Nitrogen cycling in the soil–plant system along a precipitation gradient in the Kalahari sands

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Abstract

Nitrogen (N) cycling was analyzed in the Kalahari region of southern Africa, where a strong precipitation gradient (from 978 to 230 mm mean annual precipitation) is the main variable affecting vegetation. The region is underlain by a homogeneous soil substrate, the Kalahari sands, and provides the opportunity to analyze climate effects on nutrient cycling. Soil and plant N pools, ^{15}N natural abundance ($\delta^{15}N$), and soil NO emissions were measured to indicate patterns of N cycling along a precipitation gradient. The importance of biogenic N_2 fixation associated with vascular plants was estimated with foliar $\delta^{15}N$ and the basal area of leguminous plants. Soil and plant N was more ^{15}N enriched in arid than in humid areas, and the relation was steeper in samples collected during wet than during dry years. This indicates a strong effect of annual precipitation variability on N cycling. Soil organic carbon and C/N decreased with aridity, and soil N was influenced by plant functional types. Biogenic N_2 fixation associated with vascular plants was more important in humid areas. Nitrogen fixation associated with trees and shrubs was almost absent in arid areas, even though Mimosoideae species dominate. Soil NO emissions increased with temperature and moisture and were therefore estimated to be lower in drier areas. The isotopic pattern observed in the Kalahari (^{15}N enrichment with aridity) agrees with the lower soil organic matter, soil C/N, and N_2 fixation found in arid areas. However, the estimated NO emissions would cause an opposite pattern in $\delta^{15}N$, suggesting that other processes, such as internal recycling and ammonia volatilization, may also affect isotopic signatures. This study indicates that spatial, and mainly temporal, variability of precipitation play a key role on N cycling and isotopic signatures in the soil–plant system.

Keywords: N_2 fixation, N isotopes, NO emissions, plants, soils, wet and dry years

Introduction

Insufficient nutrient supply is expected to limit ecosystem-level carbon (C) uptake and storage in many systems (Rastetter *et al*., 1997; Walker & Steffen, 1997).

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Photosynthesis is strongly affected by nitrogen (N) availability because the photosynthetic machinery accounts for more than half of the N in the leaves (Lambers *et al*., 1998). Thus, knowledge of N cycle in ecosystems is crucial to investigate the effects of global change on vegetation and C cycle. The dry savannas of the Kalahari sands occupy extensive areas of infertile sandy soils (>90% sand) (Thomas & Shaw, 1991). Even though the soils are infertile across the whole region, broad-leaf savannas (representative of nutrient poor areas) occupy the more moist northern areas and fine-leaf savannas (representative of more nutrient-rich

areas) occupy the arid southern parts of the Kalahari basin (Scholes & Parsons, 1997), owing to the decreasing precipitation from the north (1000 mm) to the south (<200 mm) of the basin (Scholes & Parsons, 1997; Ringrose *et al.*, 1998). Thus, a transect across the Kalahari provides us with an opportunity to investigate N cycling along a precipitation gradient.

In dry savannas, water and nutrients affect productivity, alternating in importance over time (Scholes, 1990), and nitrogen may be seasonally limiting (Bate, 1981). The distribution of nutrients in arid areas is spatially and temporally heterogeneous, with N located mainly in the soil surface and under the shrub base (Garner & Steinberger, 1989; Schlesinger *et al.*, 1996; Whitford *et al.*, 1997; Schlesinger & Pilmanis, 1998). Nutrients and litter from bare patches of ground are conveyed by unobstructed winds, rain splash, and runoff (Coppinger *et al.*, 1991; Parsons *et al.*, 1992) until they are captured by plant stems and root systems. Widespread, shallow root systems absorb and transport nutrients and water toward the plant, causing their fur-ther depletion from the soil in the spaces between plants (Garner & Steinberger, 1989; Návar & Bryan, 1990).

Nitrogen isotope signatures reported for precipitation gradients in the Atacama desert, South America, Hawaii, and the deserts of southern Africa and the southwestern US, have shown an enrichment of ^{15}N in soil, plant, and animal samples associated with arid regions (Shearer *et al.*, 1978; Heaton *et al.*, 1986; Heaton, 1987; Sealy *et al.*, 1987; Vogel *et al.*, 1990; Evans & Ehleringer 1993, 1994; Swap *et al.*, 2003). This enrichment suggests different bio-geophysical processing and cycling of N caused by decreased rainfall, with a more open N cycle (larger losses relative to turnover) as annual precipitation decreases (Austin & Vitousek, 1998; Austin & Sala, 1999; Handley *et al.*, 1999; Schulze *et al.*, 1999). The mechanisms causing this enrichment are not clear, but current hypotheses relate $\delta^{15}N$ to the extent to which N flows from organic to inorganic pools, which are available to gaseous and leaching losses. Any factor (aridity, tillage, extreme pH, fire or grazing) that decreases the proportional flux of ecosystem N into organic matter or increases the flux from organic to mineral pools pushes the system toward ^{15}N enrichment (Handley *et al.*, 1999).

Nitrogen inputs to a system can occur by atmospheric deposition (Swap *et al.*, 1992; Garstang *et al.*, 1998) or biogenic N_2 fixation (reduction of atmospheric N_2). Nitrogen fixation is a desirable attribute in arid areas only when N is the major limiting nutrient, because of the high costs involved in the N_2-fixing process (Sprent, 1985). Most southern African savannas are dominated by legumes (Fabaceae), which are commonly found to fix N_2. Members of the subfamily Caesalpinioideae, which dominate the more humid sites of the Kalahari sands, are generally not N_2 fixers, while members of the subfamily Mimosoideae such as *Acacia* sp., dominant in the driest sites, are commonly found to nodulate (Scholes & Walker, 1993; Sprent, 1995; Scholes *et al.*, 2002). ^{15}N natural abundance ($\delta^{15}N$) has been used to assess N_2 fixation, as it is generally higher in plants whose only N source is soil N, rather than atmospheric N_2 (Shearer *et al.*, 1983; Schulze *et al.*, 1991). The N_2 fixation activities in the Namib desert varied along an aridity gradient, being generally higher in lowland, drier savannas (100 mm annual precipitation) than in highland, more humid savannas (400 mm annual precipitation) (Schulze *et al.*, 1991). This and the fact that the driest savannas of the Kalahari sands are dominated by Mimosoideae species lead us to hypothesize that symbiotic N_2 fixation is more prevalent in drier sites of the Kalahari transect.

Cyanobacteria are also capable of fixing atmospheric N_2, and they are widely distributed in semiarid and arid soils throughout the world, including the Kalahari desert (Shushu, 2000). Estimates of N_2 fixation by soil crusts vary widely, ranging from grams to 100 kg of N_2 fixed per ha per year (Ischiei, 1980; Skarpe & Henrikson, 1986; Zaady *et al.*, 1998; Aranibar *et al.*, 2003). Even though rates of acetylene reduction do not provide accurate quantitative estimates of N_2 fixation rates by soil crusts in field settings (Aranibar *et al.*, 2003 and references therein), they are good indicators of relative N_2 fixation rates (Evans & Belnap, 1999). Cyanobacterial N_2 fixation was expected to be more important in drier sites of the Kalahari transect, due to a higher light availability.

