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REGULAR ARTICLE

Combined effects of soil moisture and nitrogen availability variations on grass productivity in African savannas

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Abstract Savannas cover about 20% of the Earth's land area and 50% of Africa. As an indispensable component of savanna, grasses play an important role in these ecosystems. A better understanding of grass productivity and its controlling factors in savanna ecosystems could therefore be a key to understand the functioning of savannas and predict savanna responses to future climatic changes. In this study, a stable isotope fertilization experiment was conducted to determine how factors limiting grass production in savannas differ across regional climate gradients. The study was conducted on the geomorphically homogenous Kalahari Transect (KT), which offers an ideal setting to study nutrient and vegetation dynamics independently of confounding soil effects. The results show that the grasses assimilated the added fertilizer at all the sites but they did not respond to nitrogen fertilization for both dry and wet years, and at both dry and wet ends of the Transect. Although prior studies have proposed a switch between water and nitrogen limitations between arid and mesic savannas, our results suggest that nitrogen availability may not limit grass productivity across the whole KT. Thus, although the traditional classifications as nutrient poor (broad-leaf) and nutrient rich (fine-leaf) savanna ecosystems may still be useful, it does not necessarily imply the existence of nitrogen limitation in the nutrient poor area; in fact, it is more likely that the herbaceous species found in the more humid sites (nutrient poor sites) are already adapted to lower nitrogen availability.

Keywords Arid · Botswana · Fertilization · Kalahari Transect · ^{15}N · Savanna · Semi-arid · Stable isotopes · Tracer · Water · Zambia

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Introduction

Savannas cover about 20% of the Earth's land area (Scholes and Walker 1993) and can be found in large areas of Africa, Australia, North and South America, across a wide range of climatic conditions. As an important and distinct biome, savannas produce

approximately 29% of global terrestrial net primary productivity (Grace et al. 2006). They are currently considered a carbon (C) neutral system (Veenendaal et al. 2004; Williams et al. 2007), although savannas have the potential to become either large sinks or sources of C, depending on changes in climate, disturbance regime, land management, and the timescales under consideration (Williams et al. 2007; Thomas et al. 2008; Wang et al. 2009a). To understand the response of savannas to changes in these "external" drivers it is therefore essential to understand the factors controlling their productivity. Water availability, disturbance (e.g., fire, grazing) and nutrient availability are considered to be the three major factors determining the structure and function of savanna ecosystems (Walker et al. 1981; Sarmiento 1984; Scholes and Walker 1993; Scholes and Archer 1997; Sankaran et al. 2004; Okin et al. 2008; Wang et al. 2009b), but it remains unclear how the relative importance of water and nutrient limitations, especially that of nitrogen (N), varies with the mean climatic conditions (Sankaran et al. 2005).

Most attempts at regional synthesis of savanna structure and function are typically conducted at single sites or without experimental manipulation (Scholes and Walker 1993; Toit et al. 2003; Sankaran et al. 2005; Scanlon et al. 2007; Okin et al. 2008; Wang et al. 2009b). In addition, many studies have focused exclusively on the distribution and abundance of woody vegetation. However, the critical role that C_4 grass expansion played in governing the origin of the savanna biome during the Miocene (Beerling and Osborne 2006), the significant grass contributions to soil carbon at drier sites (Wang et al. 2009a) and the important role grass played in soil nutrient heterogeneity in arid environments (Li et al. 2009) highlight the importance of understanding large-scale controls on grass productivity when attempting to predict the responses of savannas to future climatic changes. By analyzing the results of over 50 fertilization experiments in arid and semi-arid grassland ecosystems across continents, Hooper and Johnson (1999) tested the hypothesis that N limitation increases with rising soil water content in dryland ecosystems. It was concluded that water and N co-limit the aboveground net primary productivity (ANPP) along regional rainfall gradients. Building on this result and other evidences, Epstein et al. (2006) further proposed that N limitation does not vary along precipitation gradients and that other environmental factors (e.g., light) may constrain grass productivity in sub-humid grassland ecosystems. In the case of savanna ecosystems, there is experimental evidence (Ludwig et al. 2001) that N may limit grass productivity in a dry savanna during wet years.

In the present study, the Kalahari Transect (KT) in southern Africa is used as a representative ecosystem to investigate the interactions between the water and nutrient limitations on the productivity of grass vegetation in savannas. The KT is one of a set of IGBP (International Geosphere-Biosphere Programme) "megatransects" (Koch et al. 1995; Scholes et al. 2002) identified for global change studies. The KT traverses a dramatic aridity gradient on relatively homogenous soils (deep Kalahari sands), offering an ideal setting to study nutrient and vegetation dynamics without confounding soil effects (Wang et al. 2007a). Modeling results based on remote sensing data (Scanlon and Albertson 2003) and leaf-level physiological data (Midgley et al. 2004) both suggest the existence of two distinct regimes of vegetation productivity-rainfall relationships across the KT. In the northern, wetter portion of the Transect, a large fraction of the soil water content is lost in sandy soils as leakage through the bottom of the root zone. Because water losses are associated with leaching outputs of N, it has been argued that the productivity of these mesic and sub-humid savannas may be limited by nutrient availability (D'Odorico et al. 2003). In contrast, productivity in the southern, drier portion of the Transect is hypothesized to be limited by precipitation, which is sufficiently low to cause limited soil water availability (Scanlon and Albertson 2003).

In this study, a stable isotope fertilization experiment was conducted at selected sites along a regional-scale transect to directly examine the existence of a switching point from water-limited to N-limited conditions that has been hypothesized to exist with increasing mean annual precipitation (MAP) along the Kalahari rainfall gradient.

Materials and methods

Study sites

The field experiment was conducted at four sites (Tshane, Ghanzi, Pandamatenga and Mongu) along

the KT rainfall gradient (Fig. 1a). The detailed site descriptions including soil physical (e.g., soil texture, soil color) and chemical characteristics (e.g., soil C/N, soil mineral N) can be found in Wang et al. (2007a). Here only the major site characteristics are summarized. The northernmost site was situated in Mongu, Zambia, with a MAP around 879 mm. Vegetation in Mongu is woodland savanna dominated by the tree species *Brachystegia spiciformis* Benth and the common observed grass species is *Eragrostis spp*. (unidentified, Appendix Fig. 7). The other three sites were situated in Botswana at Tshane (southernmost site), Ghanzi and Pandamatenga (Fig. 1a). The MAP in these three areas ranges from 365 mm to 700 mm, respectively. The Tshane and Ghanzi sites are classified as open savannas dominated by *Acacia* species such as *Acacia luederizii* Engl. and *Acacia mellifera* Benth., and grass species, such as *Eragrostis lehmanniana* and *Schmidtia pappophoroides*. The Pandamatenga site is classified as woodland savanna dominated by tree species (e.g., *Kirkia Africana*). The common observed grass species are *Panicum maximum*, *Schmidtia pappophoroides* and *Pogonarihria squarrosa*.

