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Flooding and its influence on diazotroph populations and soil nitrogen levels in the Okavango Delta

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Effects of flooding on soil nitrogen (N), and asymbiotic nitrogen fixing bacterial (diazotroph) populations of the Okavango Delta were investigated. Diazotrophs from the rhizosphere of dominant annual and perennial grasses of the Okavango Delta were isolated on N-free composite media and identified applying morphological and biochemical criteria and Restriction Fragment Length Polymorphism (RFLP). *Azotobacter* species were found associated mostly with the grasses *Andropogon guyanus* and *Vetevaria nigritiana* (10^3CFU g^{-1} rhizosphere soil). Annual grasses such as *Eragrostis inamoena, Setaria sphacelata* as well as perennials showed insignificant populations (<10^1CFU g^{-1} soil). On the contrary, *Azospirillum* spp. was found associated with most of the grasses. Highest population densities (above 10^4MPN g^{-1} soil) were observed in rhizospheres of *E. inamoena*, *Cympogon excavatus, Sporobolus acinifolis, Eragrostis lapila* and *Eragrostis rigidor* and lowest population densities (below 10^2MPN g^{-1} soil) in rhizospheres of *Andropogon guyanus* and *Panicum repens*. Flooding increased the *Azotobacter* populations, while *Azospirillum* spp. populations were significantly reduced. Nitrogen-fixing sulphur reducers were observed in the rhizosphere of annuals (*A. guyanus* and *P. repens*) and only in perennials growing in flooded soils. No *Beijerinckia* species were found associated with any of the grasses. The highest soil nitrogen levels were detected in flooded soils (<0.20% N) while no N was detected in the rhizosphere soil of grasses growing in very dry soils (<5% moisture content). The results indicate that in the Okavango Delta, total soil nitrogen varies with flooding regime. With flooding, the diazotroph population shifts towards *Azotobacter* and N-fixing sulphur reducers while *Azospirillum* spp. are widespread in non-flooded soils. RFLP analysis of the 1 450bp amplicon using the restriction endonuclease *Alu1*, showed three different banding profiles, suggesting the occurrence of three different species of *Azospirillum.*

Introduction

The Okavango Delta is a large land-locked alluvial fan of low gradient, situated in the semi-arid Kalahari basin in north-western Botswana. It is the second largest inland delta in Africa after the Niger Delta (Stanistreet and McCarthy 1993). The delta water is supplied by the Okavango River, which originates from the highlands in central Angola. The Okavango Delta consists of perennial flooded swamps subjected to inundation throughout the year in all or most years, seasonally flooded floodplains inundated on a seasonal basis and the intermittently flooded areas inundated in high floods only. Vegetation in the floodplains consists mostly of densely populated grass species communities, which vary depending on water gradient, flood regime and grazing pressure. These grass communities grow on sandy soils (>85% sand) with low cation exchange capacity (<5meq per 100g soil) (Staring 1978). The combined effect of high sand texture, low cation exchange capacity and seasonal flooding and drying is thought to result in leaching of essential elements such as nitrogen, which in turn could lead to poor plant growth (Mengel and Kirby 1982). However, the area has high herbaceous plant biomass productivity and high species diversity (Bonyongo and Bredenkamp 2000, Ellery and Ellery 1997).

Trivial amounts of inorganic nitrogen have been detected in the soil profile (Mubyana *et al.* 2003). Therefore, other sources of nitrogen for plant growth must exist. In other areas, diazotrophs associated with some grasses and cereals have been shown to contribute significantly to the total nitrogen budget of the plant (Kolb and Martin 1988, Baldani *et al.* 1986, Elmerich *et al.* 1992). Thus it is highly likely that diazotrophs may be a possible source of nitrogen for the Okavango Delta floodplain grasses. Although many studies have been conducted on plants of the Okavango Delta, so far none have looked at diazotrophs associated with the

grasses in the area. Thus the main objectives of this study were to carry out a survey of diazotrophs associated with dominant grasses of the Okavango Delta, and also to assess the effect of flooding on soil nitrogen content on the diazotroph populations.

