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Nitrogen isotope composition of soils, C_3 and C_4 plants along land use gradients in southern Africa

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Abstract

This paper provides values for nitrogen isotopic abundances of southern African soils and plants along land use gradients of varying aridity. The $\delta^{15}N$ values of soils and plants were generally higher in sites with greater land use intensity, except in the most arid site where $\delta^{15}N$ decreased with land use intensity. The enrichment in ^{15}N with land use intensity agrees with the expected effects of grazing and cultivation on N cycling processes, including increased volatilization of ammonium, exports of plant material, and decreased N_2 fixation by the destruction of cyanobacterial soil crusts. Gross mineralization and nitrification rates were more affected by local heterogeneity in the soils than by aridity or land use. In general, C_3 plants had significantly higher $\delta^{15}N$ than C_4 plants from the same location, suggesting different N use by the two plant types. This study suggests that land use intensity affects N cycling processes that may result in different and opposite changes of ecosystem $\delta^{15}N$, as those observed between the most arid, and the other semi arid sites analyzed. In addition to the ^{15}N enrichment caused by the loss of gaseous and plant ^{14}N, changes in tree and grass cover may affect soil $\delta^{15}N$ by the differential uptake of soil N with different isotopic abundances.
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1. Introduction

The natural abundances of stable isotopes in ecosystem components are viewed as recorders that can be used to reconstruct ecological processes or to trace ecological activities (West et al., 2006). Nitrogen stable isotopes, in particular, provide valuable insights about the processes of N-cycling that affect soils, vegetation, animals and the atmosphere. The natural abundance of ^{15}N in animal tissue (i.e., feathers, hair and ivory) can be used to trace migratory patterns, because the diet of an animal varies isotopically across climatic and

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ecological gradients (Cerling et al., 2006; Hobson, 1999; Sealy et al., 1987; van der Merwe et al., 1990; Vogel et al., 1990). The nitrogen preserved in samples of bones or ivory can indicate climatic fluctuations of the past (Stevens and Hedges, 2004). These applications are based partially on the general pattern of ^{15}N enrichment with increasing aridity found in soils and plants across the globe (Austin and Sala, 1999; Handley et al., 1999; Heaton et al., 1986; Schulze et al., 1998). The $\delta^{15}N$ values of soils and plants reflect several physical and enzymatic processes that discriminate against ^{15}N. For example, denitrification and volatilization of ammonia result in preferential losses of gaseous ^{14}N, enriching the residual nitrate in the soil in ^{15}N; biological N_2 fixation discriminates against ^{15}N, causing a decrease in the abundance of ^{15}N in the plants (Blackmer and Bremner, 1977; Robinson, 2001; Schulze et al., 1991). The pattern of ^{15}N enrichment of plants and soils observed with increasing aridity is thought to reflect the degree of "openness" (N losses relative to internal N cycling) of the N-cycle (Handley et al., 1999). Furthermore, the ^{15}N enrichment in relatively undisturbed arid sites of southern Africa was more pronounced during years of above average precipitation than those with below average precipitation and for C_3 plants than for C_4 plants (Aranibar et al., 2004; Swap et al., 2004). Human activities, including grazing, fires and cultivation, are present in large areas of Southern Africa and other arid areas of the world and are likely to affect N isotope abundances of ecosystems. However, previous isotope studies have shown that grazing in Australian semi-arid savannas had relatively small effects on the $\delta^{15}N$ values of grasses, and no effect on trees (Cook, 2001), and that fires on South African savannas had no apparent effect on the $\delta^{15}N$ values of plants and soils (Aranibar et al., 2003).

Southern Africa is characterized by strong climatic gradients, the co-dominance of C_3 and C_4 plants, the presence of large herbivores that migrate across the subcontinent, and the long historic presence of humans who have been practicing traditional pastoralism and crop agriculture for millennia (Cole, 1986; Scholes and Walker, 1993; Schulze et al., 1996). Stable isotopes are particularly useful in the region for studies of migration of elephants and other animals, atmospheric transport (i.e. to distinguish aerosols of anthropogenic, biogenic or wildfire origins), anthropology, and ecosystem carbon and nutrient cycles (Aranibar et al., 2004; Billmark et al., 2005; Bird et al., 2004; Midgley et al., 2004; Swap et al., 1996). Slash and burn agriculture, known as Chitemene agriculture, is commonly practiced in the more humid areas of the Kalahari sands (Chidumayo, 1997; Stromgaard, 1984). In the drier Kalahari areas, dominated by cattle husbandry, overgrazing by cattle results in an invasion by woody species that cannot support significant numbers of domestic or native grazers. This process, known as bush encroachment, is observed within 2 km of villages and water-extracting boreholes (Ringrose et al., 1996). Nitrogen fixing soil crusts decrease in biomass, cover and diversity in overgrazed areas (Shushu, 2000). Urea and dung deposition directly elevate soil inorganic nitrogen levels in the proximity of boreholes and cattle pens (Seagle et al., 1992; Tolsma et al., 1987; Turner, 1998), thereby increasing atmospheric losses through volatilization or denitrification. These processes are expected to increase $\delta^{15}N$ values of the ecosystem through preferential loss of ^{14}N.