Fertilization experimental setup

The experiment consisted of a randomized block design with four 21 m×13 m plots at each of the four sites along the KT. Each plot was divided into four 10 m×6 m subplots with 1 m buffer zone between each subplot (Fig. 1b). Owing to the logistical issues with the operation of field sites at these remote locations, it was not feasible to construct fences around the plots and all the plots were open to potential grazing activity. The presence of cows was observed in the Tshane area, while the other three sites were subject to wildlife grazing. There is evidence of high levels of grazing pressure by cattle in Tshane area (Moleele and Mainah 2003). Because we aimed to capture the heterogeneous nature of the savanna ecosystems, not all the blocks at each site were under the exactly same conditions (e.g., contained the same number and same species of trees). But we tried to make conditions of all the subplots within each block similar at each study site. In August 2004, four treatments (N addition, P addition, N + P addition and control) were randomly (e.g., selection of which subplot got which treatment was random) applied to the subplots using a backpack electric sprayer. For the N and N + P treatments, 783 kg/ha of $Ca(NO_3)_2$ (133 kg N/ha) was evenly applied to each subplot, and 292 kg/ha of $Ca(H_2PO_4)_2$ (33 kg P/ha) was applied to the P and N + P subplots, whereas the control subplots were supplied with water only. These application concentrations and forms of fertilizer were chosen on the basis of data reported by Ludwig et al. (2001) for fertilization experiments in a Tanzanian dry savanna. For each treatment plot (60 m^2), the fertilizers were dissolved into 10 L water. The fertilizer solution was applied to the soil surface and was observed to infiltrate into the soil with only a negligible interception by the sparse canopy of grass leaves. The N and N + P additions were enriched with ^{15}N ($Ca(^{15}NO_3)_2$) to an isotope signature of 10.3 ‰ (hereafter referred to as the "first year" treatment). The added ^{15}N tracer was used to assess whether the target plants were able to assimilate any of the fertilizer. Such tracer is critical for many sandy soils because of their high nutrient leaching potentials and it is also important in this particular experiment since fertilizer was applied three months before the starting of the growing season. The nitrate fertilizer form ($Ca(NO_3)_2$) was used also to facilitate the interpretation of isotope data later on since ammonium will go through nitrification and induce fractionation. In August 2005, the inner 3× 5 m^2 portion of each subplot was re-fertilized using the same nutrient concentration, but different isotopic enrichment, in that the N and N + P additions were enriched with ^{15}N ($Ca(^{15}NO_3)_2$) to a signature of 100 ‰ (hereafter referred to as "second year" treatment), in order to increase the ability to discern N uptake patterns with a stronger label (Fig. 1b). Although this report concentrate on the effects of N availability on grass productivity along the KT aridity gradient, values of foliar N/P ratios were discussed in relation to their potential implications on the interactions between N and P limitation.

Soils and vegetation samples were collected from the sites for two wet seasons subsequent to the nutrient additions. Because forb species are obvious only in the driest site (Tshane) of the Transect, they were not included in our analysis. In February-March, 2005, five random locations in each treatment subplot were selected using randomly-generated coordinates. These random locations (includes both open canopy conditions and under canopy conditions, which should cancel out any canopy effects) were used for vegetation harvesting and soil sampling. Newly-formed (i.e.,