Nitrogen losses from ecosystems can occur in the form of NO (nitric oxide), NO_2 (nitrogen dioxide), N_2O (nitrous oxide), NH_3 (ammonia) emissions from soils, and N emissions from plants (Wildt *et al.*, 1997; Hereid & Monson, 2001). In this study, we were only able to focus on NO fluxes, which have been found to be important in savanna systems. Globally, savannas have significant NO emission rates averaging between 0.6 and 56 ng N m^{-2} s^{-1}, with the median around 10 ng N m^{-2} s^{-1} (Johansson *et al.*, 1988; Sanhueza *et al.*, 1990; Williams *et al.*, 1992; Yienger & Levy, 1995; Levine *et al.*, 1996; Parsons *et al.*, 1996; Meixner *et al.*, 1997a; Scholes *et al.*, 1997). NO emissions are higher during the hot, wet season than during the warm, dry season (Levine *et al.*, 1996; Scholes *et al.*, 1997; Otter *et al.*, 1999), because of soil temperature and moisture effects. NO emissions generally increase with soil temperature and increase with soil moisture to an optimal point, after which they decline. Nitrification rates and soil availability of NO_3^- and NH_4^+ are also

related to NO flux (Cárdenas *et al.*, 1993; Parsons *et al.*, 1996; Meixner *et al.*, 1997b; Scholes *et al.*, 1997; Martin *et al.*, 1998; Serça *et al.*, 1998; Roelle *et al.*, 1999).

In this study, N cycling along a precipitation gradient is investigated using foliar and soil $\delta^{15}N$, C, and N contents, and NO emissions from soils. According to current hypotheses, $\delta^{15}N$ should be related to the 'openness' of the N cycle, with more losses relative to turnover resulting in ^{15}N enrichment. We analyzed key N cycling processes that might modify isotopic signatures along a rainfall gradient. With increasing aridity, we anticipated the following: ^{15}N enrichment for soils and plants; lower soil organic C and N; increased symbiotic and non-symbiotic N_2 fixation; and decreased NO losses from the system. The processes and pools analyzed are compared with the isotopic signatures along the precipitation gradient, to indicate whether $\delta^{15}N$ relates to the 'openness' of the N cycle in southern Africa.

Materials and methods

Study sites

The sites were located in a uniform soil substrate, the Kalahari sands, at least 5 km from significant human settlements and major roads (Scholes *et al.*, 2002). The mean annual precipitation ranged from 970 mm in the North to 230 mm in the South (Table 1). Plant and soil samples were collected in the wet season of 1995 and 1999 from Lukulu, Senanga, Maziba (Zambia), Sandveld (Namibia), and Vastrap (South Africa). Additional soil samples were collected in 1999 from several sites in Botswana. In the wet season of 2000, soil and plant samples were obtained from Mongu (Zambia), Pandamatenga, Maun, Okwa River Crossing, and Tshane (Botswana). El Niño occurrence during 1994-95 resulted in below-normal rainfall across much of the region. Drought was also widespread in southern Africa during the 1999 rainy season (NOAA, 2002). The opposite occurred during the 2000 wet season, which brought the worst devastating rains and floods in nearly 50 years to the southeastern portion of the African continent (NOAA, 2002). The sites in Botswana had received above average rainfall immediately before the campaign, associated with the tropical cyclone Eline (Otter *et al.*, 2002). The mean annual precipitation for all sites was estimated from long-term precipitation records (Swap *et al.*, 2003; Griffiths, 1972; Bekker & De Wit, 1991; and data from the Botswana Meteorological Department for the Sua Pan, Botswana). The number of samples and analysis differ for each of the sites because different researchers participated in different field campaigns (Table 1). Soil texture was estimated for the sites visited in 2000, using the hydrometer method. Values of pH were measured in a 1 : 2 soil-to-water suspension (Table 2).

Analysis of $\delta^{15}N$, %C and %N of soil and vegetation

Young and mature plants with the C_3 photosynthetic metabolism (trees, shrubs and forbs) were randomly sampled, selecting several leaves (10–15, depending on the leaf size) at a similar height on the canopy for individuals of the same functional group (trees, shrubs and forbs). All the leaves from the same individual were composited into one sample, oven dried at 60 °C until constant weight, and ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) to pass a 40 mesh screen. Surface soils located under and between tree/shrub canopies were sieved (2 mm) and air dried in the field, treated with HCl to remove carbonates, and

Table 1 Experimental design, including the years when the sites where studied, mean annual precipitation, number of plants and soils sampled for $\delta^{15}N$ at each site, and soil sampling depth

Site and year sampled	Mean annual precipitation (mm)	Number of plants sampled for $\delta^{15}N$	Number of soils sampled for $\delta^{15}N$	Depth of soil samples (cm)
Lukulu (1995)	970	27	8	0–10
Mongu (2000)	879	31	6	0–5
Senanga (1995)	810	10	4	0–10
Maziba (1995)	740	17	6	0–10
Pandamatenga (2000)	698		9	0–5
Maun (2000)	460	15	4	0–5
Sandveld (1999)	410	3	2	0–20
Okwa (2000)	407	17	12	0–5
Tshane (2000)	365	21	9	0–5
Vastrap (1995–1999)	230	1	3	0–20
Additional sites in Botswana (1999)	270–685		18	5–20

Table 2 Soil texture and pH values of Kalahari sand sites (empty cells indicate that data were not available)

Site and mean annual precipitation (mm)	pH	% clay	% silt	% sand
Lukulu (970)	6.3			
Mongu (879)		0.6	1.9	97.5
Senanga (810)	5.2			
Maziba (740)	5.4			
Pandamatenga (698)		1.1	2.1	96.8
Maun (460)	6.1	3.1	1.4	95.5
Sandveld (410)	5.6			
Okwa (407)	5.1	1.6	2.4	95.9
Tshane (365)	5.2	2.0	0.0	98.0
Vastrap (230)	6.6			
Vastrap (soils with carbonates) (230)	8.8			

Sampling sizes varied from 2 to 4, including soils under and between tree/shrub canopies.

oven dried at 60 °C until constant weight in the laboratory. The number of replicates and the soil sampling depth for each site are described in Table 1. The δ^{15}N, %C, and %N of soils and plants were determined with a Micromass Optima (GC Instruments, Manchester, UK) isotope ratio mass spectrometer (IRMS) coupled with an elemental analyzer (EA), with an overall precision better than 0.3‰. The data are reported relative to a standard (atmospheric N_2) defined to be 0‰, and expressed in δ notation as

$$\delta_{\text{sample}}(‰) = \left(R_{\text{sample}}/R_{\text{standard}} - 1\right) \times 1000,$$

where δ_{sample} represents δ^{15}N, and R is the molar ratio of the heavier to the lighter isotope for the standard or sample (Hoefs, 1997). Additional plant and soil samples from Maun, Okwa, and Tshane were analyzed only for %C and %N with a CE Elantech gas chromatograph elemental analyzer, providing more replicates for these sites (39, 33 and 28 for Maun, Okwa and Tshane, respectively) than for Mongu and Pandamatenga (6 and 9 samples, respectively). These soils were collected under and outside plant canopies (trees, shrubs, grasses, and forbs). This methodology is described in detail in Feral *et al.* (2003).

