

Effects of Herbivory, Fire and N₂-fixation on Nutrient Limitation in a Humid African Savanna

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ABSTRACT

The quantities and spatial distribution of nutrients in savanna ecosystems are affected by many factors, of which fire, herbivory and symbiotic N₂-fixation are particularly important. We measured soil nitrogen (N) pools and the relative abundance of N and phosphorus (P) in herbaceous vegetation in five vegetation types in a humid savanna in Tanzania. We also performed a factorial fertilization experiment to investigate which nutrients most limit herbaceous production. N pools in the top 10 cm of soil were low at sites where fires were frequent, and higher in areas with woody legume encroachment, or high herbivore excretion. Biomass production was co-limited by N and P at sites

that were frequently burnt or heavily grazed by native herbivores. In contrast, aboveground production was limited by N in areas receiving large amounts of excreta from livestock. N₂-fixation by woody legumes did not lead to P-limitation, but did increase the availability of N relative to P. We conclude that the effects of fire, herbivory and N₂-fixation upon soil N pools and N:P-stoichiometry in savanna ecosystems are, to a large extent, predictable.

Key words: *Acacia* woodland; cattle; grassland; grazing; N:P ratios; nitrogen fixation; phosphorus; stoichiometry; tallgrass.

INTRODUCTION

In savanna ecosystems, grasses and trees co-exist, forming a mosaic that is neither grassland nor forest. The vast majority of savannas owe their existence to fire, and without it would eventually be replaced by forests (Bond and others 2005). However, in drier climates the balance between woody

and grass cover and productivity appears to be mainly determined by water availability (Aranibar and others 2004; Sankaran and others 2005). Compared to humid savannas, these dry savannas are nutrient-rich (Bell 1982; Huntley 1982), though at some sites the productivity of herbaceous vegetation is limited by the availability of nitrogen (N) (Ludwig and others 2001; Snyman 2002; Augustine and others 2003). In contrast, humid savannas are often on heavily leached soils and are characterized by low phosphorus (P) availability (Sanchez 1976); plant growth in these ecosystems may be limited by shortages of N, P or both (Högberg 1986; Medina 1987), although which nutrient is limiting in any particular case has rarely been investigated.

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Many factors affect the quantities and spatial distribution of nutrients in savanna soils, with fire, herbivory and atmospheric N_2 -fixation being particularly important. In Figure 1, we present a conceptual framework to show how these factors are likely to affect soil N pools and N:P-stoichiometry.

- (1) Fire can cause considerable losses of all nutrients to the atmosphere, with losses of N being much greater than those of other nutrients (Cook 1994; Kauffman and others 1995); as a result, repeated fires are likely to promote a shortage of N, and many savannas are thought to be primarily N-limited.
- (2) Herbivores can affect nutrient conditions in various ways. The first relates to the spatial pattern of habitat use for feeding and excretion. Some herbivores re-distribute nutrients by feeding in certain areas while depositing their excreta in others (Edwards and Hollis 1982; Jewell and others 2007), thus causing local depletion or enrichment of nutrients. In