Materials and Methods

Study site and sampling strategy

Sampling was done at the Weir site situated 2km east of Nxaraga Lagoon, in the Okavango Delta (23–24°E, 19–24°S). The Weir site was chosen because of its high diversity in grass species of the Delta. The site consisted of a moisture gradient with three distinct flooding regions, namely: 1) the primary floodplain located immediately adjacent to the riverbed that is flooded annually, 2) the secondary floodplain normally flooded in the years of average rainfall and 3) the tertiary floodplain flooded only in years of exceptionally high rainfall (Biggs 1979). The riparian woodland occupied the tertiary floodplain.

The first sampling conducted during August 2001 at the peak of the flooding period concentrated on grasses in the secondary and tertiary floodplains as those in the primary floodplain were inundated. Five replicated samples of each of the 11 grass species were collected from 1m^2 quadrants randomised in each floodplain. The second sampling conducted at the end of November 2001, when the floods had receded, included additional secondary and tertiary floodplain grasses. However, because of the high diversity of grasses available for sampling in November, only 3 replicate samples of 16 grass species were collected from 1m^2 quadrants in each floodplain. The grasses' entire root systems together with the adhering soil collected from the different floodplains were placed in sterile plastic bags and immediately transported to the laboratory in a cooler box. A list of grass species sampled and their location in the different floodplains is presented in Table 1.

Laboratory analyses

Excess soil was removed from the grass roots by gently shaking. The remaining soil adhering to the root hairs was categorised as the rhizosphere soil. Moisture contents of the excess soil samples were determined gravimetrically immediately on arrival at the laboratory (Anderson and Ingram

Table 1: Soil nitrogen levels and moisture regime in the rhizosphere of the grasses at the time of sampling

Grass species	% soil N ± SD x 10^{-2} *	Moisture regime#	Flood regime	Soil pH
Sampling time 1 (August)				
A. meridionalis (Hackel) Clayton	0.001 ± 0.82^{a}	dry	3°	6.20
Andropogon guyanus (Hackel) Clayton	0.134 ± 0.04^{d}	flooded	2°	7.42
Cynodon dactylon (L.) Pers	0.016 ± 0.70^{a}	moist	3°	8.25
Eragrostis inamoena K.Schum	0.046 ± 1.20^{abc}	moist	2°	7.81
Eragrostis rigidor 2 Pilger	0.001 ± 1.32^{a}	dry	3°	6.26
Eragrostis rigidor Pilger	0.022 ± 1.10^{a}	moist	3°	6.20
Imperata cylindrical (L.) Raeuschel	0.051 ± 1.96^{abc}	moist	2°	7.41
Panicum repens 2 L.	0.177 ± 5.94^{d}	submerged	2°	8.30
Panicum repens L.	0.117 ± 4.44^{cd}	submerged	2°	6.24
Setaria sphacelata (Schumach.) Moss	0.038 ± 1.85^{ab}	moist	2°	7.06
Veteveria nigritiana (Benth.) Stapf.	0.020 ± 1.21^{a}	dry	2°	6.86
Sampling time 2 (November)				
A. meridionalis (Hackel) Clayton	<0.001 ± 0.00^{a}	dry	3°	6.30
Andropogon guyanus (Hackel) Clayton	0.024 ± 0.67^{a}	dry	3°	6.45
Cympogon excavatus (Hochst.) Stapf	0.010 ± 0.52^{a}	dry	3°	7.14
Eragrostis lapula Nees	0.000 ± 0.53^{a}	dry	3°	6.82
Eragrostis rigidor Pilger	0.003 ± 0.46^{a}	dry	3°	6.40
Eragrostis superba Per.	<0.001 ± 0.00^{a}	dry	3°	6.20
Eragrostis superba Per.	0.027 ± 1.87^{a}	dry	3°	7.86
Panicum colaratum L.	0.059 ± 0.01^{abc}	moist	2°	6.61
Setaria sphacelata (Schumach.) Moss	0.001 ± 0.00^{a}	dry	2°	7.06
Setaria verticilata (L.) Beauv	0.112 ± 2.76^{cd}	dry	2°	6.78
Sporobolus acinifolis Stapf	<0.001 ± 0.00^{a}	dry	3°	6.27
Sporobolus africanus (Poic.) Robyns Tournay	<0.001 ± 0.00^{a}	dry	3°	6.52
Sporobolus spicatus 2 (Vahl) Kunth	<0.001 ± 0.00^{a}	dry	3°	8.56
Sporobolus spicatus (Vahl) Kunth	<0.001 ± 0.00^{a}	dry	3°	7.52
Stipagrostis uniplimus (Lichtenst. ex Roemer & Schultes)	<0.000 ± 0.00^{a}	dry	3°	6.82
Urochloa trichopus (Hochst.) Stapf	0.107 ± 1.09^{bcd}	moist	3°	9.08