Analysis of N_2 fixation

Nitrogen fixation activity in plants was indicated by foliar δ^{15}N, C/N, and taxonomic classification. Plants that derive all their N from the atmosphere may have δ^{15}N and C/N values of -2‰ and 15–17, respectively (Hobbie *et al.*, 1998). In this study, species of legumes (from the Fabacea family) with δ^{15}N values lower than 2‰, if lower than non-legumes from the same site, and low C/N (<24) were considered species with indications of N_2 fixation. The presence of N_2 fixation was determined for each site individually, considering the δ^{15}N and C/N of each legume species and the δ^{15}N of non-legumes at each particular site. However, the δ^{15}N values reported in Table 3 are averages of all individuals of the same species across all the sites, to show the taxonomic distribution of the suspected N_2 fixation activity. Other plants from different families where N_2 fixation is not present were not considered N_2 fixers, even if they had similar C/N and δ^{15}N values as N_2-fixing legumes. Some legumes with high δ^{15}N values (5 to 10‰) have been previously assumed to be N_2 fixers because the δ^{15}N was lower than non-legumes (Schulze *et al.*, 1991). However, in this study, plants with δ^{15}N higher than 2‰ and similar to non-legumes of the same site are assumed to derive most of their nitrogen from sources other than atmospheric N_2. Although some N_2 fixation could be present in these plants, it cannot be assessed by the δ^{15}N methodology, and its importance for total ecosystem N inputs is considered insignificant. The basal area of species with associated N_2-fixing acti-vity was obtained from Scholes *et al.* (2002), Appendix 1.

Analysis of soil NO emissions

NO fluxes ($F(T_s, \theta)$; ng N m^{-2} s^{-1}) were modeled using measured soil temperature and moisture data and laboratory NO emission data using the following equation:

$$F(T_s, \theta) = F_0 \times G(T_s) \times H(\theta),$$

where F_0 is the reference flux, which takes into account the soil diffusivity, bulk density, and the production and consumption rates. $G(T_s)$ is an exponential curve fitted to the temperature data describing the flux in terms of soil temperature, while $H(\theta)$ is a function fitted to the data (see Fig. 9) to describe the NO flux in terms

Table 3 Foliar $\delta^{15}N$ and C/N of plant species indicating the taxonomic distribution of N_2 fixation associated with vascular plants in the Kalahari sands

Species	Family or subfamily	Sampling sites	$\delta^{15}N$, ‰	C/N
Non-N_2-fixing species				
Acacia sp. (*A. luederitzii, A. mellifera, A. erioloba, A. erubescens, A. haematoxylon, A. fleckii*), $n = 13$	F. Mimosoideae	Maun, Okwa, Tshane, Vastrap, Gobabis	7.5 (0.8)	15.4 (1.3)
Bauhinia sp. (*B. petersiana* and *B.* sp.), $n = 5$	F. Caesalpinioideae	Mongu, Maun, Okwa	4.9 (0.3)	16.0 (1.3)
Boscia albitrunca, $n = 3$	Capparaceae	Maun, Okwa, Tshane	12.8 (1.6)	13.9 (0.3)
Brachystegia sp. (*B. longifolia, B. spiciformis*), $n = 7$	F. Caesalpinioideae	Lukulu, Senanga, Maziba, Mongu	3.0 (0.3)	20.2 (1)
Colophospermum mopane, $n = 5$	F. Caesalpinioideae	Maun	3.9 (0.5)	19.4 (0.9)
Combretum molle, $n = 1$	Combretaceae	Mongu	3.5	21.8
Commiphora tenuipetiolata, $n = 1$	Burseraceae	Okwa	7.5	12.9
Copaifera baumiana, $n = 2$	F. Caesalpinioideae	Maziba, Mongu	2.1	34.8
Cryptosepalum exfoliatum, $n = 2$	F. Caesalpinioideae	Lukulu	2.7	19.8
Diospyrus batocana, $n = 5$	Ebenaceae	Senanga, Maziba, Mongu	2.5 (0.4)	41.3 (1.4)
Diplorhynchus condylocarpon, $n = 1$	Apocynaceae	Lukulu	1.8	22.2
Grewia sp. (*G. flava, G. flavescens*), $n = 5$	Tiliaceae	Maun, Okwa, Tshane	6.4 (1.1)	19.6 (2.2)
Hannoa chlorantha, $n = 2$	Simaroubaceae	Mongu	3.8	29.8
Hymenocardia acida, $n = 3$	Euphorbiaceae	Lukulu	1.4 (0.6)	18.4
Lonchocarpus nelsii, $n = 2$	F. Papilionoideae	Maun, Okwa	5.4	7.8
Mimusops zeyheri, $n = 1$	Sapotaceae	Lukulu	0.0	38.4
Monotes glaber, $n = 3$	Dipterocarpaceae	Senanga, Maziba	3.4 (0.3)	36.0 (3.2)
Ochna pulchra, $n = 2$	Ochnaceae	Lukulu, Mongu	1.6	29.4
Parinari curatellifolia, $n = 2$	Chrysobalanaceae	Lukulu, Mongu	1.7	36.0
Paropsia brazzeana, $n = 1$	Passifloraceae	Mongu	5.8	20.7
Pseudolachnostylis maprouneifolia, $n = 1$	Euphorbiaceae	Mongu	2.4	27.1
Rhus tenuinervis, $n = 1$	Anacardiaceae	Tshane	10.2	18.4
Strychnos pungens, $n = 2$	Loganiaceae	Maziba	3.1	38.7
Syzygium guineense, $n = 1$	Myrtaceae	Lukulu	1.2	28.4
Terminalia sericea, $n = 3$	Combretaceae	Maun, Okwa, Sandveld	4.8 (0.7)	25.1 (3.8)
Ximenia caffra, $n = 1$	Olacaceae	Maun	4.5	33.3
Unidentified understory forb (nonlegumes), $n = 4$		Maun, Okwa, Tshane	7.0 (1.2)	16.9 (0.9)
Unidentified, $n = 1$	Rubiacaee	Mongu	2.6	27.3
Unidentified understory shrub, $n = 1$	Fabaceae	Mongu	3.0	17.3
Unidentified, $n = 1$		Tshane	11.1	16.8
Species with indications of N_2 fixation				
Baphia massaiensis, $n = 1$	F. Papilionoideae	Mongu	1.4	17.2
Burkea africana, $n = 3$	F. Caesalpinioideae	Lukulu, Mongu	−0.7 (0.4)	22.2 (1.6)
Chamaecrista sp., *Vigna* sp., other forb legumes, $n = 5$	F. Caesalpinioideae and Papilionoideae	Maun, Okwa	−0.1 (0.3)	10.5 (0.7)
Dialium engleranum, $n = 1$	F. Caesalpinioideae	Lukulu	2.1	24.3
Erythrophleum africanum, $n = 4$	F. Caesalpinioideae	Lukulu, Senanga, Maziba	0.9 (0.6)	23.2 (2.3)
Guibourtia coleosperma, $n = 3$	F. Caesalpinioideae	Lukulu, Mongu	0.9 (0.3)	18.8 (1.7)
Indigofera sp., $n = 2$	F. Papilionoideae	Mongu	0.6	15.3
Pterocarpus angolensis, $n = 3$	F. Papilionoideae	Maziba	0.3 (0.6)	14.8 (0.7)
Tephrosia sp. (*T. polystachia, T.* sp.), $n = 6$	F. Papilionoideae	Mongu, Tshane	1.8 (0.8)	9.2 (0.8)