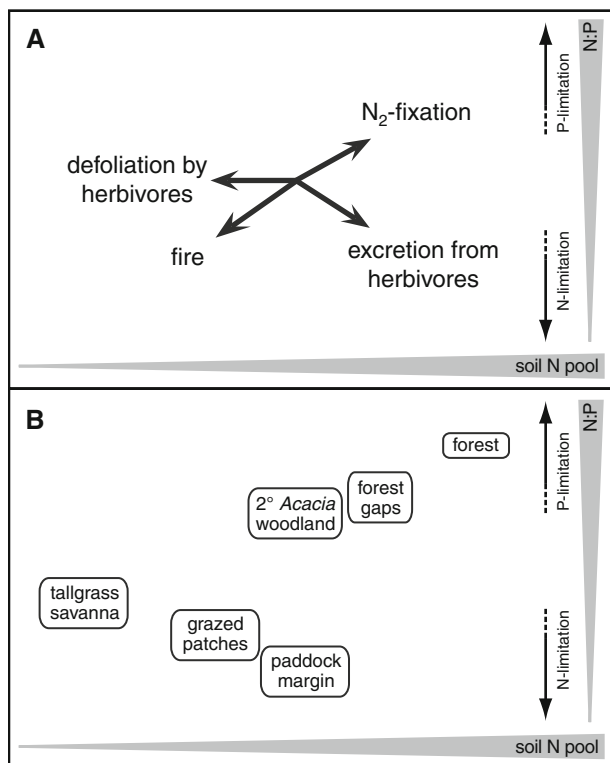


Figure 1. Conceptual framework of the effects of fire, herbivory and N_2 -fixation on soil N pool, N:P-stoichiometry and the type of nutrient limitation (A), and hypothesized status of N pools and N:P ratios of five vegetation types differentially affected by fire, herbivory and N_2 -fixation in relation to the presumed climax forest vegetation (B).

contrast, others feed and return their excreta in the same, intensively used areas, thereby potentially maintaining nutrient rich patches in otherwise nutrient-poor vegetation (Lamoot and others 2004). Second, a significant proportion of the N in dung and urine may be lost through ammonia volatilization and leaching (Ruess and McNaughton 1988; Frank and Zhang 1997; Augustine 2003), leading to a local excess of P relative to N (providing that the dung and urine are excreted in the same areas, which is commonly the case; Edwards and Hollis 1982). Third, herbivores may have a positive net effect on the soil N pool by reducing the amount of biomass exposed to fire, and thereby reducing the associated losses to the atmosphere (Hobbs and others 1991; Holdo and others 2007). Hence, depending on the patterns of grazing and excretion, herbivory has a neutral effect on the ratio of N:P availabilities (defoliation) or potentially shifts the nutrient balance towards N-limitation (excretion) (Figure 1).

- (3) Woody legumes, especially of the genus *Acacia*, are conspicuous elements of many savannas, and the majority are thought to fix N_2 symbiotically. The abundance and distribution of these trees is significantly affected by the activities of herbivores. For example, grazing by domestic livestock is often responsible for extensive encroachment of savanna grasslands by woody species (Brown and Archer 1989; Hudak 1999; Tobler and others 2003), of which legumes are a dominant component (Archer 1995; Cramer and others 2007). It has been shown that much greater amounts of N accumulate in the bushland than in the open grassland it replaces; this accumulation could in turn lead to P-limitation under *Acacia* canopies (Figure 1).

There have been many agricultural studies of nutrient limitation in tropical savanna ecosystems (for example, Weinmann 1938; Brockington 1961; Norman 1966), including several that investigated the effects of either fire or domestic herbivory (Barger and others 2002; Augustine 2003). However, we are unaware of any study of a savanna ecosystem designed to investigate the interactive effects of fire and native and domestic herbivores. Also, most studies have focussed on N and sometimes P, and the extent to which cations such as potassium may also limit plant production is not known. However, the concentrations of cations in savanna vegetation are typically low, and are

known to be a factor influencing food selection by large herbivores (McNaughton 1988, 1990).

The aim of this study was to investigate the type of nutrient limitation in different types of humid savanna vegetation in Saadani National Park, Tanzania. We selected five contrasting types of vegetation which from our conceptual framework (compare Figure 1B) we predicted would differ with respect to soil N pool and N:P-stoichiometry. These vegetation types were: forest gaps, tallgrass savanna, *Acacia* woodland, surroundings of former cattle paddocks and lawns grazed by wild ungulates. In each of these vegetation types, we measured the soil N pools and performed a factorial fertilization experiment to determine patterns of nutrient limitation.