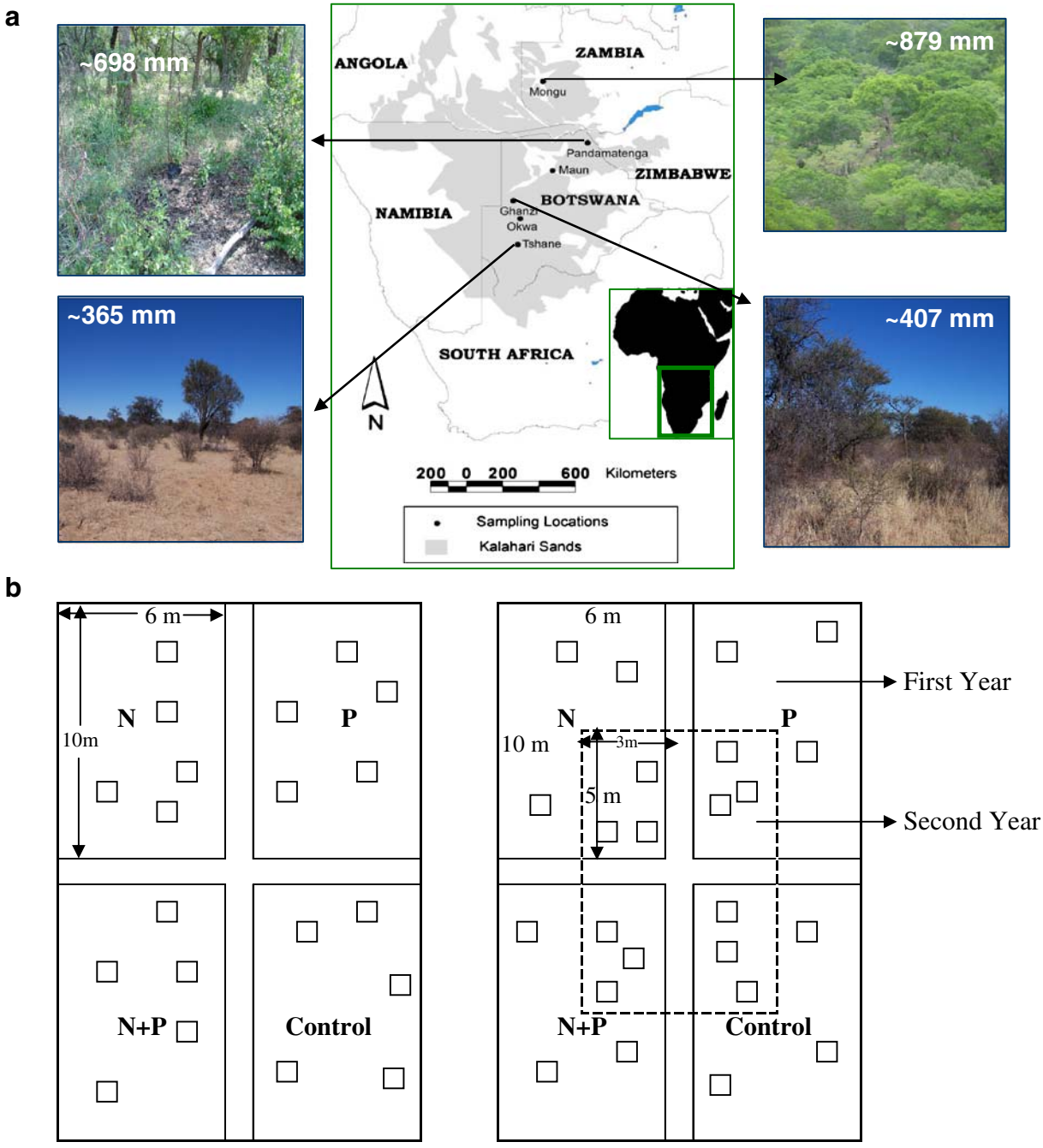

Fig. 1 Study sites along the Kalahari Transect and the habitat characteristics. The values in each photo are the mean annual rainfall for each site (**a**). **b** The experimental layout and sampling scheme in 2005 (*left*) and 2006 (*right*)

post-treatment green tissues) grass biomass was estimated by harvesting five 1×1 m^2 plots from selected locations for each treatment subplot at each site. Portions of each sample of grass biomass from each of the 1×1 m^2 plots were retained for nutrient and isotope analysis. In January–February 2006, grass biomass and subsamples for nutrient and isotope analysis were collected in a similar way as in the 2005 wet season, i.e., by harvesting three 1×1 m^2 plot from each treatment (for both first year and second

year treatment) at each site. In addition to vegetation samplings, three soil samples were taken at two different depths (0–5 cm and 30–35 cm) from each treatment subplot (including the first year and second year fertilizer additions) with a sand auger of 8 cm in diameter. The vegetation samples collected at Mongu in 2006 were lost in shipment and were unable to be evaluated for the grass biomass, nutrient content and isotope composition.

Soil respiration was measured in the fertilization plots in the 2005 and 2006 wet seasons using an EGM-4 CO_2 analyzer (PP Systems International, Inc. Amesbury, MA, USA) before the grass and soil sampling. To minimize the effect of differences (between sites) in soil water availability due to unexpected precipitation, one liter of water was poured into a soil collar (40 cm in length × 20 cm in width × 40 cm in height) (about 10 cm was inserted into the soil) and the soil respiration measurements were accomplished right after the complete infiltration of the added water ("wet" measurement). In the wet season of 2005, one measurement of soil respiration per treatment and per site was made in an open area, and another one under a tree canopy. Because no significant differences were detected between open-canopy and under-canopy soil respiration measurements made in 2005, these two microsites were not differentiated in 2006 with only three random locations being chosen for soil respiration measurements in each treatment subplot (including the first year and second year fertilizer additions).

Field rainfall data were not available for the 2005 and 2006 wet seasons at the KT sites considered in this study, due to instrument failure. Rainfall data (Fig. 5b, inset) were obtained from satellite measurements from Tropical Rainfall Measuring Mission (TRMM) of NASA. For this study the 3B43 monthly data from precipitation radar were used (e.g., Huffman et al. 2007). Although satellite rainfall estimation provides good indication of relative rainfall quantity across all the KT sites, the accuracy of the estimations is difficult to assess. Fortunately we also had field soil water content (volumetric water content) data collected from the 2004 wet season to the 2006 wet season, which more closely reflected the conditions of plant available water. The soil water content data were continuously monitored at two-hour intervals using DECAGON ECH_2O probes. The ECH_2O probes are known for being sensitive to soil texture and bulk density (Czarnomski et al. 2005) and may not provide an accurate estimate of the soil water content. However, the relatively uniform soil texture/structure found across the whole Transect (Wang et al. 2007a) allowed us to use the ECH_2O probe data in relative terms, i.e., to compare soil water content levels among sites. The soil water content data reported in this study were calculated as weighted averages along the soil profile (for each soil profile soil water content was monitored at the depths of 0.10 m, 0.30 m, 0.50 m, and 1.00 m). A mean seasonal value was reported for each of the two growing seasons (2005 and 2006). More details on the mean soil water content data for wet season 2005 and 2006 used in this paper can be found in D'Odorico et al. (2007).

Chemical analyses

Plant and soil samples for isotope and elemental analysis were oven-dried (at 60°C) in the laboratory. Soil samples were sieved (2 mm mesh size) and homogenized by mortar and pestle, and plant samples were homogenized by grinding to powder using an electric mill. Total organic C and N contents were measured using an Elemental Analyzer (EA, Carlo Erba, NA1500, Italy). Stable N isotope analyses were performed using a Micromass Optima Isotope Ratio Mass Spectrometer (IRMS) connected to the EA (GV/Micromass, Manchester, UK). The N stable isotope compositions are reported in the conventional form (‰):

$$\delta^{15}\mathrm{N}(‰) = \left[\left(^{15}\mathrm{N}/^{14}\mathrm{N}\right)_{\mathrm{sample}} \Big/ \left(^{15}\mathrm{N}/^{14}\mathrm{N}\right)_{\mathrm{standard}} - 1\right] \times 1000$$

where $(^{15}N/\ ^{14}N)_{sample}$ is the N isotope composition of a sample, and $(^{15}N/\ ^{14}N)_{standard}$ is the N isotope composition of the standard material. The standard material for stable N isotopes is atmospheric molecular N (N_2). Reproducibility of these measurements is typically better than 0.2‰ (Wang et al. 2007b).

Statistical analyses

Two-way ANOVA for randomized block design with site and treatment as two main factors were used to test differences among soil $\delta^{15}N$, plant $\delta^{15}N$, plant %N and grass biomass (SAS v. 9.1 PROC MIX). To

further explore the treatment effect at each particular site, subsequent one-way ANOVA within each site were then performed to test the differences between treatments for each response variable; to this end, mean separations were achieved by Tukey *post hoc* test at α=0.05.