Values represent mean and standard error (in parentheses) for each plant genus or species across all the sites. The sites where the plants were sampled and the taxonomic classification (family) are indicated. Subfamilies are also included for plants from the family Fabacea (F.).

of soil moisture. These equations are described in detail in Otter *et al*. (1999).

Measured soil moisture and temperature

Soil temperature and moisture were measured at 5 cm at Mongu in a manner similar to that described in Pinheiro *et al*. (2001), and at 2.5 and 7.5 cm at Maun and Okwa (Scanlon & Albertson, 2003). For comparison purposes, the values obtained at 2.5 and 7.5 cm at the Botswana sites were averaged to obtain an approximate value at 5 cm. No measurements of soil temperature or moisture were made at Pandamatenga, so estimates were obtained by averaging the values from Mongu and Maun (the sites on either side of Pandamatenga). No soil temperature and moisture measurements were taken at Tshane; therefore, NO fluxes could not be modeled for this site. However, laboratory NO data were still collected for comparison purposes.

Laboratory soil NO measurements

Three replicates of the top 5 cm of soils located between plant canopies were collected from each site, sieved (2 mm), sealed in plastic bags, and kept at 5 °C until laboratory analysis. The gravimetric soil water content was determined by conventional methods. Laboratory bulk densities were determined by weighing the amount of sieved soil in a steel tube of known volume (as described in Otter *et al*., 1999). The actual field bulk density values were obtained from the FAO global soils map (FAO, 1995).

Laboratory NO emissions were measured using a dynamic soil incubation system, similar to that used in Otter *et al*. (1999), and briefly described here. Pressurized air was supplied at 20 psi to a gas purification system (four traps consisting of glass wool, activated charcoal, silica gel, and molecular sieve) and a humidifier. Purified air passed through a mass flow controller (MFC) (Tylan, MA, USA, model FC-2805) that supplied air to two (one control and one sample) chambers (design described in Otter *et al*., 1999) at a rate of 2.5 L min^{-1}. Each chamber outlet was connected, via a switching valve, to a NO chemiluminescence analyzer with a NO_2 photolytic converter (Monitor Laboratories, Monitor Labs Inc., Englewood, CO, USA model 8840) and a CO_2/H_2O analyzer (Licor 6262, Li-cor Inc., Lincoln, NE, USA). Before the air entered the NO analyzer, it passed through a Perma Pure drying tube system (Gas Dryer MD, Perma Pure Inc., Toms River, NJ, USA). Calibrations were performed with a NO standard (11.3 ppm, Air Products, Johannesburg, South Africa). Soil NO flux rates were determined by monitoring the concentration of NO at the inlet and outlet of each chamber. The NO production rate (P in ng N s^{-1} kg $soil^{-1}$), uptake rate constant (*k* in $m^3 s^{-1}$ kg $soil^{-1}$), compensation mixing ratios (m_c in ppbv), and the soil moisture and temperature relationships were determined using the laboratory incubation system, as described in Otter *et al*. (1999).

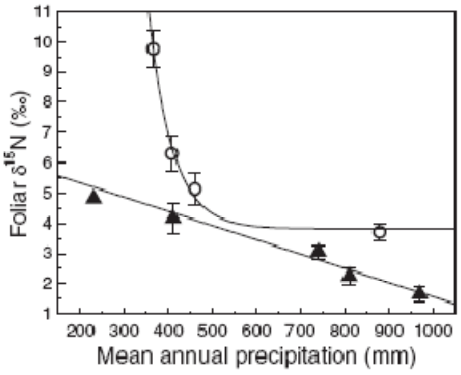

Fig. 1 $\delta^{15}N$ of non-N_2-fixing plants (total number of samples = 95) collected in dry (1995–1997, triangles) and wet (2000, circles) years along a precipitation gradient. Equation for 1995–1997: $y = -0.0047X + 6.2722$; $r^2 = 0.5$; $P < 0.0001$. Equation for 2000: $y = 4698\exp(-0.0183 1X) + 3.817$; $r^2 = 0.57$; $P < 0.0001$. Each symbol represents the average $\delta^{15}N$ value at a site, and error bars denote standard errors of the mean when three or more samples were available. The lines from the two periods (1995–1999 and 2000) are significantly different ($P = 0.017$).

Data analysis

Statistical analyses were performed with the software Prism 3.03. The isotopic data along a precipitation gradient were analyzed with the coefficient of determination (r^2) of the regression lines. Although only the average values per site are given in Figs 1 and 2 for clarity, the values of all the samples from Table 3 (excluding plant species with indications of N_2 fixation) were included in the statistical analyses. Analyses of covariance (ANCOVA) were performed to test whether the slopes or intercepts of two regression lines (during wet and dry years) were significantly different. An *F* test was carried out in cases where the data did not appear to be linear (for soils and foliar $\delta^{15}N$ from the year 2000) in order to test whether a higher order relationship yielded a significantly lower sum of squares. In all cases, the *P*-values (two-tailed) are reported. The differences among soil nutrients (C and N) associated with plant functional types were analyzed with Student's *t*-tests.