* Means followed by the same letter are not significantly different from each other at 5% level

Plants completely submerged = submerged, flooded: water at soil surface level + = flooded, soil moisture 35–10% = moist, soil moisture <10% = dry

2° = secondary floodplain, 3° = tertiary floodplain

1993). The other soil was air-dried and then passed through a 2mm mesh sieve. Total N was determined using an automated N analyser (LECO Model FP 328). Ethylene diaminetetraacetate (EDTA) was used as the standard in the determination of the %N. Active soil acidity was determined in a 1:2 soil:water suspension as outlined by Anderson and Ingram (1993). The pH in the supernatant was measured using an Accumet®/Fisher Scientific Model 50pH meter (London, UK) with a combination glass electrode. pH readings were recorded to two decimal places.

Isolation, enumeration and purification of diazotrophs

Nitrogen-fixing bacteria (diazotrophs) were cultivated on composite media, which included all essential growth factors commonly used for culturing a wide assortment of such microorganisms. Weighed 5g samples of the rhizosphere soils were placed into 95ml sterile Ringer's solution containing 0.05% Tween 80 to create a homogenate that was then diluted stepwise in sterile ringer's solution to 10^{-6}.

Diazotrophs of the genus *Azospirillum* were isolated on selective N-free malate medium (Dobereiner *et al.* 1976), using the procedure outlined by Knowles (1982). Enumeration of *Azospirillum* and N-fixing S reducers in the rhizosphere soil homogenates was done by Most Probable Number (MPN) using published procedures (Okon *et al.* 1977, Watanabe *et al.* 1979, Knowles 1982). Here, the samples were incubated at 25°C for 5–7days, after which the test tube caps were replaced with rubber serum stoppers and the tubes assayed for acetylene reduction as described below. Formation of a well-defined pellicle 1–4mm below the meniscus and an ability to reduce acetylene was regarded as positive growth for *Azospirillum* and other microaerophillic N-fixing micro-organisms. The data was interpreted using the MPN table for use with 5-tube dilutions (Cochran 1950).

Purification of *Azospirillum*, was done by scooping the pellicle using a sterile inoculation loop, and then streaking it onto *Azospirillum* solid malate medium (Dobereiner *et al.* 1976) amended with 200mg l^{-1} streptomycin sulphate, 250mg l^{-1} cyclohexamide, 200mg l^{-1} sodium desoxycholate, 15mg l^{-1} 2,3,5-triphenyltetrazolium chloride, 1g l^{-1} congo red and 15g l^{-1} bacteriological agar. Colony types resembling those described by Bashan and Levanony (1985), Dobereiner *et al.* (1976) and Rodriguez-C'aceres (1982) were selected on the basis of shape, colour and microscopic form. The isolated colonies were then streaked on agar slants of fresh solid malate medium amended with the antibiotics and stored at 4°C.

Diazotrophs of the genus *Azotobacter* were enumerated and isolated from the rhizosphere soil on *Azotobacter* glucose solid medium and *Azotobacter* mannitol solid medium (Brown *et al*. 1962, Knowles and Barraquio 1994) and incubated at 25°C for 3 weeks. The isolates obtained were purified by transferring them onto sterile fresh solid medium. Once purified, the isolates were then maintained on *Azotobacter* media slants (Knowles and Barraquio 1994).

Isolation of *Beijerinkia* spp. was done in N-free liquid Beijerinkia medium (Strijdom 1966) as described by Knowles (1982).

