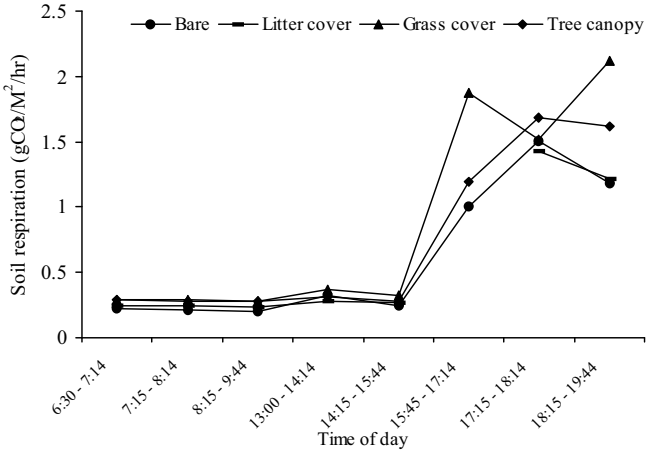

Fig. 2 Effect of time of day on soil respiration.

slightly higher in the wet season. Instead they differed with time of the day. In all the results indicated that soil respiration was higher in the wet season in comparison to the dry season (**Fig. 1**). Mean morning soil respiration was also always lower than afternoon soil respiration (**Fig. 2**).

Microbial diversity in the habitats

Microbial diversity studies in the different Mopane woodland soils habitats showed that soil bacterial populations were generally low. Total bacterial counts enumerated on full strength nutrient agar were low most of the times, especially during the dry season where they were below 10^2 CFU/g soil (**Table 5**). However, bacteria enumerations on 1/10 strength nutrient agar revealed that there were oligotrophic bacterial populations in the Mopane woodland soils. Although not significantly higher (<1 $\log_{10}$ unit), generally the oligotrophic bacterial populations in the habitat without cover (bare) were lower than in habitats with cover in both the wet and dry season (**Table 5**). No detectable differences in oligotrophic populations between the seasons were observed. Thus the influence of season on the oligotrophic bacterial population was non significant, as the populations in the different habitat did not differ with season (**Table 5**).

Table 5 Seasonal variation in microbial populations in the different Mopane habitats.

Soil habitat	Log $_{10}$ CFU / g soil)	
	Wet season	Dry season
Oligotrophic bacterial populations		
Bare	4.46 ± 0.37	4.56 ± 0.07
Litter cover	4.90 ± 0.32	5.04 ± 0.24
Grass cover	5.02 ± 0.20	4.64 ± 0.07
Tree canopy	4.91 ± 0.25	4.75 ± 0.24
Actinomycetes		
Bare	4.84 ± 0.21	4.64 ± 0.17
Litter cover	4.77 ± 0.11	4.86 ± 0.18
Grass cover	4.77 ± 0.15	4.79 ± 0.19
Tree canopy	4.80 ± 0.21	4.80 ± 0.19

Actinomyetes were also found to occur widely in all the Mopane woodland soil habitats both in the wet and dry season. However, the effect of habitat on actinomycetes populations was not significant. The actinomycete populations did not seem to be influenced by the seasons as there were non significant differences in the actinomycete populations between the wet and dry seasons. Samples from the Mopane woodland soils did not show any bacteriovorous protozoa, however the control samples from the Biological Science Nature Reserve in Gaborone indicated those samples to contain as high as 10^3 MPN protozoa per gram soil.

Nitrifying bacteria (ammonium and nitrite oxidisers) populations in the Mopane woodland soils were fairly high and varied with season and habitat. However, because of major variations between replicates, these variations were not statistically significant and did not show a specific pattern as such could not be correlated with soil respiration (**Table 6**). It was also observed that ammonium oxidizers were significantly higher during the wet season. In addition no significant difference in the populations of ammonium oxidizers at bare habitats without litter was observed between the seasons.

Soil fungal biomass carbon as determined using the Rossy-Cholodney method varied between habitats (**Table 7**). In both seasons, fungal biomass carbon did not vary between sampling times within the seasons. However between seasons, the wet season had significantly higher fungal biomass carbon than during the dry season (**Table 7**). The wet season was characterised by high fungal biomass carbon which coincided with high soil respiration. On the contrary,

Table 6 The influence of season on the Most Probable Number of nitrifiers in the different Mopane habitats.