The variations of $\delta^{15}N$ in ecosystems can potentially be used to constrain estimates of the geographic origin of plants, animals, and atmospheric aerosols, in forensic, ecological, anthropologic and atmospheric transport studies. In order to use $\delta^{15}N$ values for these and other applications, it is necessary to obtain a description of the spatial and temporal isotope variability of ecosystem components, including different plant types. This study presents the variations of $\delta^{15}N$ of southern African soils and plants associated with traditional pastoralism and crop agriculture, and the differences between the $\delta^{15}N$ of C_3 and C_4 plants from the same sites. Additionally, gross mineralization and nitrification rates are presented as they are the main processes that control N availability in the soil. Disturbed and undisturbed study sites were selected in the same precipitation regimes along a precipitation gradient in the Kalahari sands known as the Kalahari transect (Shugart et al., 2004). Observations at these sites were made to achieve the objective of analyzing the effect of human activities on the $\delta^{15}N$ values of ecosystems. An enrichment of ^{15}N in areas of cultivation and grazing was expected, because these activities tend to fractionate the nitrogen within the system thus, favoring exports of ^{14}N in the harvested or consumed plants. Fires, urine, and dung deposition by herbivores in higher intensity grazing lands, and the physical disturbance of soil layers by herbivores, also enhance atmospheric losses of ^{14}N. This study, along with previous isotope investigations in the region that reported isotopic values of plants, soils (Aranibar et al., 2004; Bird et al., 2004; Swap et al., 2004), and aerosols (Billmark et al., 2005; Swap et al., 1996) of relatively undisturbed sites, contributes to a sub continental characterization of

ecosystem isotopic signatures, which will be useful for studies on animal migration, paleoclimates, atmospheric transport, and other applications of stable isotopes.

2. Methods

2.1. Study sites

Four sites, located along a mean annual precipitation (MAP) gradient, were selected as part of the SAFARI2000-Kalahari Transect wet season campaigns (Otter et al., 2002): Tshane (365 mm MAP), Okwa (407 mm MAP), Maun (460 mm MAP), and Mongu (879 mm MAP). These sites were chosen to be representative of a large area, relatively undisturbed, and located in a uniform soil substrate, the Kalahari sands. The vegetation, ecophysiology and isotope composition of these sites have been described in previous studies (Aranibar et al., 2004; Feral et al., 2003; Midgley et al., 2004; Otter et al., 2002; Scanlon, 2004; Shugart et al., 2004; Swap et al., 2004). For this study, additional sites were selected that had different intensities of land use (grazing and previous cultivation) but have similar long term MAP as one of the main research sites of the Kalahari Transect (Table 1). These sites were assigned values of human land use intensity (LUI) relative to the main research sites of the Kalahari transect at the same MAP, to describe the effects of land use on δ^{15}N for each of the four precipitation regimes. The site least affected by human land use at each level of MAP was given an LUI value of 1, and the other sites were given increasing LUI values relative to the least affected site of the same MAP (Table 1).

A brief description of the land use gradients at each precipitation regime follows:

2.1.1. Tshane and Tshane village (365 mm MAP)

The main research site, Tshane (LUI = 1), is located at approximately 10 km from the village of Tshane. The vegetation was mixed woodland savanna, with *Acacia leuderitzii* as the most predominant species. The canopy cover was 13.8%, and the tree height of the tallest trees was 7 m (average height of the tallest 10% of the trees; Scholes et al., 2002). The site that represents a higher LUI, "Tshane Village" (LUI = 2), is located at a distance of approximately 500 m from the village of Tshane. This site was heavily grazed, mainly by goats. The trees were considerably taller than at the site with lower LUI, "Tshane", but the grass cover was nearly absent. Several *Acacia* species (*A. erioloba* and *A. leuderitzii*, among others), *Dichrostachys cinerea* and *Ziziphus mucronata* comprised the tree layer, and *Grewia flava* and *A. hebeclada* formed a thick shrub layer, characteristic of "bush encroached" areas. Grasses were only present under shrubs, where animals could not reach them.

Table 1
Sampling sites characteristics and number of samples analyzed at each site

Site	MAP (mm)	Land use type (–)	LUI (–)	Number of soil samples (–)	Depth of soil samples (cm)	Number of C_3 plant samples (–)	Number of C_4 plant samples (–)	Gross mineralization and nitrification rates (–)
Tshane	365	None	1	9	0–5	14	9	Yes
Tshane village	365	Domestic grazing	2	8	0–5	12	0	Yes
Okwa	407	None	1	12	0–5	14	3	Yes
Cattle station	407	Domestic grazing	2–5	7	0–5	14	14	No
Wildlife	460	Wildlife grazing	1	9	0–5, 0–20 and 0–40	4	4	Yes
Maun	460	Domestic grazing	2	4	0–10	10	8	Yes
Maun village	460	Domestic grazing	3	13	0–5, 0–15 and 0–35	5	6	No
Mongu	879	None	1	6	0–5'	16	0	Yes
Old Field	879	Fallow field after cultivation	2	9	0–5	17	0	Yes

MAP is mean annual precipitation; LUI is human land use intensity of the site relative to the main research site of equal MAP.