Acetylene reduction assays

Purified bacterial isolates were tested for acetylene reduction activity on semi-solid (0.75% agar) malate medium and assayed using a gas chromatograph equipped with a flame ionization detector and a 1.5m long by 3.2mm diameter Poropak Q column. The carrier gas used was N_2. The source of air and fuel for the flame was H_2 (Knowles and Barraquio 1994).

Identification of diazotrophs

Morphological characterisation, carbon source utilisation, and Gram stain of all the isolates were determined as described by Gerhardt *et al.* (1981). Biochemical identification involving API 20E microtube identification system were carried out and interpreted according to the API 20E analytical profile index (bioMerieux Inc. USA). Other supplementary tests for the identification of *Azospirillum* as described by Rennie (1981) were carried out. Reference strains of *Azospirillum brasilense* ATCC 29145, *A. lipoferum* ATCC 29708, *Azotobacter vinelandii* ATCC7489 and *A. chroococum* ATCC 480 were also run along the isolates in the identification. The identity of *Azospirillum* isolates was confirmed by carrying out restriction fragment length polymorphism analysis of the 16S rDNA as described by Grifoni *et al.* (1995). Genomic DNA was extracted using a modified method of Marmur and Doty (1961). PCR was carried out with an Applied Biosystems GeneAmp PCR system 2400 thermocycler using a PCR core kit (Roche Diagnostics) according to the manufactures instructions. The amplified DNA was digested with *Alu* I restriction endonuclease (Roche Diagnostics) and the fragments were separated in 0.8% agarose gel dissolved in 0.4M Tris-acetate and 0.01M EDTA. The gel was stained with ethidium bromide, and the DNA visualised by UV transillumination. The restriction fragment profiles generated were compared to those described by Grifoni *et al.* (1995).

Statistical analysis

An analysis of variance tested differences in the abundance of different groups of N-fixing bacteria, as well as soil N and moisture contents, in the rhizosphere of the grasses in the different floodplain. Significantly different treatment means were separated using a Tukey honest significant difference test.

Results and Discussion

More than 150 different types of bacteria were isolated from the roots of floodplain grasses on the different N-free media. Based on colony type and microscopic observations of these, only 103 could be purified. Tentative identifications using acetylene reduction assay, morphological and biochemical tests showed that 40 of these isolates were species of *Azotobacter* and 41 belonged to the species within the genus *Azospirillum*, whereas the rest were unidentifiable. Only those bacterial isolates, which yielded acetylene reduction rates above 0.766mmoles ml^{-1} hr^{-1} were designated as N fixers and subjected to further analysis.

All the *Azospirillum* isolates used malic acid as the sole source of carbon, and also formed well-defined pellicles 2–5mm below the meniscus. They were all gram negative, catalase and oxidase positive, grew on Mackonkey solid media (Difco), but were not lactose fermenters. The morphological, biochemical and RFLP profiles of the most abundant *Azospirillum* isolates (60% occurrence) are presented in Table 2. These isolates tested positive for nitrate reduction to nitrogen gas (N_2) on the API 20E strips and classified further to species level using amplified ribosomal DNA restriction fragment length analysis. All produced an expected 1 450bp amplicon, except the isolates B21SS and A52ERD, which did not produce any PCR product (results not shown). The first restriction profile banding pattern (Figure 1, lane 2) concurred with that observed in *Azospirillum brasilence* using the same restriction endonuclease (Grifoni *et al.* 1995). The second pattern (Figure 1, lane 3) was consistent with that of *Azospirillum lipoferum* (Grifoni *et al.* 1995). The last profile banding patterns in lanes 4–7 were typical of the isolates B33CE, B34CE, B13SAF and B22PR10 but not as yet documented for any distinct species.

Due to the presence of oligonitrophiles and other nitrogen scavengers, three transfers had to be made before pure *Azotobacter* colonies could be obtained. Two morphologically different *Azotobacter* colonies were distinguished on the *Azotobacter* solid media based on their colour and texture. Although most of the *Azotobacter* isolates used mannitol as their carbon source, a few of the Azotobacter isolates from the rhizosphere of *P. repens* and *V. nigritiana* were also able to use glucose as the sole source of carbon.