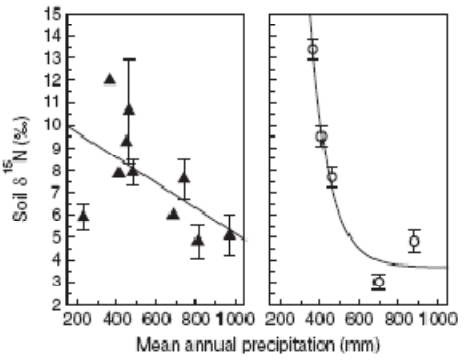

Fig. 2 Bulk soil $\delta^{15}N$ for samples collected during dry (1995–1997–1999, triangles) and wet (2000, circles) years. Equation for 1995–1999: $y = -0.0056X + 10.785$; $r^2 = 0.2$; $P = 0.0006$; Equation for 2000: $y = 448.2\exp(-0.01053X) + 3.686$; $r^2 = 0.85$, $P < 0.0001$. Each symbol represents the average $\delta^{15}N$ value at a site, and error bars denote standard errors of the mean when three or more samples were available. The slope of the 2000 line is significantly different from that of previous years ($P < 0.0001$).

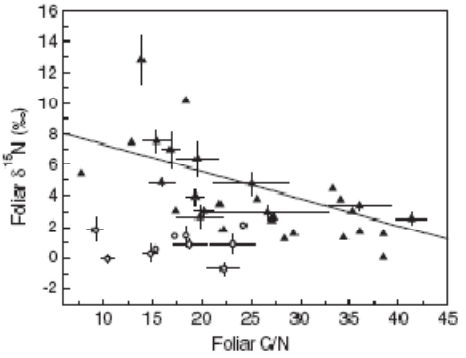

Fig. 3 Foliar $\delta^{15}N$ and C:N ratios of N_2-fixing (circles) and non-N_2-fixing (triangles) plants. $r^2_{\text{nonfixing plants}} = 0.29$, $P_{\text{nonfixing plants}} < 0.0001$. Each symbol represents the average $\delta^{15}N$ and C/N value of one species, and the error bars denote standard errors of the mean when three or more samples were available.

Results

The $\delta^{15}N$ of non-N_2-fixing plant and soil samples were correlated with mean annual precipitation. The ANCOVA analyses indicated that the regression lines from dry (1995–1999) and wet (2000) years were significantly different ($P = 0.017$ for plants and $P < 0.0001$ for soils) (Figs 1 and 2). The F test indicated that 2000 data were better fitted by exponential than linear equations with

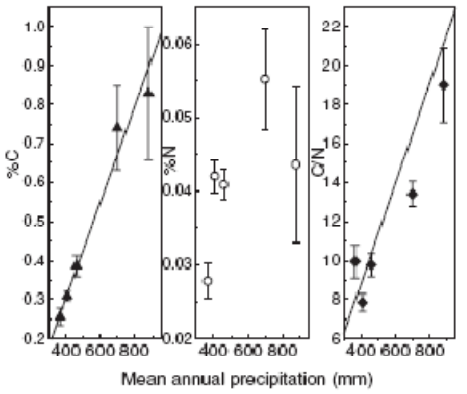

Fig. 4 Soil %C (triangles), %N (circles), and C/N (diamonds) along a precipitation gradient. Each symbol represents the average value at a site, and error bars denote standard errors of the mean. %C: $r^2 = 0.46$, $P < 0.0001$; %N: $r^2 = 0.09$, $P < 0.001$ (function not shown); C/N: $r^2 = 0.41$, $P < 0.0001$. Values for the three more arid sites were obtained from Feral *et al.* (2003).

$P < 0.0001$ for both plants and soils (exponential relations yielded $r^2 = 0.57$ for plants and $r^2 = 0.85$ for soils, with $P < 0.0001$ for both, plants and soils). Data from 1995 to 1999 were best represented by linear equations ($r^2 = 0.5$, $P < 0.0001$ for plants; and $r^2 = 0.2$, $P = 0.0006$ for soils). The $\delta^{15}N$ of plants and soils collected in 2000 were higher than those collected in previous years at sites with similar mean annual precipitation. The $\delta^{15}N$ of soils located under and between tree canopies did not show any pattern (data not shown). In addition, there was a negative relation between $\delta^{15}N$ of non-N_2-fixing plants and their C/N ($r^2 = 0.29$, $P < 0.0001$) (Fig. 3).

Soil C decreased with decreasing precipitation ($r^2 = 0.46$, $P < 0.0001$) (Fig. 4 and Feral *et al.*, 2003). The higher standard errors of the two more humid sites (Mongu and Pandamatenga) reflect the lower number of samples analyzed for C and N in these sites (6, 9, 39, 33, and 28 samples were available for Mongu, Pandamatenga, Maun, Okwa, and Tshane, respectively). Soil N seems to decrease with aridity if the most humid site is not considered, but the coefficient of determination was low ($r^2 = 0.09$, $P < 0.001$). Soil C/N was correlated with precipitation ($r^2 = 0.41$, $P < 0.0001$), but the trend was not consistent in the more arid sites (Fig. 4). Soils associated (under or close to the canopy) with different plant functional types (trees, shrubs, forbs, and grasses) had significantly different C/N ($P < 0.05$) (Fig. 5). In general, soils associated with forbs had the lowest C/N, and those associated with grasses the highest, following the general pattern of foliar C/N (Fig. 6). No clear

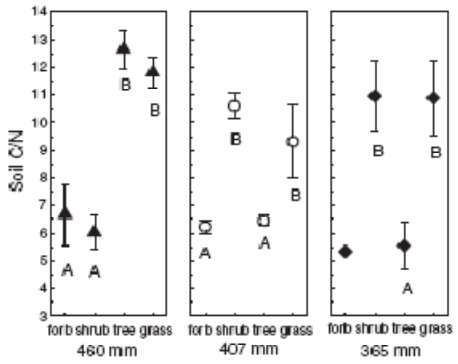

Fig. 5 Average C:N ratios of soils located under or near plant functional types (forb, shrub, tree, grass) in three sites along a rainfall gradient: Maun (460 mm, triangles), Okwa (407 mm, circles), and Tshane (365 mm, diamonds). Error bars denote standard errors of the mean when three or more samples were available. Different letters indicate significant differences ($P < 0.05$) among functional types for each site.

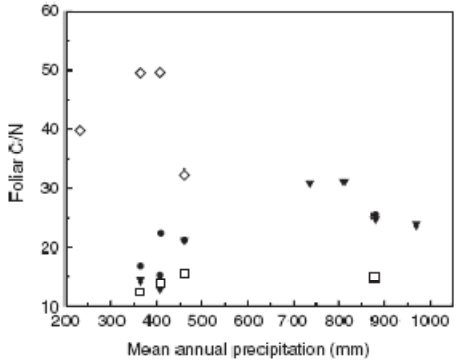

Fig. 6 Foliar C/N of trees (triangles), shrubs (circles), forbs (squares), and grasses (diamonds) along a precipitation gradient. Each symbol represents the average C/N for each site.

pattern was found between foliar C/N and precipitation for each plant type or all samples combined (Fig. 6).