METHODS

Study Area

The study area is located in Saadani National Park on the Tanzanian coast (5°43'S, 38°47'E). The northern part of the national park was operated as a cattle ranch from 1954 to 2000 with up to 13,000 head of cattle on approximately 460 km². The southern part was a game reserve from 1969 until it became a national park in 2002. The area is grazed by wild herbivores including warthog, waterbuck, reedbuck, buffalo, wildebeest, giraffe and elephant. Densities and diversity of wild herbivores are much higher in the southern part of the park compared to the former ranch area (Treydte and others 2005). Mean annual temperature recorded at the former ranch complex is 25°C (1973–1998). Annual precipitation from 1957 to 1998 has ranged from 610 to 1,700 mm, with a mean of 1,040 mm. The wet season lasts from March until June, and there is a short rainy season from mid-October to mid-November. The driest months are January and February and August and September, and during these periods fires are common, many of them being started deliberately by the local people. The relatively nutrient-poor soils consist of greyish, fine or loamy sand in the flats, and reddish, loamy sand over clay on slopes and hilltops (Klötzli 1980). For more detailed description of the study area see Tobler and others (2003).

The vegetation of the area is dominated mainly by bushland and grassland, but there are extensive areas of evergreen forest. The annual precipitation is sufficient to allow for 100% woody cover (Sankaran and others 2005), and the fact that the vegetation is now mainly savanna is probably due to recurrent fires of anthropogenic origin (Bond

and others 2005). We selected a representative site in each of the following five vegetation types:

- (1) Gaps in remnant forest. These grass-dominated patches are surrounded by remnant forest and connected to the open savanna by narrow grass corridors. Because fires rarely reach these areas (a conclusion based on aerial photographs and personal observations), they served as a reference for the more frequently burnt vegetation types. The site selected was dominated by the grasses *Sporobolus pyramidalis* and *Schoenefeldia transiens*, and the sedge *Abildgaardia triflora*. The vegetation was 50-cm tall with flowering tillers reaching to 1.5 m; the average basal cover was 85%.
- (2) Tallgrass vegetation. This type of vegetation is known to be the most frequently burnt due to its high biomass (Frost and Robertson 1987), and we predicted that it would have the greatest cumulative nutrient losses, especially of N. The site chosen was dominated by the grasses *Hyperthelia dissoluta*, *Diheteropogon amplexans* and *Andropogon schirensis*. Vegetation was 2-m tall, and basal cover was 70%.
- (3) *Acacia* woodlands. This open woodland has developed from tall grass communities as a result of cattle grazing (Tobler and others 2003); compared to the tall grass savanna, we expected it to be relatively N-rich because of symbiotic N₂-fixation. At the chosen site, there were scattered trees of *Acacia zanzibarica* 6-m high and with a basal area of 1.3 m²/ha. *A. zanzibarica* trees were nodulated (P.G. Cech, personal observation), and δ¹⁵N data indicated that they derived approximately 58% of their N from fixation (Cech 2008). The understory was dominated by the grasses *Heteropogon contortus*, *Panicum infestum* and *Digitaria milaniana* and the sedge *A. triflora*. This layer was 60-cm tall, and basal cover reached 90%.
- (4) Paddock margins. The former paddocks ('bomas'), which were used until 2000 to hold the cattle overnight, and their surroundings, were very nutrient enriched. We predicted these areas would be relatively P-rich due to large N-losses from ammonia volatilization. The site selected was dominated by the grasses *Eragrostis superba* and *D. milaniana* and the sedge *Cyperus bulbipes*. Vegetation height was 60 cm and basal cover 100%.
- (5) Grazed lawns. Grazed patches (sensu Archibald and others 2005) are localized areas of intensive grazing that are not continuously maintained by wild herbivores. We explicitly assumed that

wild herbivores not only eat the vegetation, but also deposit dung and urine in these patches. In support of this assumption, we found much more dung in grazed lawns than in the other vegetation types where dung counts were made (tallgrass savanna, *Acacia* woodland, medium height savanna) (Cech 2008). Therefore we expected grazed patches to be generally nutrient-rich (though less so than the paddock margins) and with relatively high levels of P compared to N. We selected an area where vegetation—dominated by the grasses *D. milanjiana*, *D. amplexans* and *P. infestum*—had been maintained at less than 10-cm height by wild herbivores for at least 12 months (P.G. Cech, personal observation). During the rainy season prior to the start of our experiment, however, grazing pressure decreased, and at the start of the experiment the vegetation was 40-cm tall and had a basal cover of 95%.