Results

For the 2005 sampling (February–March) the foliar $\delta^{15}N$ signatures were significantly higher in the N and N + P addition treatments than in the control and P addition plots at all sites except for Pandamatenga (F=21.28, p<0.01 in two-way ANOVA, Table 1, Fig. 2a). No significant differences in foliar $\delta^{15}N$ signatures were detected between the control and P addition, nor between the N and N + P addition plots (p>0.05, Table 1, Fig. 2a). For the 2006 sampling (February–March), differences in foliar $\delta^{15}N$ signatures among the four treatments exhibited a pattern similar to the one in the 2005 sampling except for Pandamatenga, where foliar $\delta^{15}N$ signatures in N and N + P addition treatments were significantly higher than in the control treatment (F=50.47, p<0.01 in two-way ANOVA, Table 1, Fig. 2b). Based on mixing ratio calculations of isotope signatures with soil organic matter and fertilizer as two end-members (e.g., Wang et al. 2009a), the percentage of N uptake from the fertilizer (versus from original soil mineral N) in the wettest area (Mongu, 43%) was similar to the driest area (Tshane, 38%), and the highest N uptake percentage from the fertilizer was found at an intermediate precipitation regime (Ghanzi, 68%). This percentage showed a similar pattern for the 2006 sampling, although with much lower values (8%, 14% and 2% in Tshane, Ghanzi and Pandamatenga, respectively).

For the surface soils (0–5 cm), there were significant differences in $\delta^{15}N$ signatures between the four locations (F=66.51, p<0.01 and F=85.33, p<0.01 for First Year and Second Year of 2006 sampling respectively, Table 1), however, for the 2006 sampling no significant differences in soil $\delta^{15}N$ were found among the four treatments (N, N + P, P and control) at any of the four locations, either for the second year (p>0.05, Fig. 3a) or the first year treatments (p>0.05, Fig. 3b). There were similar patterns for soil in the deeper layer (30–35 cm) (p>0.05, data not shown). In addition, the interaction between location, treatment and soil depth was not statistically significant (p>0.05, data not shown).

There were significant differences in foliar %N among the four sites (F=51.52, p<0.01 and F=42.45, p<0.01 for First Year and Second Year of 2006 sampling respectively, Table 1), however, no significant differences were found among treatments in the wet seasons of 2005 or 2006 (p>0.05, Fig. 4). Both in 2005 and 2006 the foliar %N peaked at Ghanzi, a location with intermediate MAP between the two ends of the KT. The foliar N/P ratios ranged between 9 and 19 in 2005 and decreased between the control,

Table 1 The two-way ANOVA results (F-values) on various response variables with site and treatment as two main effects. First Year and Second Year refer to the outer and inner portion of each subplot and more details please refer to the Method section and Fig. 1b

	Site effect (S)	Treatment effect (T)	S*T
Foliar $\delta^{15}N$ (2005 Wet season)	155.90**	21.28**	4.82**
Foliar $\delta^{15}N$ (2006 Wet season First Year)	104.58**	3.55*	0.54
Foliar $\delta^{15}N$ (2006 Wet season Second Year)	159.20**	50.47**	10.94**
Foliar %N (2005 Wet season)	93.72**	1.86	1.79
Foliar %N (2006 Wet season First Year)	51.52**	1.31	1.04
Foliar %N (2006 Wet season Second Year)	42.45**	0.49	0.41
Soil $\delta^{15}N$ (0–5 cm, 2006 Wet season First Year)	66.51**	0.21	0.16
Soil $\delta^{15}N$ (0–5 cm, 2006 Wet season Second Year)	85.33**	2.45	4.12**
Vegetation biomass (2005 Wet season)	55.07**	1.21	0.30
Vegetation biomass (2006 Wet season First Year)	96.48**	0.05	0.27
Vegetation biomass (2006 Wet season Second Year)	55.51**	0.57	1.45

*p<0.05, **p<0.0001

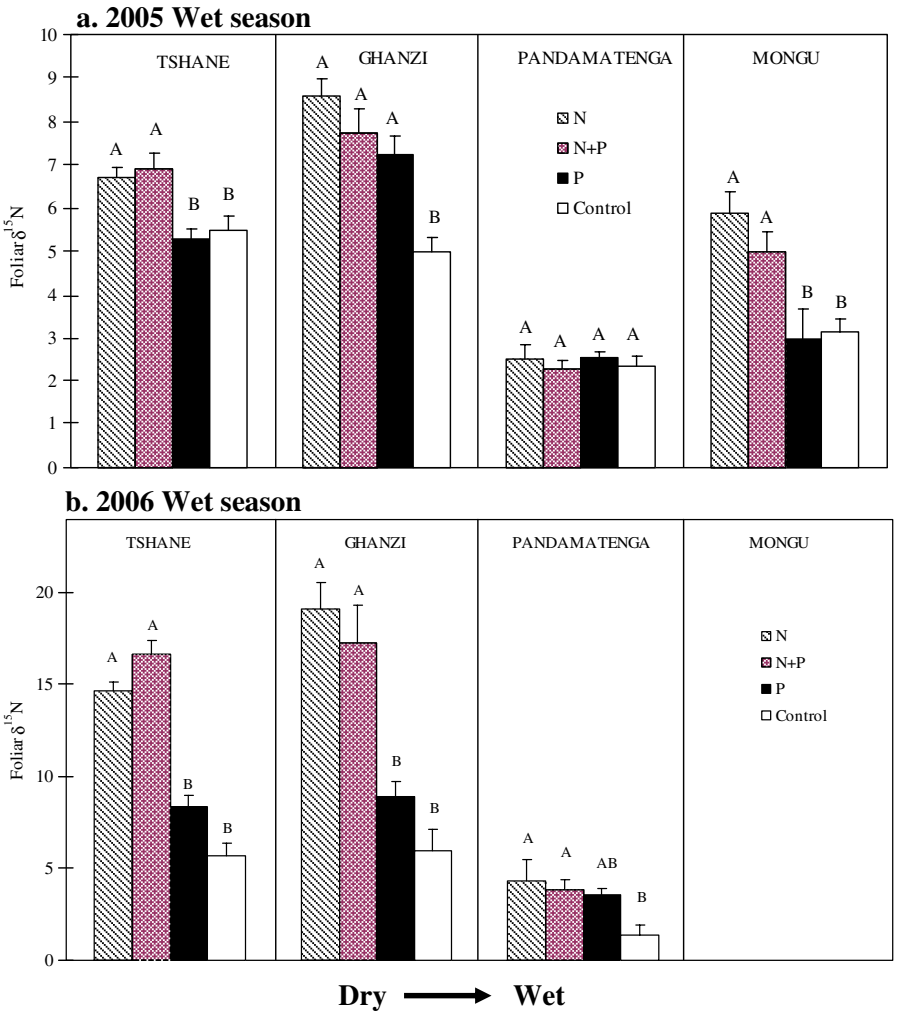

Fig. 2 Foliar $\delta^{15}N$ signatures (‰) in each treatment at each site for the wet season 2005 (**a**) and 2006 (**b**). *Different capital letters* indicate different means for the four treatments at each location (α=0.05). The *bars* represent mean values and the *error bars* represent standard errors