Soil habitat	NH_4^+ oxidisers¥		NO_2^- oxidisers¥	
	Wet season	Dry season	Wet season	Dry season
Bare	759	788	105	551
Litter cover	1119	600	144	218
Grass cover	788	759	204	676
Tree canopy	1358	630	204	149

¥Most Probable Number per gram soil

Table 7 Seasonal variation in fungal biomass carbon (µg C/g soil) in different habitats.

Soil habitat	Wet season	Dry season
Bare	153.19 a	11.84 a
Litter cover	266.09 b	26.99 b
Grass cover	282.14 b	34.40 b
Tree canopy	162.05 a	44.16 b

lower fungal biomass carbon was recorded in the dry season. During the wet season, the difference in fungal biomass in habitats with cover and those without cover were not always statistical significant, but they were always lower in the habitat without cover.

DISCUSSION

Effect of abiotic factors on soil respiration

The Mopane woodland soils of north western Botswana were found to be highly sandy with most habitats having sand contents above 95% (**Table 1**). As such, these soils have an inherent low moisture and nutrient holding capacity. With the rains in the wet season, soil moisture increased. However, soil moisture content variation within seasons was minimal; 2.01 to 2.69% in the wet season and 0.68 to 0.88% in the dry season (**Table 3**). The increased soil moisture content in the wet season coincided with the increase in soil respiration as the wet season showed significantly higher soil respiration than the dry season (**Table 3**). In this study, the effects of soil moisture content on soil respiration within seasons were minimal. Variation in soil respiration within each season was not significantly correlated with soil moisture content. However, significant variation in soil moisture content existed between seasons (**Table 2**). This variation coincided with fluctuation in soil respiration, suggesting a strong link between variation of soil respiration and soil moisture between seasons. A strong seasonality in soil respiration is highly pronounced in areas with a seasonal pattern of rainfall (Cuevas 1995). Thus seasonality in soil respiration in the Mopane woodland was linked to the seasonal pattern of rainfall in the area. These results also agree with observations by other researchers that an increase in water potential coincides with increased microbial respiration and the resultant soil respiration (Moore 1986). Soil moisture also plays a significant role in soil respiration as observed in Australian tropical semi-arid woodland where soil moisture content was observed to be the major factor controlling soil respiration (Holt *et al*. 1990). Furthermore, fluxes of CO_2 have been found to show a general increase with soil moisture content in a range of soils (Howard and Howard 1993). When the relationship between seasonal variation of soil respiration, soil moisture and soil temperature were explained by multiple linear regression analysis, soil moisture accounted for 82% of the variance, with temperature accounting for only 7% in the dry tropics in eastern Australia (Holt *et al*. 1990). In the present study the regression equation employed for the three variables (habitat, soil moisture content and soil temperature), showed that these explained over 76% of the variance. Soil moisture alone accounted for 73% of the variance with temperature and habitat providing only 48% of the variance. Like in most forest ecosystems (Kelliher *et al*. 1999), variation in soil moisture between seasons in the Mopane woodland was strongly associated with the seasonality of the soil respiration.

Increase in soil temperatures have been shown to result in increased soil respiration when moisture is not a limiting factor in a range of soils (Howard and Howard 1993). Other studies have shown that although temperature has some influence on soil respiration it only exerts a decisive influence when there is sufficient soil moisture (Lloyd and Taylor 1994). In the Mopane woodland soils studied, soil temperature did not differ significantly between seasons. The mean daily temperatures ranged from 32.1 to 33.9°C in the wet season and 27.5 to 32.9°C in the dry season. Therefore it was not easy to link variation of soil respiration between seasons to soil temperature. Meanwhile the temperatures varied from 25.3 to 31.9°C in the mornings and from 26.7 to 40.5°C in the afternoons (**Table 4**). The effects of morning and afternoon temperatures differed significantly in the Mopane woodland. Lower temperatures recorded in the mornings coincided with lower soil respiration rates. The afternoons with higher temperatures were characterized by higher rates of soil respiration. This therefore suggested a positive relationship between the diurnal soil respiration rate and soil temperature in these soils (**Fig. 2**). This relationship has not been observed in all ecosystems (Holt *et al*. 1990) however, it has been reported in some (Blanke 1996; Fang *et al*. 1998).