2.1.2. Okwa and grazed sites near a cattle station (407 mm MAP)

The vegetation of the main research site with lower LUI, Okwa (LUI = 1), was *Acacia* shrubland, with 32% canopy cover and a tree height (of the 10% tallest trees) of 2.3 m (Scholes et al., 2002). Approximately 20 km south of the main research site, a location near a cattle station was selected. This location supported its own population of cattle and also received additional cattle from other boreholes. Vegetation at the cattle station was marked by the strong presence of *Acacia* spp. (*A. mellifera*, *A. leuderitzii*, *A. erioloba*, *A. fleckii*, *A. hebeclada*), as well as the unpalatable annuals *Crotalaria sphaerocarpa* and *Tribulus terrestris*. With the intention to observe a gradient in grazing pressure, sampling was done at distances of 100 m, 1 km, 2 km, and 3 km (LUI = 5, 4, 3, and 2, respectively) from the cattle pens. However, the last of these locations was approximately a kilometer of the road, it was used by cattle from other boreholes, and showed signs of intense grazing, possibly even higher than the site located only 2 km of the pens (Feral et al., 2003).

2.1.3. Maun, Wildlife, and Maun village (460 mm MAP)

The vegetation of the main research site, Maun (LUI = 2), was mopane woodland, with 36% canopy cover and a mean height of the tallest 10% of trees of 7 m (Scholes et al., 2002). Two additional sites were sampled close to the main research site. The "Wildlife" site (LUI = 1) had a lower intensity of domestic grazing, but a higher intensity of wildlife grazing than the Maun location. This site is located approximately 26 km from the town of Maun, inside the Northern Botswana Veterinary Fence system, where domestic cattle were not present. Wildlife feeding and other damage (uprooted trees and excavation by elephants) affected the vegetation. This location was dominated by *Colophospermum mopane* (also known as *Hardwickia mopane*) trees of similar height as in the Maun main research site.

The other site of this land use gradient, "Maun village" (LUI = 3) was chosen approximately 1 km off the main road and represents land under a more intense use regime. This site was also near several homesteads and hosted a presently unused cattle pen, indicating a likely history of heavy grazing. At present, a number of donkeys wander largely unattended. Their abundance, grazing habits, and impact on the vegetation are unknown. The proximity of the site to a road and nearby houses makes it vulnerable for the collection of fuelwood and building materials. The field is largely depleted of mopane trees and had a dense coverage of *Pechuel-loeschea leubnitzae*, a strongly aromatic, invasive perennial of the Compositae family (Feral et al., 2003).

2.1.4. Mongu and Old Field (879 mm MAP)

The vegetation of these sites was typical Miombo woodlands, with *Brachistegia spiciformis* as the dominant tree. The canopy cover of the main research site, "Mongu" (LUI = 1), was 65% and the mean height of the tallest 10% of the trees was over 10 m (Scholes et al., 2002). An additional site, "Old Field" (LUI = 2), was selected north of the main research site. This site was long-abandoned, fallow and more open than the Mongu main research site, and represents one of many areas around the Mongu research site that have been substantially disturbed by deforestation and clearing of the land for agriculture. Given the generally low fertility of Kalahari sand soils, few of the fields are likely to be permanently cultivated. Most are abandoned after a few years and are rapidly re-invaded by shrubs such as *Baphia massaiensis* and *Bauhinia petersiana* (Frost, 2000).

2.2. Sampling methods and chemical analysis

The sampling strategy was designed to obtain information about N cycling processes along precipitation and land use gradients at a single time over a large region, eliminating the effects of seasonality on the N cycle. For this reason, only 3–4 days of the 2000 wet season were spent at each location along the precipitation gradient (Mongu, Maun, Okwa, and Tshane). Samples were collected and processed at each land use gradient during those days, although with some logistical problems. Not all the chemical analyses could be accomplished at all of the sites, and the number of samples obtained varied for different sites (Table 1). Both young and mature plants with C_3 (trees, shrubs, and forbs) and C_4 (grasses) photosynthetic metabolic pathway were randomly sampled at each site, selecting several leaves (10–15, depending on the leaf size) at a similar height in the canopy for individuals of the same functional group (trees, shrubs, forbs and grasses).