Several Caesalpinioideae species located in the humid sites of the transect showed typical values of N_2-fixing plants (average by species = −0.7 to 2.1‰, Table 3) and lower than non-legumes of the same site, suggesting associations with N_2-fixing microorganisms. All the Papilionoideae species, except the shrub *Lonchocarpus nelsii*, showed indications of N_2 fixation, which agrees with previous studies (Lim & Burton, 1981; Sprent, 1995) (Fig. 3 and Table 3). None of the

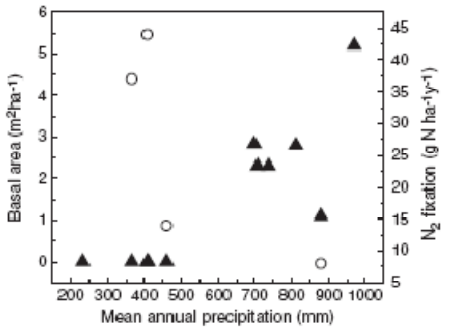

Fig. 7 Basal area of N_2-fixing tree legumes (triangles) and N_2 fixation rates of soil crusts (circles) along a rainfall gradient. Soil crust N_2 fixation rates were obtained from Aranibar *et al.* (in press).

Mimosoideae species showed any indication of N_2 fixation. All the *Acacia* species studied had similar values compared with the non-legume species (7.5±0.8‰) and much higher than the N_2-fixing Papilionoideae species at the same sites (0.6 to 1.8±0.8‰). The basal area of the species with indications of N_2 fixation along the rainfall gradient points to lower N_2 fixation rates with increasing aridity (Fig. 7).

The NO production rates in soils collected in the two northernmost sites (Mongu and Pandamatenga) were more than double those of the southern regions (Table 4). NO consumption rate constants tended to increase and compensation mixing ratios decreased from north to south. There was no significant correlation between the production and consumption rates and the soil NH_4^+ and NO_3^- concentrations reported for these sites (Feral *et al.*, 2003), but the compensation points tended to increase with NH_4^+ concentrations ($r^2 = 0.92$).

Soils with moisture content of less than 10% WFPS (<2.5% gravimetric) did not show any response to temperature. NO fluxes increased with soil temperature, but to different degrees across the sites (Fig. 8). The responses for Mongu, Maun, and Okwa soils were similar, with NO fluxes starting to increase at about 10 °C to a flux of 80–105 ng N m^{-2} s^{-1} when temperatures approached 35 °C. Soils from Mongu showed a decline in the flux as temperatures increased above 40 °C. The flux from Pandamatenga soils on the other hand only started to increase at about 25 °C. NO fluxes from soils collected in Tshane showed little response to temperature with a very slow increase to about 10 ng N m^{-2} s^{-1}.

NO fluxes from Tshane soils did not show any response to a change in soil moisture (Fig. 9), while the NO flux from the Pandamatenga soils showed a slight

Table 4 NO production rates, NO consumption rate constants, compensation mixing ratios (as measured in the laboratory), and soil mineral nitrogen content at five sites along the Kalahari transect; samples were collected and measured during March 2000

Site and mean annual precipitation (mm)	NO production rate, P (ng N s^{-1} kg^{-1} soil)	NO consumption rate constant, k ($\times 10^{-3}$ m^{-3} s^{-1} kg soil)	Compensation mixing ratio, mc (ppbv)	Soil NH_4 content (μg g^{-1})*	Soil NO_3 content (μg g^{-1})*
	1.72	0.34	873	24.0±3.7	1.0±0.4
Mongu (879)					
	2.36	1.85	223	10.8±1.8	1.8±0.4
Pandamatenga (698)					
	0.85	2.71	88	12.3±1.4	1.9±0.5
Maun (460)					
	0.90	5.06	31	8.7±3.3	1.6±0.2
Okwa (407)					
	0.19	1.03	32	11.2±2.2	1.4±0.3
Tshane (365)					

*From Feral *et al.* (2003).

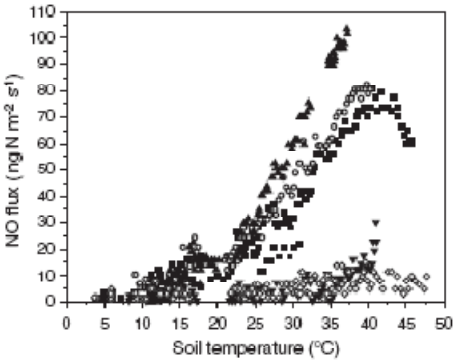

Fig. 8 Soils from Mongu (squares), Maun (circles), and Okwa (up triangles) show a strong positive relationship between NO flux and soil temperature. The NO flux from the Pandamatenga (triangles) and Tshane (diamonds) soils only begin to increase when soil temperatures are higher than 30 °C.

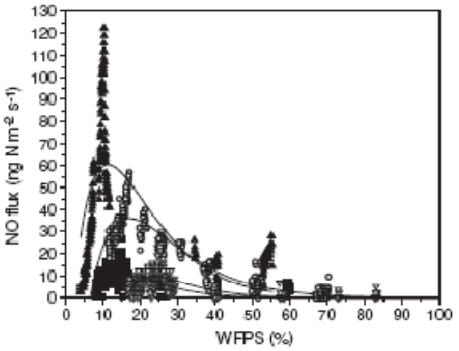

Fig. 9 NO flux increases to a maximum at a soil moisture content of 10%, 14%, 17%, and 23% at Okwa (up triangles), Mongu (squares), Maun (circles), and Pandamatenga (triangles), respectively. The straight lines show the fitted moisture function described in Otter *et al.* (1999):

$$H(\theta) = \exp\left[\frac{(a\,\theta_2 - \theta_{opt})(\theta - \theta_{opt})}{(\theta_{opt} - \theta_1)(\theta - a\,\theta_2)}\right] \times \frac{(\theta - \theta_1)}{(\theta_{opt} - \theta_1)},$$

where a is a moisture curve-fitting parameter, θ is the gravimetric soil moisture (%), θ_{opt} is the soil moisture at which maximum NO emissions occur, θ_1 is the soil moisture at which $F_T = 0$ for $\theta < \theta_{opt}$ and θ_2 is the soil moisture at which $F_T = 0$ for $\theta > \theta_{opt}$.

increase (to 16 ng N m^{-2} s^{-1}) at a WFPS of 23%. The maximum NO flux from Mongu, Maun, and Okwa soils occurred between 10 and 17% WFPS, which is slightly below the field capacity of the Kalahari soils. Figure 9 shows that the measured maximum NO fluxes for the Okwa, Maun, and Mongu soils were 125, 58, and

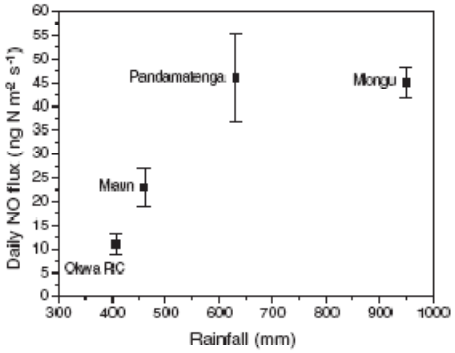

Fig. 10 Average daily NO flux from each site plotted against the annual rainfall indicates that NO fluxes decrease as aridity increases ($r^2 = 0.898$).