The distribution and abundance of different diazotrophs in the rhizosphere varied according to the different type of grass species and along the flood regime. Most probable number (MPN) enumerations revealed that *Azospirillum* was the most abundant diazotroph on the roots of most of the grasses studied with populations of up to 10^4MPN g^{-1} soil in the rhizosphere of *C. dactylon*, *E. inamoena*, *E. rigidor* (Table 3). The least populations of *Azospirillum* (10^2MPN g^{-1} soil) were found in the rhizosphere of submerged grasses such as *P. repens* and *A. guyanus*. *Azotobacter* species were also isolated from the roots of the dominant species of grasses, which were inundated at the time of sampling. The most abundant *Azotobacter* populations (10^3CFU g^{-1} soil) were enumerated in the rhizosphere of *A. guyanus*. *Azotobacter* was also isolated from the rhizosphere of *P. repens* and *V. nigritiana* with populations of 10^2CFU g^{-1} soil, and *S. sphacelata* with a population of 10^1CFU g^{-1} soil (Table 3). The dominance of *Azotobacter* in flooded as opposed to non-flooded soils could be attributed to less O_2 in flooded soils. High O_2 is known to interfere with the nitrogenase enzyme activity (Marshal and Venderleyden 2000).

Nitrogen-fixing sulphate reducers were also detected in the rhizosphere of flooded grasses. These were characterised by the darkening of the bottom of the tube in N-free medium used to enumerate *Azospirillum*. Although fewer grass species seemed to have N-fixing sulphate reducers in

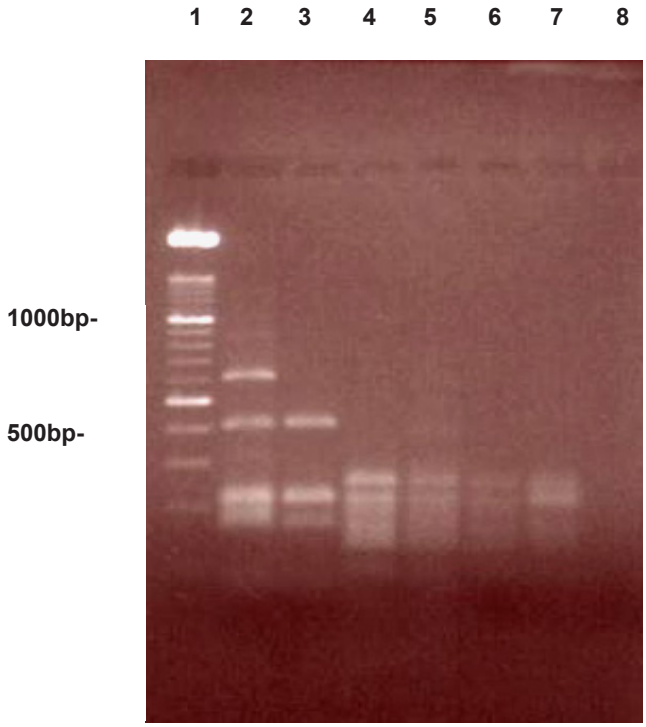

Figure 1: Agarose gel electrophoresis of amplified 16S rDNA of seven tentative *Azospirillum* isolates digested with restriction endonuclease Alu1. Lane 1: 100bp ladder maker; Lanes 2–8: B12AM, B13SSP, B33CE, B34CE, B13SAF, B22PR10 and B12CCD10

Table 2: Morphological, biochemical and RFLP profiles of some of the *Azospirillum* isolates

Isolate ID	Description on different media	RFLP Profiles	API 20E Biochemical profile group
B22SSP2	Pink and red colony on CR media, pink on YEN, col. dia. 2mm, 2mm from meniscus	No 1 450bp amplicon	Profile H, not identified. 5% occurrence
B21URO, B34CE, B33CE, B13SAF, B22PR10, B21SS(2)	Scarlet on CR, 2mm col. dia., cream on GYEN, 2mm from meniscus, except for B22PR10, is 10mm from meniscus	1 450bp amplicon 250bp, 240bp, 200bp and 180bp	Profile A, 60% occurrence, except for B21SS, which is isolate C
B12AM, A43CDP	2–3mm below meniscus, scarlet colonies on CR, cream on GYEN	1 450bp amplicon 630bp, 450bp, 240bp and 180bp	Profile A, 60% occurrence
A43IC, B13SSP	2–5mm below meniscus, rich red colonies on CR, Cream on GYEN	1 450bp amplicon 450bp, 240bp, 180bp	Profile A, 60% occurrence
B12CCD10	10mm below meniscus, Scarlet on CR, col. dia. 2mm	1 450bp amplicon but no bands after restriction digest	Profile G, similar API to A. 10% occurrence