Other studies have shown diurnal soil respiration to be controlled by soil temperature and independent of diurnal soil moisture content in a range of soils (Parker *et al*. 1983). Possibly because an increase in soil temperature leads to an increase in the pool size of carbon respired by soil microbes (Zogg *et al*. 1997). This increase in substrate pool size at higher temperatures results in a shift in the microbial community position associated with soil warming as dominant communities at higher temperatures have the ability to metabolize substrates not used by members of microbial community dominant at low temperatures (Zogg *et al*. 1997). This may be the case in the Mopane woodland soils of Maun.

Effect of biotic factors on soil respiration

In this study different rates of soil respiration were recorded in different habitats. The bare habitat had significantly lower rates of soil respiration compared to habitats with cover. Habitats under Mopane tree canopy recorded the highest rates of soil respiration (**Tables 3** and **4**) irrespective of variations in temperature and moisture. This was evident in mornings as well as afternoons and in the overall respiration recorded during the two seasons. These findings are comparable to the results obtained in a pine plantation where soil respiration under palmetto was significantly higher than that from open floor in slash pine plantation (Fang *et al*. 1998).

In this study, fungal biomass carbon was higher in soils under cover or canopy which may indicate higher total fungal populations. This may explain the difference in soil respiration in soils without cover and those under cover. A link exists between basal respiration and microbial biomass and organic carbon; as some researchers have observed maximum microbial populations in forest soils with high litter fall and the lowest population when litter fall was minimal (Yiqing *et al.* 2005). The influence of cover has also been noted in earlier studies in the same region. Mubyana-John *et al.* (2007) showed that similar soils under grassland cover had higher organic matter and higher microbial biomass than Mopane woodland soils in the same region. Thus the Mopane woodland soils were characterized by higher microbial activity in habitats with litter cover giving rise to higher rate of soil respiration. Valentini *et al*. (2000) also suggested that habitats with cover are highly likely to have a higher soil respiration and that the major contributors to soil respiration in forest soils such as Mopane woodland soils are root and microbial. In bare habitat soils with no litter cover the contributors to soil respiration are mostly

likely to be chemical oxidation and minimal contribution arises from microorganisms and soil fauna (Ryan and Law 2005). Since there were very few or no fine roots in bare soils, soil respiration may have originated mainly from microbial respiration especially fungi. Buried Rossy-Cholodney slides in bare soils without litter cover showed presence of less fungal hyphae when compared to habitats with cover. This provided additional evidence of microbial activity in these habitats and their possible contribution.

This study has also showed that in Mopane woodland soils, habitat can be used as a predictor of variations in soil respiration. The linear regression model showed that 36% of the time, habitat alone accounted for the variation in soil respiration. The regression coefficient also showed that the effect of habitat was significant ($p < 0.05$). One way ANOVA with habitat as an independent and soil respiration as the dependent factor, showed the effects of habitat on morning, afternoon and mean daily soil respiration to be significant at $p < 0.05$.