The first four sites were located on sandy soil in the area of the former cattle ranch. The grazed lawn was on a similar sandy soil in the southern part of the national park where wild herbivores were much more abundant.

Experimental Design and Treatments

The experiment was started at the end of the wet season in June 2006. At each site we set up an experimental plot in a patch of homogeneous vegetation. Plots comprised 30 blocks of 1 m² separated from each other by 1 m buffer zones, and were surrounded by a 1.5-m tall fence to exclude small and large mammal herbivores. Blocks were randomly assigned to one of six nutrient treatments: control, N, P, N and P (N + P), cations (+cations) and all nutrients (N + P + cations). 'Cations' was a combination of the macronutrients K, Ca and Mg, and the micronutrients Fe, Mn, Zn, B, Cu and Mo. The rationale for this treatment was to determine whether production was limited by a nutrient other than N or P. N was supplied as NH₄NO₃ at 20 g/m², P as Na₂HPO₄ at 5 g/m², potassium as KCl at 5 g/m², calcium as CaCl₂ at 5 g/m² and magnesium as MgSO₄ at 1.4 g/m². These supply levels were sufficiently high to offset growth limitation without being toxic (Augustine and others 2003), and are similar to those used in other fertilization experiments in comparable ecosystems (reported in Table 3). The micronutrients, in the form of oxides or mineral salts of chloride or sulphate, were supplied in the same proportions relative to N as are used in Hoagland's solution. Nutrient addition was split into two applications of

1.5 l of aqueous solution; one half was added at the start of the experiment and the second 2 weeks later. Because the topsoil had become visibly drier by the second application of nutrients, infiltration was improved by wetting the soil with 1.5 l of water before adding the nutrient solution. Control blocks received an equivalent amount of water only.

Harvest and Chemical Analyses

Before the first application of nutrients, blocks were clipped to 3-cm height and the harvested biomass was weighed. Regrowth was harvested after 46 days and again after 172 days, and dried to constant weight.

Biomass samples from control blocks and those receiving the complete nutrient dressing were ground, and analyzed for N and P concentrations after Kjeldahl digestion. Total N and P concentrations in the digests were measured by means of a continuous flow injection analyzer (FIASStar, Foss Tecator, Höganäs, Sweden). A second subsample was extracted with 0.5 M HCl and analyzed for K, Ca and Mg concentrations, using atomic absorption spectrometry (Hunt 1982) (SPECTRAA 240 FS, Varian AG, Zug, Switzerland).

Soil samples (2.8-cm diameter cores, top 10-cm soil) were taken at the start of the experiments (one mixed sample of five cores per site), and at the second harvest (one core per unfertilized control block; five replicates per site). The mixed samples were used for K, Ca and Mg analyses; total C and N as well as extractable N and P were determined on soil cores taken at the second harvest. Total C and N were measured on a dry combustion analyzer (CN-2000, LECO Corp., St. Joseph, Minnesota, USA). Soil extractable PO₄²⁻ was determined by extraction of 5 g of fresh soil with 50 ml Bray-2 solution (Bray and Kurtz 1945). Exchangeable NH₄⁺ and NO₃⁻ were determined by the extraction of 10 g fresh soil in 50 ml 0.2 M KCl solution. Extraction was done within 12 h of collection of the soil cores. KCl-extracts were acidified with 5% H₂SO₄ for conservation until analysis. Concentrations of PO₄²⁻, NO₃⁻ and NH₄⁺ in the extracts were measured colorimetrically using a continuous flow injection analyzer (FIASStar, Foss Tecator, Höganäs, Sweden). K, Ca and Mg were measured by atomic absorption spectrometry from 1 M ammonium acetate extracts (Carter 1993) (SPECTRAA 240 FS, Varian AG, Zug, Switzerland).