and the N + P or P treatments for all the sites except at Mongu (Table 2). The foliar N/P ratios in 2006 were similar to those in 2005 (Table 2) except at Pandamatenga, where the N/P ratios were found to dramatically decrease between 2005 (drier than average) and 2006 (close to the long-term mean precipitation for this site).

At all sites there was no significant increase in aboveground grass biomass for both one (p>0.05, Fig. 5a) and two (p>0.05, Fig. 5b) growing seasons after the nutrient additions. The grass biomass decreased from the dry to the wet end of the Transect in 2005 and increased in 2006 (Fig. 5). The soil water content/rainfall pattern in 2005 was abnormal, in that soil water content values at the dry end of the Transect were higher than at the wet end (Fig. 5a insert; see also D'Odorico et al. (2007)) and rainfall values at the dry end of the Transect were comparable to the wet end. In 2005 rainfall at the two wetter sites was only two-thirds of the MAP (Fig. 5b insert). Soil water content/rainfall pattern in 2006 was "normal" at the wetter two sites, in that it was consistent with the long-term precipitation patterns (Shugart et al. 2004), though at the two drier sites the 2006 rainfall was higher than the MAP (Fig. 5b insert). At all sites the aboveground grass biomass did not respond to nutrient addition in either year (Fig. 5). Changes in biomass were most significant for the wetter site (i.e., Pandamatenga), where soil water content doubled from 2005 to 2006 while grass biomass increased more than seven fold (Fig. 5).

In general, there was no significant response of soil respiration to fertilization both one (p>0.05, Fig. 6a) and two (p>0.05, Fig. 6b) growing seasons after the

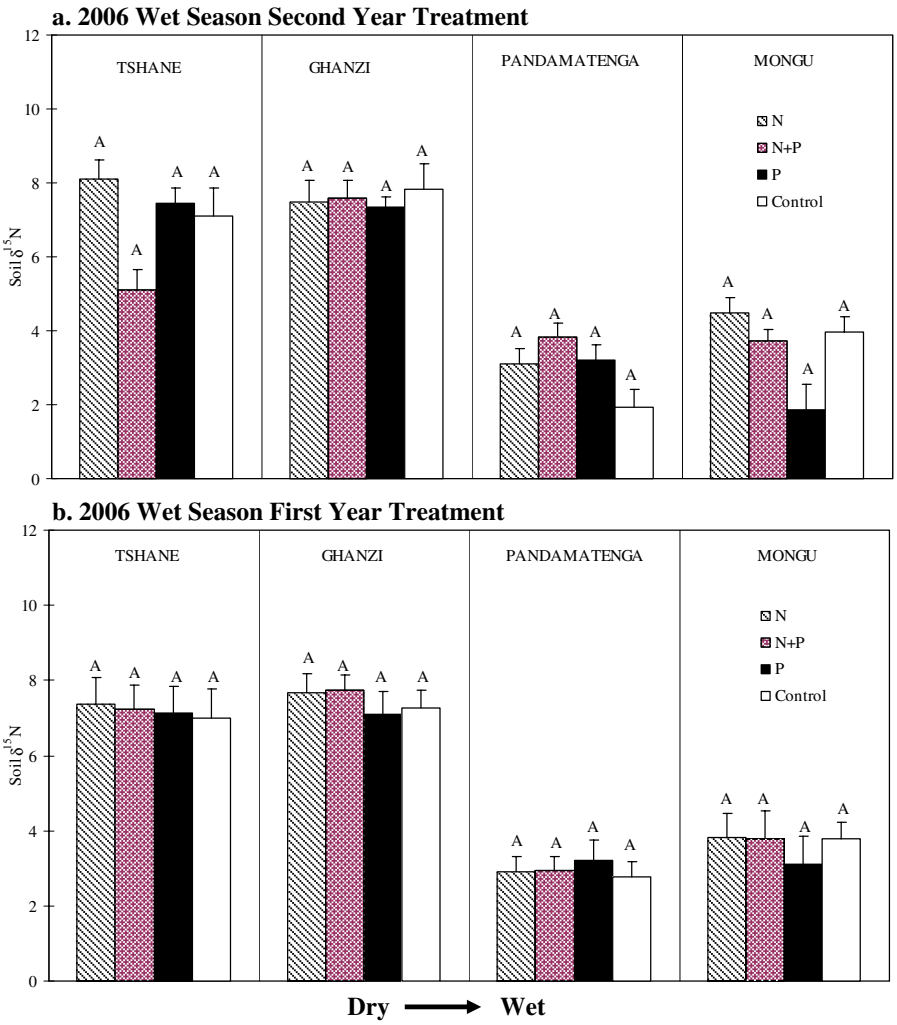

Fig. 3 Soil $\delta^{15}N$ signatures (‰) (0–5 cm) in each treatment at each site for the first year treatment (**a**) and second year treatment (**b**) in 2006. *Different capital letters* indicate different means for the four treatments at each location (α=0.05). The *bars* represent mean values and the *error bars* represent standard errors

nutrient addition. There was significantly higher soil respiration in the N treatment at Ghanzi and in the N + P treatment at Mongu for under canopy measurements in 2005 (Fig. 6a). The same trends did not appear in the 2006 measurements (p>0.05, Fig. 6b). In both years, soil respiration tended to peak at a location (Pandamentaga) characterized by intermediate values of MAP between the ends of the rainfall gradient (Fig. 6).