From the C_3 plants, only the non-N_2 fixing species (as described in Aranibar et al., 2004) were selected for this study. All the leaves from the same individual were combined into one sample, and initially air-dried in the field, followed by oven drying at 60 °C until constant weight. The leaves were then ground in a Willey mill to pass a 40-mesh screen (425 μm). Surface soils (0–5 to 0–40 cm) were randomly collected at all the sites, and the obvious plant debris was removed from the soils. The samples were then dried in a fashion similar to the leaves, followed by sieving (2 mm). The soils were then treated with HCl (30%) to remove carbonates. The soils and leaves were analyzed for $\delta^{15}N$ using an Optima isotope ratio mass spectrometer (GV Micromass, Manchester, UK) coupled to an elemental analyzer, with an overall precision better than 0.3‰. The isotopic data are reported relative to a standard, and expressed in the δ notation as:

$$\delta^{15}N_{samples}(‰) = (R_{sample}/R_{standard} - 1) \times 1000,$$

where $\delta^{15}N_{sample}$ represents the natural abundance of ^{15}N in the sample relative to a standard, and R is the molar ratio of the heavier to the lighter isotope for the element in either the standard or sample. The standard, defined to be 0.0‰, is atmospheric N_2 (Hoefs, 1997). All the isotope data are available online (http://daac.ornl.gov) from the Oak Ridge National Laboratory Distributed Active Archive Center (Aranibar and Macko, 2005).

The $\delta^{15}N$ of nitrate (NO_3^-) and ammonium (NH_4^+) were analyzed according to the procedure of Velinsky et al. (1989) for composite ($n = 6$ for each site) soils from Mongu, Maun, Okwa and Tshane. The soils were extracted in the field with KCl (2 M). A KCl solution (150 ml) was added to the soil (50 g), which was then shaken, allowed to settle and filter-sterilized through a 0.45 μm Gelman Supor filter. The extracts were refrigerated at 5 °C during the field campaign and frozen on return to the laboratory until analysis. A Labconco Rapid Kjeldahl System (RapidStill II) was used for steam distillation of ammonia (NH_3). The KCl extracts were treated with NaOH (5 N) elevating the pH to greater than 10 and converting all the NH_4^+ to the NH_3 form, which was then steam distilled and trapped with an HCl (0.03 N) acid trap. The NH_4^+ was bound by absorption onto a zeolite molecular sieve (dried at 160 °C for 1 h) at the optimum binding pH of 5.5–6. The pH was continuously monitored and maintained at the pH of 5.5–6 through addition of either HCl (0.03 N) or Na_2CO_3 (0.05 N). The zeolite molecular sieve was vacuum-filtered, and the remaining solution was treated with Devarda's alloy (0.75 g for 100 μM of N) to reduce the NO_3^- to NH_4^+, which was distilled as described above. Two zeolite bindings were completed for each, NH_4^+ and NO_3^- analysis, which were then vacuum filtered onto ashed (550 °C for 1 h) glass fiber filters, oven dried at 60 °C overnight and analyzed for $\delta^{15}N$ with an Optima isotope ratio mass spectrometer. Standard solutions of NH_4Cl ($n = 4$) and KNO_3 ($n = 5$) were prepared in the range of concentrations observed in the samples (from 2.5 to 40 μM for NH_4^+ and from 7.5 to 200 μM for NO_3^-) and were analyzed. The standard deviations of the standards' $\delta^{15}N$ were 1.8‰ for ammonium and 1.5‰ for nitrate.

Gross mineralization and nitrification rates were evaluated using the ^{15}N–NH_4^+ and ^{15}N–NO_3^- pool dilution techniques, respectively (Anderson and Poth, 1998; Davidson et al., 1991), for the Mongu, Old Field Mongu, Maun, Maun Wildlife, Okwa, Tshane, and Tshane Village sites. Both, mineralization and nitrification rates were determined under and between woody plants at all of the sites except Mongu, where the tree cover was high and without the heterogeneity of the other sites. The top 5 cm of soil was sampled ($n = 6$, for each soil type and for soils under and between canopies), combined, with the coarse roots being removed. The composite samples were each weighed into Whirlpak bags (20 g of soil each). Four ml of ^{15}N–NH_4^+ (0.4 mM and 30 ^{15}N atom %) were added to six bags for determination of gross N mineralization, and four ml of ^{15}N–NO_3^- (0.5 mM and 30 ^{15}N atom %) were added to six bags for measurement of nitrification. The amount of solution added was meant to wet the soils to the previously determined field capacity of Kalahari sand soils. Three replicates of the bags were incubated under about 5 cm of soil for 48 h. Three additional bags were kept for gravimetric determination of soil moisture by oven drying at 60 °C. At the end of the incubations, 60 ml of a 2 M KCl solution were added to each of the bags, shaken, allowed to settle and filtered through a 0.45 μm Gelman Supor filters into Whirlpak bags. The KCl extracts were refrigerated in the field at <5 °C, and frozen on return to the laboratory until analysis for nutrient levels and isotope content was accomplished. Ammonium was determined colorimetrically by an automated indophenol method, and $NO_3^- + NO_2^-$ by copperized cadmium reduction in combination with diazotization. All were analyzed using an Alpkem "Flow Solution" autoanalyzer, equipped with a model 510 spectrophotometer. The soil extracts (50 ml, containing

approximately 30 μg of N) were placed in sterile, disposable plastic cups and MgO was added. Ammonium was trapped by diffusion onto $KHSO_4$-soaked filters for a 6-day period, as described by Brooks et al. (1989). The levels of atom % ^{15}N were determined in the isotope laboratory at the University of California, Davis, using a system similar to previously described for the natural abundance levels.