Rainfall, temperature and relative humidity were recorded with tipping bucket rain gauges and data

loggers (HOBO RG3-M, HOBO H8 Pro, Onset Computer Corp., Bourne, Massachusetts, USA) at two locations: one in the northern and one in the southern part of the national park at proximity of the experimental plots. During the wet season prior to the experiment, the northern part had received approximately 630 mm of precipitation and the southern part 430 mm. From the start of the experiment until the first harvest on day 46, cumulative rainfall was 30 mm in both parts of the national park. Between the first and the second harvest on day 172, precipitation amounted to 480 mm in the north and 280 mm in the south. During the experiment, mean daily temperature and mean daily relative humidity were 24.9°C and 88% in the north, and 25.7°C and 79% in the south.

Statistical Analysis

Statistical analysis was carried out with JMP 6.0.3 (SAS Institute, Cary, USA). Soil and vegetation characteristics (including concentrations and ratios of N, P, K, Ca and Mg) of the five study sites were compared using one-way ANOVAs, with site as the fixed effect. Differences in aboveground biomass production between nutrient treatments were tested with one-way ANOVAs, with nutrient treatment as a fixed effect with six levels. Between-site differences in concentrations and ratios of N, P, K, Ca and Mg in aboveground biomass of control plots were tested with one-way ANOVAs, with site as a fixed effect. If assumptions of normality and homoscedascity were not fulfilled, data were log or square-root transformed. Multiple comparisons were made with the Tukey-Kramer HSD test ($P < 0.05$). Additionally, the effect of fertilizer treatment on nutrient concentrations in aboveground biomass was tested for each site by comparing concentrations and ratios of N, P, K, Ca and Mg between control plots and plots receiving N + P + cations, using a *t*-test assuming unequal variances.

RESULTS

Soil Nutrients

Mean values of total N in the top 10 cm soil varied among sites by a factor of more than 4, from 0.24 in the tallgrass savanna plot to 1.07 mg g⁻¹ in the forest gap (Table 1). The second highest N pool was in the secondary *Acacia* woodland, but this was only 60% of that in the forest gap. Variation among sites in the N pool paralleled variation in the topsoil C pools (Table 1), and C:N ratios were similar at all sites (mean values ranged from 14.0 to 14.7, no

significant differences) except the paddock margin where they were significantly lower (mean 11.4). Mean extractable inorganic N varied by a factor of around 2, with high values in the forest gap and the paddock margin (0.34 and 0.36 mg g⁻¹, respectively) and lowest values in the grazed patch (0.17 mg g⁻¹; Table 1). The mean concentrations of extractable P varied fivefold amongst sites, being highest in the paddock margin (0.88 mg g⁻¹), and 40% lower in the grazed patch; this in turn had significantly higher P concentrations than the other three types of site (0.15–0.18 mg g⁻¹).

Nutrient Limitation of Biomass Production

Biomass production in the control treatments ranged from 23 in the grazed patches to 100 g m⁻² in the paddock margin at the first harvest (46 days), whereas the corresponding yields at the second harvest (172 days) were 94 and 286 g m⁻², respectively (Figure 2). At the second harvest, when there was a marked growth response at all sites, the lowest mean N concentrations in regrowth were in the tallgrass savanna, whereas the highest values were in the forest gap and *Acacia* woodland (Table 2). Mean P concentrations ranged more than threefold, being lowest in the *Acacia* woodland and highest in the paddock margin. As a result, N:P ratios in the regrowth on control plots ranged widely, from 4.8 in the paddock margin to 16.7 in *Acacia* woodland (second harvest, Table 2).