Table 3: Diazotroph populations in the rhizosphere of dominant annual and perennial grasses of the Okavango Delta

Grass species	Floodplain (sampling time)	% soil moisture	*Azospirillum* spp. (MPN g^{-1})	*Azotobacter* spp. X (CFU g^{-1})	S reducers (CFU g^{-1})
A. guyanus	2° (1)	44.98	10^2	10^3	10^2
A. meridionalis	3° (1)	1.32	10^4	–	–
C. dactylons	3° (1)	11.14	10^4	–	–
E. inamoena	3° (1)	22.72	10^4	–	–
E. rigidor	3° (1)	1.07	10^4	–	–
E. rigidor	3° (1)	16.83	10^3	–	–
I. cylindrica	2° (1)	11.12	10^3	–	–
P. repens	2° (1)	47.02	10^3	10^2	10^3
P. repens	2° (1)	56.46	10^2	10^2	10^3
S. sphacelata	2° (1)	18.68	10^4	10^1	–
V. nigritiana	2° (1)	9.80	10^3	10^3	–
S. spicatus 2	3° (2)	7.09	10^3	–	–
S. sephacelata	2° (2)	5.05	10^4	–	–
E. lapula	3° (2)	5.61	10^4	–	–
A. guyanus	2° (2)	3.60	10^3	–	–
S. verticilata	2° (2)	5.10	10^4	–	–
A. meridionalis	3° (2)	1.11	10^4	–	–
E. rigidor	3° (2)	1.98	10^4	–	–
C. dactylon	3° (2)	9.08	10^4	–	–
U. trichopus	3° (2)	11.81	10^4	–	–
E. superba	3° (2)	3.56	10^4	–	–
C. excavatus	3° (2)	4.34	10^4	–	–
S. africanus	3° (2)	4.53	10^4	–	–
S. spicatus	3° (2)	5.24	10^3	–	–
P. coloratum	2° (2)	7.39	10^4	–	–

Sampling time 1: August 2002, Sampling time 2: November 2002, S reducers: Sulphate reducers, –: populations below detected levels

their rhizosphere, most of the grasses that were flooded during sampling exhibited the presence of N-fixing sulphate reducers. Highest populations of N-fixing sulphate reducers were observed in the rhizosphere of *P. repens* and *A. guyanus*, i.e. 10^3MPN g^{-1} soil and 10^2MPN g^{-1} soil, respectively. No N-fixing sulphate reducers were recorded in the grasses from the dry moisture regime (tertiary and secondary floodplains with <10% soil moisture content). This may be attributed to the ability of S-reducing bacteria to use sulphate as a terminal electron acceptor in the consumption of organic matter under anaerobic conditions, resulting in the production of hydrogen sulphide (H_2S) under anaerobic conditions (Dally *et al.* 2000). Therefore, the prevailing anaerobic conditions in the rhizosphere of the flooded grasses may have provided suitable conditions for sulphate reduction.

Apart from *Azospirillum* and *Azotobacter* spp., there were other isolates from the N-free media. Few were identified as Pseudomonads, however others could not be identified. These include those isolates from the rhizosphere of dominant perennials such as *U. trichopus*, *E. superba*, *S. verticilata*, *C. excavatus* and *S. africanus* and some dominant annuals such as *C. dactylon*, *E. rigidor*, *S. sphacelata* and *I. cylindrica*. Nitrogen-fixing pseudomonads have also been isolated from roots of grasses by other authors working in other areas (Lifshitz *et al*. 1986). Some of the unidentifiable isolates, also formed well defined pellicle in the *Azospirillum* media, but had different biochemical profiles on the API 20E strip. *Beijerinckia* was not isolated from any of the grasses sampled in this study. This may be attributed to the higher pH of the soils, which ranged from 6.20 to 9.08. Although *Beijerinkia* species may be wide spread in nature, in many studies, it has only been isolated and associated with acidic soils (pH ≤ 4.5) (Becking 1961).