In the Mopane woodland soils studied, total bacterial counts were very low. However, oligotrophic bacterial populations ranged from 3.1×10^4 to 2.1×10^5 g^{-1} soil in the dry season and 1.2×10^4 to 3.0×10^5 g^{-1} soil in the wet season (**Table 5**). The Mopane woodland soils of Maun are an extension of the Okavango delta soils. However, irrespective of having similar physical soil characteristics, these soils habour lower bacterial populations than similar woodland soils closer to the Okavango Delta (Mubyana *et al.* 2003). This was attributed to declining moisture content, resulting in very little vegetation and soil organic matter as distance from the Delta increases. When compared to other regions, these populations are also slightly lower than what was observed by Ohta and Hatori (1983) in their study using two types of sandy soils where populations of oligotrophic bacteria were observed to vary from 3.9×10^6 to 6.5×10^7 g^{-1} soil, and from 7.2×10^5 to 3.3×10^6 g^{-1} soil oven dried matter of sands denoted as sand A and sand B respectively. In the Mopane woodland soils actinomycetes populations between 3.0×10^4 to 1.4×10^5 CFU g^{-1} soil in the dry season and 3.2×10^4 to 1.4×10^5 CFU g^{-1} soil in the wet season in the top 20 cm (**Table 5**) were observed, indicating that these populations do not fluctuate much during the two seasons. The data may not agree with that from other areas as findings on actinomycetes populations determined and recorded in literature is often not comparable, with that from different soil types and land use (Williamson and Johnson 1991). The macro fauna inhabiting the area may also affect actinomycete population variations between areas due to variations in substrate. The non significant difference in populations of both oligotrophic bacteria and actinomycetes population between the two seasons (**Table 5**) suggests that these microorganisms could be well adapted to the seasonal fluctuations in temperature and moisture in the Mopane woodland. Influence of habitat on these groups of microorganisms was also not significant. The regression summary model which included these microorganisms together with nitrifying bacteria as factors explained only 30% of the variation of soil respiration and the regression coefficient model showed their influence to be insignificant. Therefore it was difficult to link the seasonal fluctuation of soil respiration with populations of these groups of microorganisms.

In the Mopane woodland soil fungal biomass carbon varied significantly with season in all habitats (**Table 7**). These variations seemed to be soil moisture content dependent and fungal biomass was affected more than the biomass of other microorganisms studied. Fungal biomass carbon ranged from 162.05 to 282.14 μg C g^{-1} soil in the wet season and from 11.84 to 44.16 μg C g^{-1} soil in the dry season (**Table 7**). Fungal populations, like all microbial populations, show a positive response to increasing water availability (Cuevas 1995). Moreover observations of higher fungal populations in the wet season than in the dry have been recorded in the Okavango region as well as in other areas (Banda 2004). In this study the wet season was characterized by high fungal biomass carbon coinciding with high soil respiration rates. Similarly the very low fungal biomass carbon observed in the dry season corresponded to very low soil respiration rates. The high soil respiration in the wet season observed in this study may be due to increased root exudates which serve as microbial substrates. The fungal biomass fluctuations may be associated with low below ground plant biomass during the dry season which may serve as a substrate as opposed to the wet season with high plant biomass (Bonyongo and Mubyana 2004). Mellilo *et al*. (1992) also noted that in the wet season, favourable moisture which permitted flourishing ground vegetation in turn added fresh substrate to the soil in the form of leaf litter and root. These conditions may have accounted for an elevation in fungal biomass carbon during the season. The biomass decline to very low levels in the dry season must have been due to deficiencies in moisture. Generally higher levels of fungal biomass carbon were recorded in habitats with cover, this was more pronounced in the dry season. This may explain the higher activity observed in these habitats as reflected by the respiration rates thereof. The close relation between high fungal biomass carbon, high moisture content and high plant litter fall implicate fungi as the major decomposers in these soils probably because fungi are more active at lower soil water potential than bacteria (Banda 2004). Moreover direct microscopic observation has shown that in the upper soil horizons, fungal biomass, mycelium, spores and yeast cells usually form 90–95% of the biomass (Zvyagintsev 1995).

When the relationship between variation of soil respiration and oligotrophic bacteria, actinomycetes, nitrifying bacteria and fungal biomass carbon were explained by a linear regression analysis, the regression equation explained at least 64% of the variance. Fungal biomass carbon alone accounted for 55% of the variance, while all other microorganisms accounting for only 30.3% of the variance. The regression coefficient revealed that the effect of fungal biomass was significant whereas that of the rest of microbes was insignificant. This suggests that fungi have a more significant influence on variations of soil respiration compared to other microorganisms studied in the Mopane woodland soils. This study confirms Yiqing (2005) findings who observed that CO_2 tends to show a positive correlation with fungal biomass.

In this study (**Table 6**) ammonium oxidizers were generally higher in the wet season. During this season there is a lot of microbial activity and mineralization releases ammonium ions from ammonium compounds and these serve as substrates for ammonium oxidizers. Nitrification of ammonium compounds such as ammonium sulphate has been observed to proceed more rapidly at 25°C at different moisture content levels. However it is also inhibited at very low or very high moisture content and soil temperatures (Cassman and Munns 1980); characteristic of these Mopane woodland soils where the moisture contents are very low in the dry season. In bare soils, nitrate formation was generally lower than in habitats with cover especially in the dry season as indicated by the high population of NO_2^- oxidizers. Presence of nitrifying bacteria in dry Mopane woodland soils is also indicative of their adaptation to the soil climate.