21 ng N m^{-2} s^{-1}, respectively, whereas the fitted moisture function indicates much lower maxima (61, 36 and 13 ng N m^{-2} s^{-1}, respectively).

There is a trend of decreasing emissions with increasing aridity (Fig. 10), with a daily average of 45, 46, 23, and 11 ng N m^{-2} s^{-1} for Mongu, Pandamatenga, Maun, and Okwa, respectively. There were insufficient data to model emissions for Tshane. During summer days, when soil temperatures increase above 30°C, emissions at Pandamatenga increase dramatically (modeled daytime average of 76.38 ng N m^{-2} s^{-1} during March, 2000), which would cause the trend to be obscured.

Discussion

The δ^{15}N values of C_3, non-N_2-fixing plants along the rainfall gradient agree with previous studies, showing an inverse relation between δ^{15}N and precipitation (Fig. 1). The relation was stronger for the Kalahari sands (this study) than for the whole Southern African region (Swap *et al.*, 2003), suggesting that soil texture also affects isotopic signatures. In addition, the relation was steeper for the 2000 samples, indicating that N cycling and δ^{15}N were considerably affected by anomalous precipitation during a single year. Soil δ^{15}N showed a similar increase with aridity, which was stronger and steeper for the 2000 soils. The difference between the dry and wet years for soils and plants (Figs 1 and 2) suggests that the unusually high water availability during 2000 could have enhanced soil microbial activity, such as N mineralization of old, heavy organic N pools, and gaseous emissions by denitrification. All these processes result in more ^{15}N-enriched soil N, which could have been absorbed by C_3 plants (Nadelhoffer & Fry, 1994). The anomalies in precipitation were more pronounced and had a higher impact in the dry ends of the transect (Tshane and Okwa), which explains the steeper relation for plant and soil δ^{15}N during 2000. Mongu, the wettest site of the 2000 campaign, did not have unusually high rainfalls (Mukelabai, personal communication; Otter *et al.*, 2002). Several mechanisms have been proposed for the overall ^{15}N enrichment with aridity, and are discussed in Handley *et al.* (1999), Swap *et al.* (2003) and references therein. It is thought that higher δ^{15}N values indicate a more open N cycle, with more losses relative to turnover as the precipitation decreases. Our analyses only partially support that hypothesis. The NO fluxes were estimated to decrease with aridity, which would result in less ^{15}N enrichment in arid sites, contrary to our findings. Other N losses in arid areas can be caused by enhanced ammonia volatilization, due to cycles of wetting and drying and high pH values (>7) associated with carbonates in the soil (Table 2). Also, the lower NO fluxes from drier sites could still represent a larger loss with respect to turnover when compared with wetter site soils. Although the decreased organic matter with aridity (Fig. 4) suggests lower internal recycling in arid areas, soil N turnover should be estimated. Reduced organic matter inputs to the soil pool in arid areas, such as litter fall, and decomposition would also contribute to the ^{15}N enrichment (Handley *et al.*, 1999). The higher N_2 fixation in humid areas observed in this study lowers ecosystem δ^{15}N. Although plants associated with N_2 fixation were not considered in the correlation between mean annual precipitation and foliar δ^{15}N, because they do not reflect soil or ecosystem δ^{15}N, these plants dilute the whole system of ^{15}N upon litter fall and decomposition. Indeed, the soil N available for non-N_2-fixing plants becomes progressively more ^{15}N depleted in humid than in dry areas. Although soil crust N_2 fixation was higher in arid than in humid areas (Fig. 7), the estimated rates were low, on the order of grams of N ha^{-1} yr^{-1}.

Nitrogen fixation associated with vascular plants was higher in more humid areas (Fig. 7), contrary to our expectations. Perhaps in humid areas, primary production can provide the energetic demands of the N_2-fixing machinery, or phosphorus availability is higher (Stock *et al.*, 1990; Sprent, 1995). In dry areas, only annual forbs of low biomass showed indications of N_2 fixation, although the dominant *Acacia* species were suspected, and often assumed to fix N_2. Cyanobacterial N_2 fixation was more important in drier areas, perhaps because competition for light is reduced (Aranibar *et al.*, 2003). Thus, the two biogenic inputs of nitrogen analyzed, soil crust, and vascular plants N_2 fixation, alternated in importance along the precipitation gradient.

It was surprising to find that the Fabaceae (legume) species of the subfamily Mimosoideae located in the driest areas did not show any indication of N_2 fixation, while species from the subfamily Caesalpinioideae of more humid areas suggested N_2-fixing activity (Table 3). This is contrary to the general pattern of N_2 fixation on legume subfamilies (Sprent, 1995), but agrees with the energetic supplies necessary to maintain N_2 fixation, which may not be enough in dry areas due to low primary production (Sprent, 1995). Some of the Caesalpinioideae species, such as *Guibourtia coleosperma* and *Dialium englerianum*, have not been previously found to nodulate, but their $\delta^{15}N$ and C/N strongly suggest that their N is recently derived from the atmosphere. The presence of symbiotic N_2 fixation in these species should be tested with conventional methods such as acetylene reduction assays. The bulk soil $\delta^{15}N$ and foliar $\delta^{15}N$ of most of the non-legumes suggest that soil N sources to plants were enriched relative to atmospheric N_2 (from 5‰ to 13‰ in the sites where vegetation samples were analyzed for N_2 fixation). However, some species (*Ochna pulchra*, *Parinari curatellifolia*, *Mimusops seheri*, *Hymenocardia acida*, *Diplorhynchus condyloncarpon*, and *Syzygium guineense*) showed $\delta^{15}N$ values similar to those of N_2-fixing legumes, although their taxonomy and in some cases high C/N did not indicate N_2 fixation activity (Table 3). These plants may have received N from neighboring N_2-fixing legumes, transferred through hyphal networks (Högberg & Alexander, 1995). On the other hand, many non-fixing legumes (*Acacia luederitzii*, *A. mellifera*, *A. erioloba*, *A. erubescens*, *A. haematoxylon*, *A. fleckii*, *Bauhinia* sp., *Brachystegia spiciformis*, *Colophospermum mopane*, *Lonchocarpus nelsii*) had low C/N and high N contents, which point to other efficient mechanisms of N acquisition besides N_2 fixation, such as mycorrhizal associations or the ability to exploit various N sources. Legumes may have had greater advantages regarding N acquisition than other plants before they evolved the N_2-fixing mechanism (Sprent *et al.*, 1993). Mimosoideae species, in particular, appear to have highly diverse nitrogen-use strategies, such as N_2 fixation, access to organic N via mycorrhizal associates, and the ability to assimilate both nitrate and ammonium (Stewart & Schmidt, 1998). The negative correlation between $\delta^{15}N$ and C/N of non-N_2-fixing plants (Fig. 3) suggests the advantage of taking isotopically heavier soil N, perhaps from old, ^{15}N-enriched organic pools or ammonium. *Boscia albitrunca*'s high $\delta^{15}N$ and low C/N supports this hypothesis (Table 3).