Samples for analysis of atom % ^{15}N in NO_3^- (nitrification study) were prepared by a two-step procedure. First, MgO was added to KCl extracts in sterile, disposable plastic cups. The cups were left open for 48 h to allow any NH_4^+ present to diffuse out of the sample. The NO_3^- present in the sample was then converted to NH_4^+ by addition of Devarda's alloy. The produced NH_4^+ was trapped and analyzed by mass spectrometry as described above. Gross nitrogen mineralization and nitrification rates were determined from measured changes in atom % ^{15}N and concentrations of NH_4^+ and NO_3^- using the model of Wessel and Tietema (1992). The gross rates are reported as mg of N (either ammonium or nitrate) produced $m^{-2}\,day^{-1}$ from the surface to 5 cm depth.

Standard errors of the N mineralization and nitrification rates were estimated by propagation of errors (Hagiwara et al., 2002), calculated with Mathematica 4.2 (Wolfram Research, Inc.). Relationships between plant and soil $\delta^{15}N$ were analyzed by linear regressions, using the coefficient of determination (r^2). Student's *t*-tests were used to test significance of the differences between the two groups (soil or foliar $\delta^{15}N$ of the C_3 and C_4 plants from sites with different land use intensities). Results were considered significant at $p \leqslant 0.05$.

3. Results

Overall, the average $\delta^{15}N$ values of soils and C_3 plants in areas with lower LUI were lower than those of areas subjected to cultivation and domestic grazing, except on the most arid site, Tshane (365 mm MAP), where the response was the opposite (Figs. 1 and 2). The differences between sites with different LUI at the same precipitation regime were significant in plants from Okwa and Maun, and in soils from Tshane, Maun and Mongu.

The highest variation of gross mineralization and nitrification rates estimated at field capacity with isotope dilution of ^{15}N marked products (ammonium and nitrate) was associated with the location of the soils under or between tree/shrub canopies (Fig. 3). Mineralization and nitrification rates were both higher under tree canopies than between canopies, except in the Maun Wildlife site (410 mm MAP and LUI = 1), where mineralization rates were similar. Land use type or intensity had no apparent or uniform effect on microbial

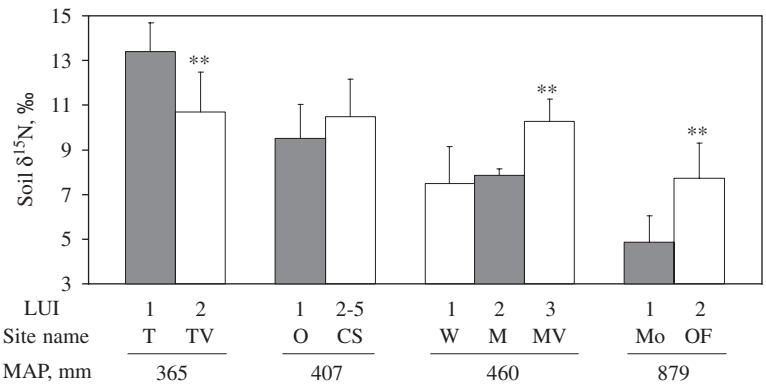

Fig. 1. Average soil $\delta^{15}N$ along four land-use gradients, located along a precipitation gradient. Filled, gray bars indicate the relatively undisturbed main research sites of the precipitation gradient selected for the SAFARI 2000 wet season campaign (data from Aranibar et al., 2004). White bars indicate additional sites with different land use intensities than the main research sites. T = Tshane, TV = Tshane Village, O = Okwa, CS = Cattle Station, W = Wildlife, M = Maun, MV = Maun Village, Mo = Mongu, and OF = Old Field. MAP is mean annual precipitation. LUI is the land use intensity with relative units, used to compare sites of a given MAP. The lowest human land use intensity for a MAP is represented with a LUI = 1, and higher LUI values represent higher intensities at the same MAP. Asterisks denote significant differences between the asterisk-marked site and the main research site of that MAP. For example, at 460 mm MAP (Maun), the site with higher LUI has a significantly higher soil $\delta^{15}N$ than the main research site (LUI = 2). For ** $p < 0.01$. Bars indicate standard deviations of the mean.