Mopane woodland soils showed negligible numbers of predatory protozoan while test samples from the Biological Sciences Nature Reserve showed the presence of protozoan ranging from 450 to 680 MPN g^{-1} soil. This indicates that the Mopane woodland soils of north western Botswana are very low in biophagic protozoa. This was not surprising as total bacterial counts on Tripticase soy agar were also very low. The very low MPN protozoa counts obtained in these Mopane woodland soils are not surprising as low counts have also been reported in other areas where bacteria counts were low as well (Gupta and Germida 1988). Closer observations also indicated that although the habitats with cover seemed to promote microbial populations, this was not always the case with the Mopane tree canopy in the dry sea-

son. Lower fungal biomass carbon and ammonium and nitrite oxidizers were recorded under the Mopane tree canopy, a factor that may be associated with competition for water between the microorganisms and the tree in these sandy soils with very low moisture holding capacity.

Role of fungi in the soil structure

Observation of stained Rossy-Cholodney slides showed that in the Mopane woodland soils fungi play a very important role in aggregation of soil particles by their hyphae. This result is in line with other findings that soils are not homogeneous but are made up of aggregates of different sizes held together by fungal hyphae (Burri *et al.* 2007). Moreover fungi have long been known to secrete polysaccharides to which microaggregates firmly adhere (Tisdall *et al*. 1997).

CONCLUSIONS

This study showed that mean daily soil respiration rates in the Mopane woodland soils are significantly higher in the wet than in the dry season. Habitat also seemed to greatly influence soil respiration as higher rates were observed in habitats with cover than those without cover. The present study also showed that diurnal variation in soil respiration in the Mopane woodland soils was temperature dependent, but temperature did not influence seasonal variation of soil respiration in these soils. Furthermore, seasonal variation on soil respiration in the Mopane woodland soils was soil moisture dependent. The populations of oligotrophic, actinomycetes and nitrifying bacteria did not seem to significantly have an effect on seasonal variation in soil respiration although these microbial groups may contribution to soil respiration. Meanwhile a strong link was established between fungal biomass carbon and soil respiration variation between seasons. Thus fungi in the Mopane woodland soils are possibly the major contributors to soil respiration, much more than bacteria by virtue of numbers and adaptation. Microbial biomass was moisture dependent and was significantly higher in habitats with cover.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Harry Openheimer Okavango Research Centre in Maun for use of their facilities and the Botswana Ministry of Education for funding the project.

REFERENCES

Anderson JM, Ingram JSI (1993) *Tropical Biology and Fertility*: *A Handbook of Methods*, C.A.B International, UK, pp 93-95

Atlas RM, Bartha R (1993) *Microbial Ecology: Fundamentals and Applications* (3rd Edn), The Benjamin Cummings Publishing Company Inc., California, USA, pp 166-169

Banda A (2004) The influence of range fire on soil microbial biomass activity, fungal population and diversity along the Boro route in the Okavango Delta. MSc thesis, University of Botswana, 115 pp

Bhalota YPR (1987) *Climate of Botswana. Part II, Elements of Climate*, Rainfall Department of Meteorological Services, Gaborone, Botswana, 104 pp

Blanke MM (1996) Soil respiration in an apple orchard. *Environmental and Experimental Biology* **36**, 339-348

Bonyongo MC, Mubyana T (2004) Soil nutrient status in vegetation communities of the Okavango Delta floodplains. *South African Journal of Science* **100**, 337-340

Burri K, Graf F, Böll A (2007) Increasing eco-engineering success with the contribution of plants and mycorrhizal fungi. *European Geosciences Union Geophysical Research Abstracts* **9**, 05537

Cassman KG, Munns DN (1980) Nitrogen mineralization as affected by soil moisture, temperature and depth. *Soil Science Society of America Journal* **44**, 1233-1237