Discussion

The foliar $\delta^{15}N$ signatures in the N and N + P additions were consistently and significantly higher than in the control and P-additions both in the 2005 and the 2006 wet seasons (Fig. 2), indicating that grasses along the KT took up and assimilated the applied N. However, the assimilated N did not induce a significant increase in foliar N content (Fig. 4), nor in aboveground biomass (Fig. 5), indicating that N may not be a limiting factor along the entire KT. Because the study sites covered a broad spectrum of tropical savanna ecosystems, characterized by a variety of rainfall regimes, these results indicate that grass productivity in savannas along the whole KT may not be prone to N limitation (Fig. 5) regardless of the MAP. These non-limitation results are along the line with findings from a rangeland fertilization experiment in South Africa (Snyman 2002), where the author found that rangeland in a semi-arid environment in good condition did not respond to N and P fertilizer. He concludes that insufficient soil water content in those semi-arid climates is therefore more often than nutrients to limit

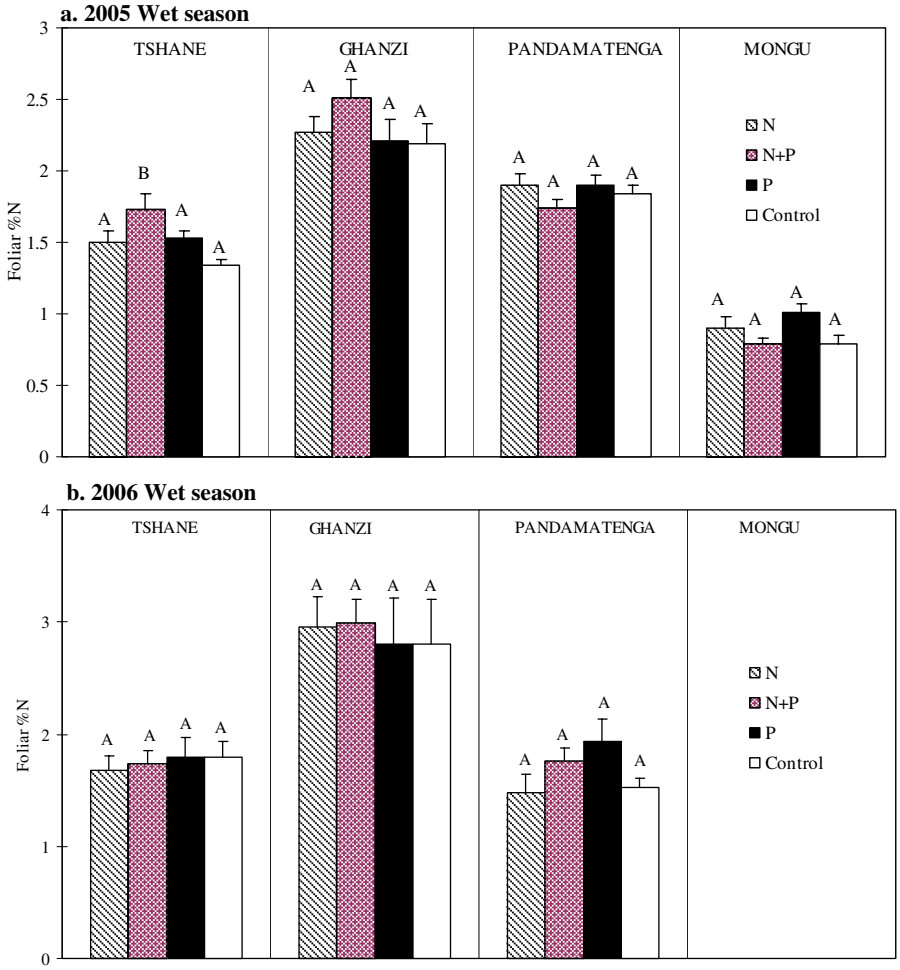

Fig. 4 Foliar %N in each treatment at each site for the wet season 2005 (**a**) and 2006 (second year) (**b**). *Different capital letters* indicate different means for the four treatments in each location (α=0.05). The *bars* represent mean values and the *error bars* represent standard errors

Table 2 Foliar N/P ratios following fertilization at each site along the Kalahari Transect in 2005 and 2006

	Treatment	Locations			
		Tshane	Ghanzi	Pandamentaga	Mongu
2005	N	–	–	–	–
	N + P	12	13	10	9
	P	13	12	14	11
	Control	19	16	15	10
2006	N	–	–	–	–
	N + P	10	10	5	–
	P	12	13	4	–
	Control	18	14	6	–

The foliar %P data were from Ries (2007)

forage production. However, the lack of a response to N fertilization is contradictory to several grassland studies (e.g., Hooper and Johnson 1999) as well as to studies from other savanna ecosystems (e.g., Ludwig et al. 2001). These results also differ from previous theoretical predictions (Cilliers et al. 1995; Scanlon and Albertson 2003; Toit et al. 2003; Midgley et al. 2004). The lack of N limitation is presumably a result of plant adaptations to these low N environments over many millennia. The low N conditions are partly related to the nature of the sandy soil (Aranibar et al. 2004; Wang et al. 2007a) existing along the KT, and to its limited water and nitrogen holding capacity. In both years, the percentage of assimilated N contributed by the fertilizer was highest at a site (Ghanzi) within the intermediate rainfall regime. Although the patterns in relative fertilizer uptake across sites is valid using mixing ratio calculations, the significant low fertilizer uptake rates in 2006 may be artificial due to the very