Active soil acidity (soil pH) in the rhizosphere soils varied from 6.20 in *A. meridionalis* to 9.09 in *Urochloa trichopus*. These variations did not seem to be related to soil moisture content nor grass species, as high variations existed in the rhizosphere of replicate samples from the same grass species. These variations may be attributed to ash deposits carried by water from upstream where burning occurs in the floodplains.

Although soil nitrogen levels in the rhizosphere varied with grass species ($P < 0.05$), the influence of moisture regime was much greater ($P < 0.01$) (Table 4). Insignificantly low soil nitrogen levels were detected in all the drier rhizosphere soils from the drier tertiary floodplain bordering the riparian woodland. These grasses included *A. meridionalis* and *E. rigidor* during the first sampling and all the tertiary floodplain grasses during the second sampling. This trend was also observed during the second sampling as all the grasses which had less than 10% moisture in the rhizosphere had significantly lower nitrogen levels in their rhizosphere when compared to those in wetter rhizosphere soils (*Andropogon guyanus*, *Panicum repens)*. Significantly higher ($P < 0.05$) levels of soil N in the wetter moisture regime (submerged and flooded) were detected (Table 1). Thus in this study, the soil nitrogen content decreased from the primary to the tertiary floodplains. Soil moisture content was positively corre-

Table 4: Probability levels for statistical significance for different parameters

Variable	Probability
Diazotroph populations	**
Diazotroph diversity	*
Moisture regime	**
Nitrogen	*
Nitrogen x moisture regime	**
Diazotroph populations x moisture regime	*
Diazotroph diversity x moisture regime	*

* Statistically significant at $P < 0.05$
** Statistically significant $P < 0.01$

lated with soil N. As the soil moisture is of flood origin in this region, which has insignificant rain (Gaborone Meteorological Services data), this may indicate that nitrogen may be of floodwaters origin. Okavango floods originate from the high lands in Angola (Ellery and Ellery 1997). On their journey to the Delta, these waters flow through floodplains often grazed by cattle and wildlife whose droppings could increase the dissolved organic residues of the water (Biggs 1979). Burning is also a common practice in the grasslands of the Delta Panhandle through which these waters flow (Biggs 1979). Thus it is not unusual for this water to carry a higher load of nutrients when compared to rainfall floodwaters origin occurring in highly impermeable clay soils (Brady 1990). Soils of the Okavango delta are highly sandy (>85% sand) with a low (<5%) cation exchange capacity (Staring 1978), and both conditions are very likely to favour the leaching of nitrogen from these soils (Brady 1990, Knops *et al*. 2002). Thus the low N in the dry soils could be due to the high sand content and drainage of the Okavango Delta. When these soils undergo the seasonal changes in flooding and drying, soil N could be leached, thus resulting in low soil nitrogen in dry soils. Although high levels of soil nitrogen have been documented to reduce biological nitrogen fixation (Kolb and Martin 1988, Elmerich *et al*. 1992), it is highly unlikely that this could be the case here, as these soils are generally low in total soil nitrogen (Mubyana *et al*. 2003).

Conclusions

Overall analyses showed that significant differences in both diazotroph diversity and population abundance exist in these floodplains, with diazotrophs such as N-fixing Sulfur reducers and *Azotobacter* more prominent during the flood season. On the contrary, *Azospirillum* species were the dominant diazotrophs during the dry season. Molecular identification of *Azospirillum* using Restriction Fragment Length Polymorphism showed three different profiles. Based on the profiles, two species were identified as *A. brasilense* and *A. lipoferum*. The third RFLP profile was unique and could not be assigned to any species of *Azospirillum*. The presence of these diazotrophs in the delta indicates that they may be a possible N source for the plants. Thus in the Okavango plains, flooding has a significant effect on diazotroph populations, diversity and soil nitrogen.

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