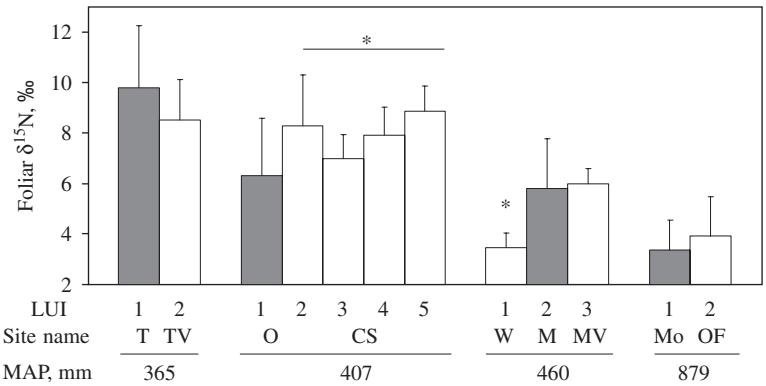

Fig. 2. Average foliar δ^{15}N of C_3 plants along four land-use gradients, located along a precipitation gradient. Filled, gray bars indicate the relatively undisturbed main research sites of the precipitation gradient selected for the SAFARI 2000 wet season campaign (data from Aranibar et al., 2004). White bars indicate additional sites with different land use intensities than the main research sites. T = Tshane, TV = Tshane Village, O = Okwa, CS = Cattle Station, W = Wildlife, M = Maun, MV = Maun Village, Mo = Mongu, and OF = Old Field. MAP is mean annual precipitation. LUI is the land use intensity with relative units, used to compare sites of a given MAP. The lowest human land use intensity for a MAP is represented with a LUI = 1, and higher LUI values represent higher intensities at the same MAP. Asterisks denote significant differences between the asterisk-marked site and the main research site of that MAP. Asterisks denote significant differences between the asterisk-marked site and the main research site of that MAP. For example, at 460 mm MAP (Maun), the site with lowest LUI (Wildlife) has a significantly lower foliar δ^{15}N than the main research site (LUI = 2). For * $p<0.05$. Bars indicate standard deviations of the mean.

activity. In Tshane (364 mm MAP), both mineralization and nitrification rates decreased with domestic cattle grazing, whereas at Mongu (879 mm MAP) nitrification was higher in the Old Field than in the site with lower LUI. In the other sites near Maun (410 mm MAP) the response was not uniform for soils located under or between tree canopies. Along the precipitation gradient, nitrification was higher than mineralization in all the sites except in Mongu, the site with highest MAP and tree cover (Fig. 3).

The C_3 plants had significantly higher δ^{15}N than C_4 plants from the same location in four of six sites analyzed (Fig. 4). The δ^{15}N values of soils and C_3 plants from the same sites were highly correlated ($r^2 = 0.86$), but soils and C_4 plants did not show a significant correlation (Fig. 5). The δ^{15}N values of nitrate were lower than those of both ammonium and bulk N at all the sites analyzed (Table 2).

4. Discussion

Land use intensity and MAP did not have a uniform effect on mineralization and nitrification rates (Fig. 3), probably because there are different processes that can enhance or inhibit microbial activity in overgrazed areas. For example, urea deposition by cattle can enhance N mineralization, but cattle grazing can compact the soil and decrease microbial activity. Mineralization and nitrification rates were generally higher under tree canopies than between them (Fig. 3). This pattern was expected because areas beneath tree canopies displayed a greater concentration of litterfall and mineral N concentration (Feral et al., 2003), and cooler temperatures in the shade of trees can encourage microbial activity (Belsky, 1994). Extremely high temperatures may be experienced outside tree canopies at these sites during the summer, and may inhibit N-mineralization and nitrification.

In general, there was an increase in δ^{15}N of soils and C_3 plants in locations with increasing intensity of land use, except in the most arid sites analyzed, Tshane and Tshane village (Figs. 1 and 2). These increases in isotope abundances may result from the effects of human activities such as domestic grazing and cultivation on the following processes of the N cycle: removal of ^{15}N depleted plant material by herbivore consumption and harvesting of crops, enhanced ammonia volatilization by the addition of urine in areas with high cattle density (Kreitler, 1975, 1979; Kreitler and Jones, 1975), destruction of N_2 fixing biological soil crusts and forbs by movement of cattle. Denitrification also fractionates against ^{15}N, but is thought to be a less significant process in the arid sandy soils of the Kalahari. However, the most arid sites of the Kalahari received the

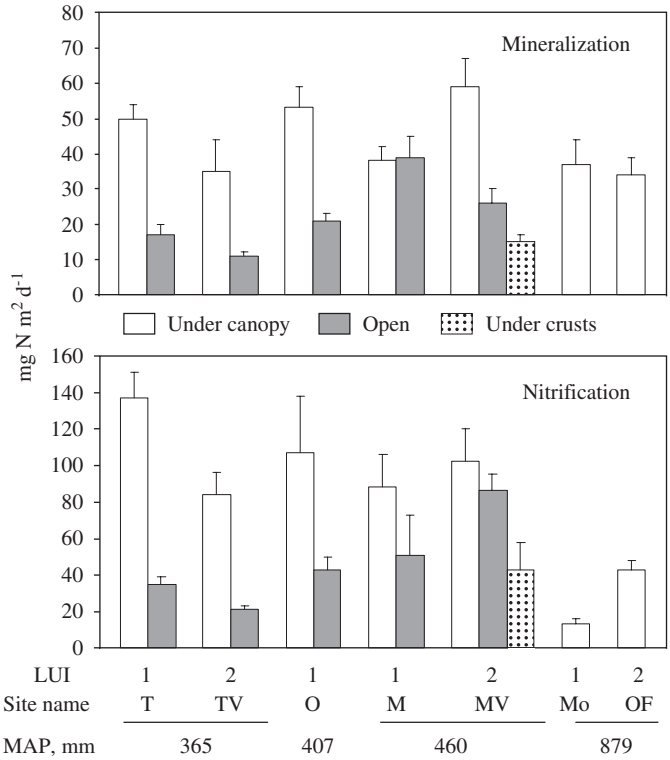

Fig. 3. Gross mineralization and nitrification rates along a precipitation gradient and land use gradients. White bars indicate rates in soils located under tree canopies. Filled, solid bars indicate soils located between tree canopies. Dots indicate soils located under biological crusts. T = Tshane, TV = Tshane Village, O = Okwa, M = Maun, MV = Maun Village, Mo = Mongu, and OF = Old Field. MAP is mean annual precipitation. LUI is the land use intensity with relative units, used to compare sites of a given MAP. 1 reflects the lowest human land use intensity for that MAP, and higher represent higher intensities. Bars denote standard errors of the mean.