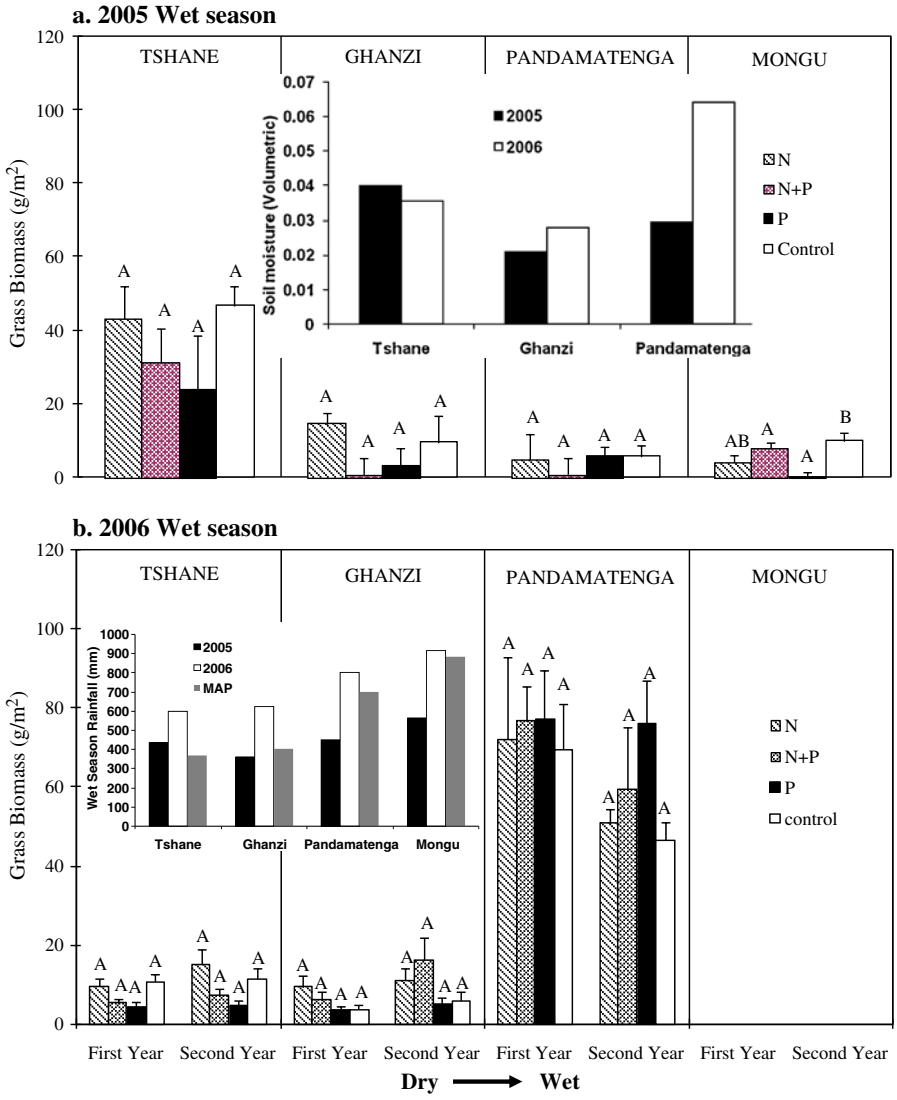

Fig. 5 Wet season plant biomass in each treatment from each location in 2005 (**a**) and 2006 (**b**). *Different capital letters* indicate different means for the four treatments in each location (α=0.05). The *bars* represent mean values and the *error bars* represent standard errors. The *insert* in **a** shows the average soil water content for the 2005 and 2006 growing seasons (D'Odorico et al. 2007), and the *insert* in **b** shows the mean annual precipitation at each site and wet season (October–April) rainfall data from satellite measurements from NASA's Tropical Rainfall Measuring Mission for 2005 and 2006

high fertilizer ^{15}N signature used. The high fertilizer ^{15}N signature may dramatically decrease after application to soil surface and this problem could be solved by sampling soil for ^{15}N analysis right after fertilizer application in future experiments. The fertilizer uptake pattern matched the one observed for foliar %N (Fig. 4), suggesting that plants with higher N content tend to be more plastic in N uptake (i.e., they more easily assimilate the additional N available in the soil). This observation supports that of Chapin (1980); Chapin et al. (1986) for wild plants that are restricted to infertile soils generally exhibiting lower maximum potential growth rates and responding less to nutrient addition than do related plants from more fertile soils. The patterns of N uptake found along the KT are therefore the result of intrinsic features of the plant communities at each site, and do not reflect different conditions of N limitation. The lack of differences in soil respiration rates between treatments (Fig. 6) may further support the hypothesis that these savanna systems may not be N limited as previous suggested (Scanlon and Albertson 2003; Aranibar et al. 2004). However, because soil respiration was measured only once in the whole growing season and there is possibility of missing microbe responses in transient states and we are cautious to conclude that soil microbe community are not suffering N limitation in these savanna ecosystems.

The decreasing trends in soil $\delta^{15}N$ signatures with increasing MAP agree with previous findings