Cochran WG (1950) Estimates of bacteria densities by means of the "most probable number". *Biometrics* **6**, 105-106

Cuevas E (1995) Biology of the below system of tropical dry forests. In: Bullock H, Mooney HA, Medina E (Eds) *Seasonally Dry Tropical Forests*, Cambridge Press, Cambridge, United Kingdom, pp 363-368

Ellery K, Ellery W (1997) *Plants of the Okavango. A Field Guide*, Tsaro Publishers, Durban, South Africa, 225 pp

Espenshade EB, Hudson JC, Morrison JL (1996) *Goode's World Atlas* (18th Edn), Rand McNally and Co., New York, USA, 370 pp

Fang C, Moncrieff JB, Gholz HL, Clark KL (1998) Soil CO_2 efflux and it's spatial variation in a Florida slash pine plantation. *Plant and Soil* **205**, 135-146

Frankland JC (1975) Estimation of live fungal biomass. *Soil Biology and Biochemistry* **7**, 339-340

Gupta SRVV, Germida JJ (1988) Population of predatory protozoa in field soils after 5 years of elemental S fertilizer. *Soil Biology and Biochemistry* **20**, 787-791

Holt JA, Hodgen MJ, Lamb D (1990) Soil Respiration in the seasonally dry tropics near Townsville, North Queensland. *Australian Journal of Soil Research* **28**, 737-745

Howard DM, Howard PJA (1993) Relationships between CO_2 evolution, moisture content and temperature for a range of soil types. *Soil Biology and Biochemistry* **25**, 1537-1546

Kelliher FM, Lloyd J, Arneth A, Lühker B, Byers JN, McSeveny TM, Milukova I, Grigoriev S, Panfyourov M, Sogatchev A, Varlaargin A, Ziegler W, Bauer G, Wong SC, Schulze ED (1999) Carbon dioxide flux density from the floor of a central Siberian pine forest. *Agriculture and Forest Meteorology* **94**, 217-232

Larionova AA, Yermolayev AM, Blagodatsky SA, Rozanova LN, Yevdokimov IV, Orlinsky DB (1998) Soil respiration and carbon balance of gray forest soils as affected by land use. *Biology and Fertility of Soils* **27**, 251-257

Lloyd J, Taylor JA (1994) On the temperature dependence of soil respiration. *Functional Ecology* **8**, 315-323

Mellilo JM, Mcguire AD, Joyce LA, Kicklighter DW, Grace AL, Moore III B, Vorosmarty CJ (1992) Interactions between carbon and nitrogen dynamics in estimating net primary productivity for potential vegetation in North America. *Global Biogeochemical Cycles* **6**, 101-124

Moore AM (1986) Temperature and moisture dependence of decomposition rates of hardwood and coniferous leaf litter. *Soil Biology and Biochemistry* **18**, 427-435

Mubyana T, Krah M, Totolo O, Bonyongo MC (2003) Influence of seasonal flooding on soil total nitrogen, organic phosphorus and microbial populations in the Okavango Delta, Botswana. *Journal of Arid Environments* **54**, 359-371

Mubyana-John T, Wutor VC, Yeboah SO, Ringrose S (2007) Fire and its influence on microbial community structure and soil biochemical properties in the Okavango Delta Botswana. *Science Research Essays* **2 (2)**, 47-54

Ohta H, Hattori T (1983) Oligotrophic bacteria on organic debris and plant roots in a paddy field soil. *Soil Biology and Biochemistry* **15**, 1-8

Omari K, Mubyana T, Matsheka MI, Bonyongo MC, Veenendaal E (2004) Flooding and its influence on diazotroph population and soil nitrogen levels in the Okavango Delta soils. *South African Journal of Botany* **70**, 734-740

Parker LW, Miller J, Steinberger Y, Whitford WG (1983) Soil respiration in a Chihuahuan desert rangeland. *Journal of Soil Biology and Biochemistry* **5**, 303-309

Parkinson D, Gray TRG, Williams SI (1971) *Methods of Studying the Ecology of Soil Microorganisms*, International Biological Programme, Handbook 19, Blackwell Scientific Publications Ltd., London, UK, 116 pp