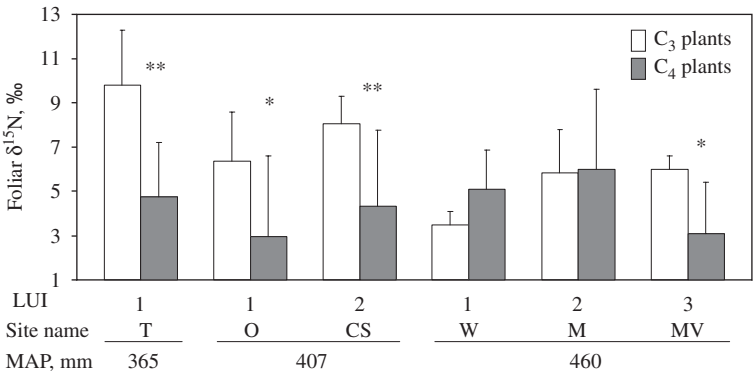

Fig. 4. Mean foliar δ^{15}N of C_3 (white bars) and C_4 (gray bars) plants at sites with different mean annual precipitation (MAP) and land use intensity (LUI). T = Tshane, O = Okwa, CS = Cattle Station, W = Wildlife, M = Maun, MV = Maun Village. Asterisks denote significant differences between the two plant types at a given site at $p<0.01$ (**) and $p<0.05$ (*).

highest amounts of precipitation recorded in decades during the year of this study (2000) (Shugart et al., 2004). This anomalous weather could have increased soil moisture and anaerobic conditions favorable for denitrification, contributing to the overall ^{15}N enrichment with increased MAP. The higher nitrification rates in the Old Field than in the Mongu site (879 mm MAP) (Fig. 3) increased nitrate availability, and could have enhanced denitrification, contributing with the higher soil δ^{15}N values of the Old Field.

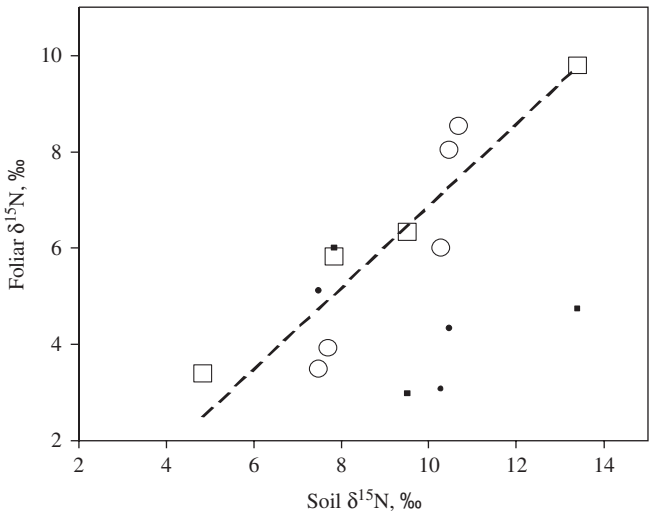

Fig. 5. Relationship between foliar and soil $\delta^{15}N$ for C_3 (open, big symbols) and C_4 (closed, small symbols) plants. Squares indicate average values of the main research sites, and circles indicate average values of sites with different land use intensities. For C_3 plants, $r^2 = 0.89$, and for C_4 plants $r^2 = 0.09$.

Table 2
$\delta^{15}N$ of bulk N (from Aranibar et al., 2004), ammonium and nitrate in Kalahari soils

Site and MAP (mm)	Soil bulk $\delta^{15}N$ (‰)	Soil ammonium $\delta^{15}N$ (‰)	Soil nitrate $\delta^{15}N$ (‰)
Tshane (365 mm)	13.4	12.3	5.2
Okwa (407 mm)	9.5	−0.3	−3.4
Maun (460 mm)	7.8	5.5	4.3
Mongu (879 mm)	4.8	5.8	1.4

The most arid land use gradient, Tshane and Tshane village (365 mm MAP), presented a response of ^{15}N to cattle grazing intensity different from the other land use gradients. The $\delta^{15}N$ values of soils and C_3 plants were higher in the site with lower LUI, which is contrary to the response expected from physical and microbial processes. This indicates the existence of other processes outside of those expected to enrich the system in ^{15}N that cause the ^{15}N enrichment of the site without overgrazing.