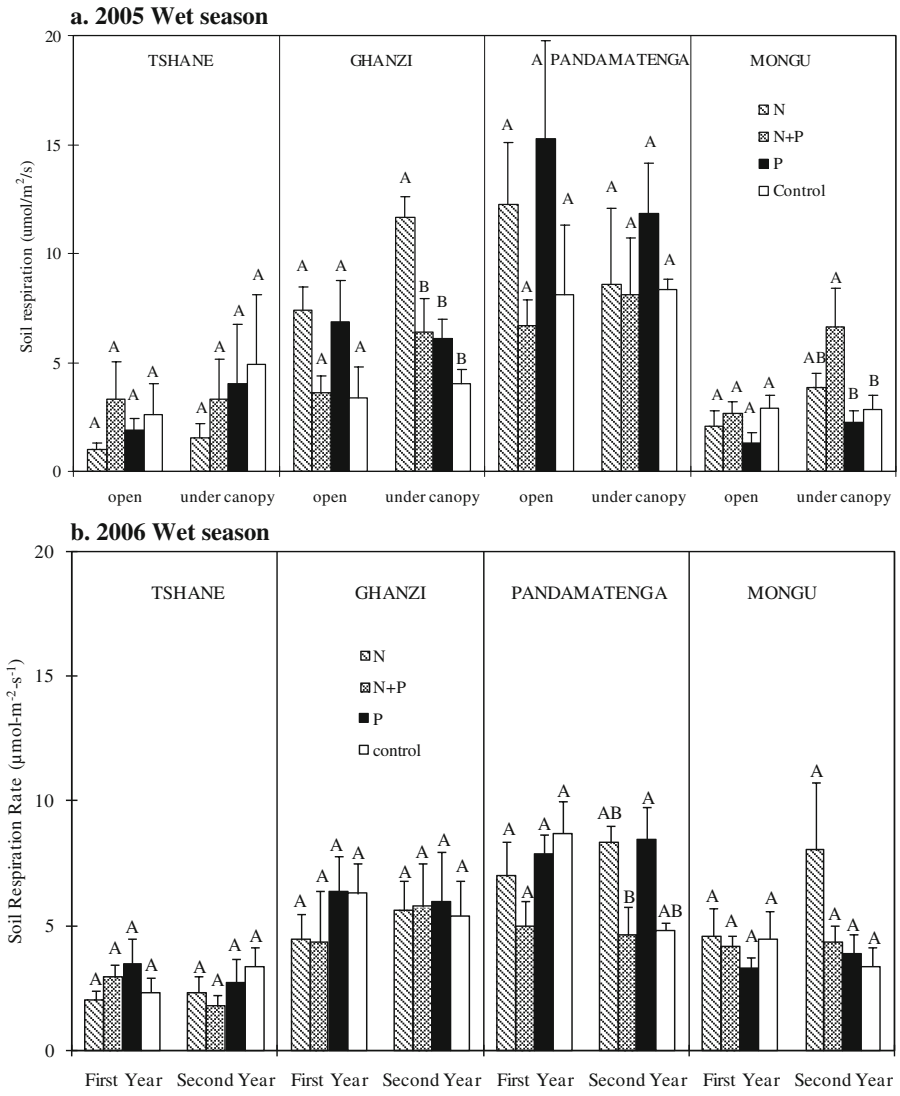

Fig. 6 Soil respirations in each treatment from each location in wet season 2005 and 2006. In 2005, the measurements were separated by open canopy and under canopy. In 2006, the measurements were separated by first year and second year treatments. *Different capital letters* indicate different means for the four treatments at each location (α=0.05). The *bars* represent mean values and the *error bars* represent standard errors

(Aranibar et al. 2004; Wang et al. 2007a) from the same ecosystems, which possibly indicate at ecosystem scales a more open N cycling at the dryer portion of the Transect (Wang et al. 2009b). The lack of differences in soil $\delta^{15}N$ signatures among the four treatments (Fig. 3) indicates that the fertilizer N represented only a minor part of the total soil N pool and indicates high nutrient leaching rates in these sites (applied fertilizer are all leached out after one growing season). One of the great difficulty in fertilizer experiments is that it is not known how much a treatment increased the availability of a particular nutrient (Hooper and Johnson 1999). Such information could be achieved by periodically measuring N mineralization rates (total %N are not effective for this purpose) or using the field ^{15}N dilution method to assess the N fluxes (Murphy et al. 2003) after fertilization, but it is difficult to be applied in extremely rural locations such as field sites of this study. However, because we applied ^{15}N as a tracer and by comparing the foliar ^{15}N signatures between control and treatments, we at least knew that target plants were able to uptake and assimilate the applied fertilizer.

The fertilizer treatments applied at each site could have altered the structure and function of grasses in a number of ways that were not addressed in this study. For example, although the results appear to demonstrate a general lack of nutrient limitation in grasses across the sites, we are unable to determine if additional N availability altered either grass allocation

patterns (root/shoot ratios) or seed production/viability. In addition, our analyses focused on community-wide responses at each site, so if adaptive responses to N availability are strongly species-specific (e.g., Snyman 2002), we would not be able to observe these effects. Lastly, the foliar N/P ratios decreased from 16–19 in the control treatment to less than 14 in the P and N + P treatments. Foliar N/P range of 14–16 is often used as a boundary value to detect N or P limitation (Koerselman and Meuleman 1996). The foliar N/P ratio changes after nutrient additions in this study, however, may indicate P luxury uptake since the grass biomass did not respond to nutrient additions along the KT (Ries 2007).

Grass biomass did not respond to nutrient addition at any site along the Transect regardless of whether "wetter" or "drier" conditions occurred. At the wetter site-Pandamatenga, where previous studies speculated about the existence of N-limitations (Scanlon and Albertson 2003; Toit et al. 2003; Midgley et al. 2004), grass biomass increased more than seven times when soil water content/rainfall increased between 2005 (below average) and 2006 (average level). These significant changes in grass biomass indicate that grass productivity is very sensitive to rainfall at the wetter end of the KT.

The grass biomass values reported in this study are much lower than those found in other southern African savannas in regions with similar rainfall regimes (e.g., Swemmer et al. 2007). We think that these differences were partly due to 1) the nature of the sandy soils existing in this region with limited water and nitrogen holding capacity, 2) the single sampling of peak biomass and 3) grazing, especially at the drier sites where grazing was reported (Moleele and Mainah 2003). Grazing is inherent to the nature of savanna ecosystems and the present study design could not include any fencing system owing to logistical issues. Rainfall and soil water content increased between 2005 and 2006, the grass biomass at the two drier sites (Tshane and Ghanzi) either did not change or decreased (Tshane), as shown in Fig. 5. We argue that this result might be explained by grazing at the drier sites. The grazers may graze down the two drier sites to the same height each year, regardless of rainfall or the total amount of biomass produced. In light of this, for the two drier sites, we cannot totally rule out the possibility that grazing took place in the fertilized plots early in the growing season when differences in productivity and grass N concentrations may appear. By the time of sampling this confounding effect may have disappeared if re-growth in the fertilized plots had lower leaf N concentrations (similar to that of the control plots). However, we think that grazing does not affect our overall N limitation conclusions because 1) the grazing activity at the two wetter sites were not high (personal communications with local farmers); 2) the grass heights of all the treatment and control subplots in the wet end were similar to that of the extensive surrounding environments during both dry (2005) and normal (2006) years, further indicating that preferential grass consumptions from the fertilized subplots did not happen; and 3) the main objective of this study was to examine whether the wet end was N-limited. Therefore the potential influence of grazing at the dry end unlikely affects the overall conclusions of this study that N is not limiting in these savanna ecosystems.

Conclusions

In summary, this large scale field manipulation experiment shows that N may not be a limiting factor in tropical savanna ecosystems. Unlike other reports (Scanlon and Albertson 2003; Midgley et al. 2004) that conjectured about a switch between water and N limitations taking place as annual precipitation exceeds a critical value, these results suggest that N limitation does not exist along the whole KT and that grass productivity at the wetter sites is very sensitive to rainfall/soil water content variations. Thus, although the traditional classifications of nutrient poor (broad-leaf savannas) and nutrient rich (fine-leaf savannas) savanna ecosystems may still be useful in the study of these ecosystems and their dynamics (Scholes and Walker 1993; Toit et al. 2003), this classification does not necessarily imply the existence of nitrogen limitation in the nitrogen poor area, in that the vegetation may already be adapted to nitrogen poor conditions.

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Appendix

Fig. 7 The field photo of unidentified grass species at Mongu, Zambia

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