Parkinson D, Paul EA (1984) Microbial biomass. In: Page AL, Miller RH, Keeney DR (Eds) *Methods of Soil Analysis Part 2*, *Chemical and Microbiological Properties* (2nd Edn) American Society of Agronomy, Soil Science of America Publishers INC. Madison, Wisconsin, USA, pp 821-830

Ringrose S (1999) An analysis of vegetation cover components in relation to climatic trends along the Botswana Kalahari transect. *Botswana Notes and Records* **31**, 33-52

Rossy G, Riccardo S, Gesue M, Wang TK (1936) Direct microscopic bacterial investigations in soil. *Soil Science* **41**, 53-66

Ryan MG, Law BE (2005) Interpreting, measuring, and modeling soil respiration. *Biogeochemistry* **73**, 3-27

Schmidt EL, Belser LW (1984) Nitrifying bacteria. In: Page AL, Miller RH, Keeney DR (Eds) *Methods of Soil Analysis Part 2*, *Chemical and Microbiological Properties* (2nd Edn), American Society of Agronomy, Soil Science of America Publishers INC, Madison, Wisconsin, USA, pp 1026-1041

Steffen W (2000) The IGBP terrestrial transects: Tools for resource management and global change research at the regional scale. In: Ringrose S, Chanda R (Eds) *Towards the Sustainable Management in the Kalahari Region; Some Essential and Critical Issues*, Directorate of Research and Development, University of Botswana, Gaborone, Botswana pp 1-12

Tisdall JM, Smith SE, Rengasamy P (1997) Aggregation of soil by fungal hyphae. *Australian Journal of Soil Research* **35**, 55-60

Valentini R, Matteucci G, Dolman AJ, Schulze ED, Rebmann C, Moors EJ, Granier A, Gross P, Jensen NO, Pilegaard K, Lindroth A, Grelle A, Bernhofer C, Grunwald T, Aubinet M, Ceulemans R, Kowaslski AS, Vesala T, Rannik U, Bersbigier P, Loustau, Guomundsson J, Thorgeirsson H, Ibrom A, Morgenstern K, Clement R, Moncrieff J, Montagnani L, Minerbi S, Jarvis PG (2000) Respiration as the main determinant of carbon balance in European forests. *Nature* **404**, 861-864

Veenendaal E, Kolle O, Lloyd J (2003) Seasonal variation in energy fluxes and carbon dioxide exchange for a broad-leafed semi-arid savanna (Mopane Woodland) in Southern Africa. *Global Change Biology* **10**, 318-328

Walkley A (1947) A critical examination of a rapid method for determining or-

ganic carbon in soils: Effects of variation in digestion conditions and inorganic soil constituents. *Soil Science* **63**, 251-263

Williams ST, Wellington EMH (1982) Actinomycetes. In: Page AL, Miller RH, Keeney DR (Eds) *Methods of Soil Analysis Part 2*, *Chemical and Microbiological Properties,* (2nd Edn), American Society of Agronomy. Soil Science of America Publishers INC. Madison, Wisconsin, USA, pp 969-987

Williamson JC, Johnson DB (1991) Microbiology of soils at opencast coal sites. II. Population transformations occurring following land restoration and the influence of ryegrass/fertilizer amendments. *Journal of Soil Science* **42**, 9-15

Wollum II, AG (1982) Cultural methods for soil microorganisms. In: Page AL, Baker DE, Keeney DR (Eds) *Methods of Soil Analysis (Part 2) Chemical and Microbiological Properties*, American Society of Agronomy, Inc. and Soil Science Society of America, Inc. Madison, USA, pp 781-802

Yiqing I, Xu M, Zou X, Shi P, Zhang Y (2005) Comparing soil organic carbon dynamics in plantation and secondary forest in wet tropics in Puerto Rico. *Global Change Biology* **11**, 239-248

Zogg GP, Zak DR, Ringelberg DB, MacDonald NW, Pregitzer KS, White DC (1997) Compositional and functional shifts in microbial communities due to soil warming. *Soil Science Society of America Journal* **61**, 475-481

Zvyagintsev DG (1995) Vertical distribution of microbial communities in soil. In Ritz K, Dighton J, Giller KE (Eds) *Beyond the Biomass*, John Wiley, Chichester, UK, pp 29-37