The C_4 plants (grasses) of this study had significantly lower $\delta^{15}N$ values than C_3 plants (trees, shrubs and forbs; Fig. 4) in four of the six sites analyzed, indicating a different N cycling for the two plant types. Grasses of other humid, semiarid and mesic sites also had lower $\delta^{15}N$ values than those of trees of the same locations (Cook, 2001; Gebauer and Schulze, 1991), and the $\delta^{15}N$ values of C_4 grasses showed a less intense response to aridity than those of C_3 plants (Swap et al., 2004). Several processes could explain the differences, including the uptake of N with different $\delta^{15}N$ values, or differences in the internal processes that metabolize N. Temporal or chemical partitioning of N sources have been observed in other ecosystems, and could contribute to the different ^{15}N values of trees and grasses observed in this and other studies (Fig. 4). Temporal partitioning in N uptake after moisture pulses has been observed in tundra and cold desert communities (Gebauer and Ehleringer, 2000; McKane et al., 2002). Species with symbiotic associations differ in their preferences for particular N sources (Eviner and Chapin, 1997). For example, the grass *Digitaria eriantha* prefers nitrate to ammonium (Wolfson and Cresswell, 1984), whereas several Australian mycorrhizae-associated trees may be able to better use amino acids and protein N sources (Stewart and Schmidt, 1999).

The isotopic analysis of nitrate and ammonium present in Kalahari soils indicate that chemical partitioning could originate different $\delta^{15}N$ values in plants. Nitrates had lower $\delta^{15}N$ values than ammonium and bulk soil N (Table 2). This difference was more remarkable at the Tshane site, where ammonium was 7‰ more enriched

than nitrate (Table 2), which may have resulted from the highest nitrification rates found in this site (Fig. 3). If C_4 and C_3 plants absorb nitrate and ammonium, respectively, then the $\delta^{15}N$ values of C_4 plants would be lower than those of C_3 plants, as it was observed in most of the sites (Fig. 4). However, the high nitrification rates of the three more arid sites (Fig. 3) suggest that microorganisms use most of the ammonium for nitrification, and it is not widely available for plants to absorb. Absorption of organic N by C_3 plants could also explain the observed $\delta^{15}N$ values. It is not known whether these Kalahari plants have the ability to use organic N, but the capacity of roots to incorporate glycine (an organic form of N) is widespread in similar Australian ecosystems (tropical savanna woodlands) including genera common to the Kalahari (*Acacia*, *Eragrostis*, *Solanum*, and *Abutilon*) (Schmidt and Stewart, 1997, 1999; Stewart and Schmidt, 1999). The high correlation between the $\delta^{15}N$ values of soils and C_3 plants (Fig. 5) supports the hypothesis of organic N uptake. On the other hand, the lack of a correlation between the $\delta^{15}N$ values of soils and C_4 grasses supports the hypothesis of a different N source (i.e., nitrate), with a $\delta^{15}N$ independent from the effects of aridity and overgrazing.

If the hypothesis of different chemical forms of N absorbed by trees and grasses holds true for Kalahari ecosystems, then the biomass of each functional type would affect soil $\delta^{15}N$ values, by removing N with different ^{15}N abundances. The presence of grasses could contribute to the successive enrichment of the remaining soil N, by grass uptake of most of the available ^{15}N depleted nitrate. The absence of grasses would have the opposite effect, leaving more nitrate available in the soil for continuous recycling or leaching to the subsoil or groundwater. This could explain the ^{15}N enrichment found in the Tshane soils with lower LUI, which presented the highest $\delta^{15}N$ values (Fig. 1), and the highest grass cover (Feral et al., 2003) of all the sites. However, this hypothesis remains to be tested experimentally. A demonstration that two plant types can preferentially use different N sources could be accomplished with ^{15}N labeling experiments. These results have implications for the use of stable N isotopes in other disciplines. For example, aridity may be reflected more clearly on $\delta^{15}N$ values of animals feeding from trees (i.e., giraffes) than on those of animals eating predominantly grasses (i.e., antelopes), and animals eating both types of plants (i.e., elephants) would reflect diet changes in their N isotope abundances. Furthermore, the removal of grasses by land use activities could potentially alter the amount of nitrate leached to the subsoil or to the groundwater, affecting groundwater quality.

5. Conclusions

This study indicates that grazing by domestic animals and cultivation can modify the $\delta^{15}N$ values of soils and plants in southern Africa. $\delta^{15}N$ values of soils and plants generally increased in areas used for domestic cattle grazing or cultivation. This pattern agrees with the expected responses of physical and microbial processes to these human activities: enhanced ammonia volatilization, decreased soil crust N_2 fixation, and reduced litter inputs through export of plant material from the system. However, these processes do not explain the isotopic signatures found at the most arid site, Tshane, in which soils were more ^{15}N enriched in the site with lower LUI than in the overgrazed location. The $\delta^{15}N$ values of C_4 plants were significantly lower than those of C_3 plants at four of six sites, suggesting potentially different N use by the two plant types. Based on the results of this study, it is proposed that C_3 and C_4 plants affect soil $\delta^{15}N$ values by absorbing different chemical forms of N.

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