Soil organic C and C/N decreased with aridity (Fig. 4), probably because of the lower biomass, increase of grasses and decrease of woody vegetation in arid areas (Scholes *et al.*, 2002; Feral *et al.*, 2003). This trend supports the idea of lower internal recycling, which would contribute with the observed ^{15}N enrichment with decreasing precipitation (Handley *et al.*, 1999). In addition, lower C/N in arid areas would enhance gaseous losses from the system through mineralization (and subsequent volatilization of the ammonium produced), nitrification and denitrification, enriching the remaining substrates in ^{15}N (Brady & Weil, 1999). However, N turnover rates should be estimated to confirm the hypothesis of higher losses relative to turnover causing ^{15}N enrichment with aridity.

Soil C/N varied with the presence of different plant types. Soil C/N under the influence (under or close to the plant canopy) of some plant functional types was significantly different than under others ($P < 0.05$). Soils associated with forbs and grasses generally had the lowest and highest C/N, respectively, following the pattern found for foliar C/N of these plant types (Figs 5 and 6). The C/N of soils associated with shrubs and trees differed across sites (Fig. 5). However, certain species seemed to have the greatest effect on soil C/N. *B. albitrunca* (a tree) and *Rhus tenuinervis* (a shrub) were associated with low C/N soils, while *Colophospermum mopane* and some *Acacias* (either trees or shrubs) were associated with high C/N soils (data not shown). Although there were not enough data to analyze species effects on soil C/N, our study suggests that plant types or individual species affect N cycling in the soil. Foliar C/N did not show a clear relation with precipitation, even when separated into plant functional types. However, if trees and shrubs are considered together (solid symbols in Fig. 6), arid areas tended to have lower C/N than humid areas, which is similar to the pattern found in soils.

The NO production and consumption rates in this study are in the same range as previously reported for savannas, grasslands, and miombo vegetation in southern Africa (Otter *et al.*, 1999). Compensation points for these vegetation types range between 3.6 and 157 ppbv (Johansson & Galbally, 1984; Kramer & Conrad, 1991; Remde *et al.*, 1993; Rudolph *et al.*, 1996), whereas in this study the ratio goes as high as 872 ppbv. Compensation points measured in the laboratory, however, can be much higher than field measurements (Slemr & Seiler, 1991). Production rates were higher and consumption rates lower in the wetter regions, suggesting that emission rates are higher in these wetter regions, which agrees with the modeled flux data (Table 4).

NO fluxes from Mongu, Maun, and Okwa showed a characteristic increase with temperature from about 10 °C (Yang & Meixner, 1997; Otter *et al.*, 1999; van Dijk *et al.*, 2000), whereas at Pandamatenga and Tshane fluxes only started to increase from 25 °C. The degree of increase of NO emissions with temperature is depen-

dent on soil moisture (van Dijk, 2000). In this experiment, soil moisture for all samples was kept constant for comparative purposes, but NO emissions from Pandamatenga and Tshane soils could show a similar response to temperature at the other sites if the soil moisture was higher. Similarly, if soil temperatures were increased above the 25°C used in the laboratory study, these two sites could show an enhanced response to soil moisture. Model simulations show that at higher soil temperatures and moistures, NO fluxes from Pandamatenga actually exceed those from other sites, which supports this concept. These results indicate that fluxes from Pandamatenga and Tshane soils respond in a similar manner to environmental change, but differ significantly from the response shown at the other three sites. Soil texture (Table 2) does not vary significantly among sites. Thus it could not be responsible for the difference. The temperature response suggests that differences in microbial populations, as microorganisms are very sensitive to temperature and each has a different temperature optimum, could have affected the observed NO fluxes.

NO emissions were maximum at soil water contents of 10–25% WFPS (2.5–6.2% gravimetric), indicating that even relatively dry soils can emit NO. Soil water content at the sites during the campaign was between 3.5% and 6.2% gravimetric, and the average daily emission rates were estimated to be between 46 and 11 ng N $m^{-2} s^{-1}$ (Fig. 10). These indicate a higher basal wet season emission rate than the 10 ng N $m^{-2} s^{-1}$ previously recorded for African savannas and forests (Meixner *et al.*, 1997; Scholes *et al.*, 1997). The Kalahari basin could therefore be an important source of NO in the region, particularly during the wet, summer season. Emissions during the dry season are expected to be low (<3 ng N $m^{-2} s^{-1}$). The average daily emission rates decreased with aridity (Fig. 10), and this trend is expected to be pronounced if the annual emissions were plotted against annual rainfall, because of the longer wet seasons in the northern regions.

Conclusions

The negative relation between precipitation and soil and plant $\delta^{15}N$ agrees with previous studies in other regions, but it was stronger for the Kalahari sands than for the whole southern Africa (Swap *et al.*, this issue). The ^{15}N enrichment with aridity was enhanced during wet years (Figs 1 and 2), probably due to increased mineralization of old organic N pools, which produced available N with high $\delta^{15}N$. Indeed, processes causing the commonly observed ^{15}N enrichment with aridity may be promoted by the higher variability and unpredictability of precipitation, instead of the lower mean annual precipitation in arid regions. The hypothesis stating that high ecosystem $\delta^{15}N$ reflects a more 'open' N cycle and higher losses relative to turnover in arid than in humid areas is only partially supported by this study. With respect to the 'openness' of the N cycle, higher N_2 fixation and NO emission in humid areas indicate a more open N cycling in humid than in dry areas, contrary to the hypothesized increased of 'openness' with aridity. Lower C/N in arid sites would enhance processes that cause N losses, contributing to the observed pattern of higher $\delta^{15}N$ with increasing aridity. The absence of N_2 fixation activity associated with woody legumes in arid sites would also cause the same pattern. However, modeled and measured soil NO emissions decreased with increasing aridity, which would enrich humid sites in ^{15}N. Indeed, most of our measurements agree with the isotopic signatures of plants and soils along a precipitation gradient, except for the soil NO emissions. Although lower organic matter stocks in arid areas indicate lower internal recycling than in humid sites, which may increase the relative importance of N losses on soil $\delta^{15}N$, estimates of N turnover are necessary to determine the effect of N emissions on soil isotope signatures. Other processes such as ammonia volatilization, inputs by atmospheric deposition, and competitive interactions between trees and grasses may also contribute to the observed isotopic pattern. Although N cycling at regional scales involves numerous and complex processes, our study shows that spatial and mainly temporal variability of precipitation play a significant role on isotopic signatures and N cycle in the soil–plant system.

Acknowledgements

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