In the forest gap plot, none of the nutrient treatments had a significant effect upon regrowth at the first harvest, but production at the second harvest was increased by the combined addition of N, P and cations (Figure 2). The increase in biomass production caused by the addition of N + P was on average equal to the increase observed by the N + P + cations treatment, but it was statistically not significantly different from the control due to the large variation (Figure 2). Strictly speaking, these results therefore lead to the conclusion that growth was co-limited by N, P and cations; however, in view of the high average response to N + P we interpret them as indicating N and P co-limitation. Tissue N and P concentrations both significantly increased at the first harvest in response to the full nutrient treatment, but not at the second harvest (Table 2).

In the tallgrass savanna site, production at the first harvest was increased in the N + P treatment (Figure 2), whereas N + P + cations increased growth even more. In contrast, at the second harvest N addition alone was sufficient to increase

Table 1. Soil and Vegetation Characteristics of the Five Study Sites in Saadani National Park

	<i>n</i>	Forest gap	Tallgrass savanna	<i>Acacia</i> woodland	Grazed patch	Paddock margin
Total N (mg/g)	5	1.07 ± 0.10 ^A	0.24 ± 0.01 ^D	0.56 ± 0.03 ^B	0.35 ± 0.03 ^C	0.47 ± 0.03 ^{BC}
Topsoil N pool (g/m ²)	5	134 ± 13 ^A	33 ± 1 ^D	78 ± 5 ^B	54 ± 6 ^C	67 ± 4 ^{BC}
Topsoil C pool (kg/m ²)	5	1.98 ± 0.19 ^A	0.46 ± 0.02 ^D	1.11 ± 0.05 ^B	0.75 ± 0.05 ^C	0.76 ± 0.05 ^C
Extractable N (g/m ²)	5	0.34 ± 0.04 ^{AB}	0.21 ± 0.01 ^{BC}	0.24 ± 0.04 ^{ABC}	0.17 ± 0.04 ^C	0.36 ± 0.04 ^A
Extractable P (g/m ²)	5	0.18 ± 0.03 ^C	0.17 ± 0.02 ^C	0.15 ± 0.02 ^C	0.57 ± 0.02 ^B	0.88 ± 0.10 ^A
Extractable N:P	5	2.18 ± 0.66 ^A	1.26 ± 0.07 ^A	1.57 ± 0.18 ^A	0.29 ± 0.07 ^B	0.43 ± 0.03 ^B
Extractable K (g/m ²)	1	10	5	5	13	12
Extractable Ca (g/m ²)	1	743	31	130	49	53
Extractable Mg (g/m ²)	1	36	5	18	12	7
Bulk density (g/cm ³)	10	1.26 ± 0.09 ^B	1.38 ± 0.12 ^{AB}	1.38 ± 0.15 ^{AB}	1.53 ± 0.15 ^A	1.42 ± 0.11 ^A
Soil water content at day 0 (g/g)	5	0.14 ± 0.02 ^A	0.17 ± 0.01 ^A	0.17 ± 0.04 ^A	0.07 ± 0.01 ^C	0.11 ± 0.01 ^B
Soil water content at day 172 (g/g)	5	0.09 ± 0.01 ^A	0.11 ± 0.01 ^A	0.09 ± 0.02 ^A	0.02 ± 0.004 ^C	0.06 ± 0.01 ^B
Total aboveground biomass at day 0 (g/m ²)	30	616 ± 39 ^{AB}	543 ± 22 ^{BC}	499 ± 19 ^C	484 ± 18 ^C	671 ± 23 ^A
Proportion of dead biomass at day 0	5	0.76 ± 0.08 ^A	0.34 ± 0.10 ^{BC}	0.37 ± 0.08 ^{BC}	0.28 ± 0.15 ^C	0.52 ± 0.05 ^B

Values are means ± standard errors.

Extractable N: extractable nitrate and ammonium (0.2 M KCl).

Extractable P: extractable phosphorus (Bray-2).

Extractable K, Ca, and Mg: extractable potassium, calcium and magnesium determined from a mixed sample of 5 pooled soil cores (1 M ammonium acetate).

Values not sharing the same letter indicate significant differences between sites (Tukey-HSD, $P < 0.05$).

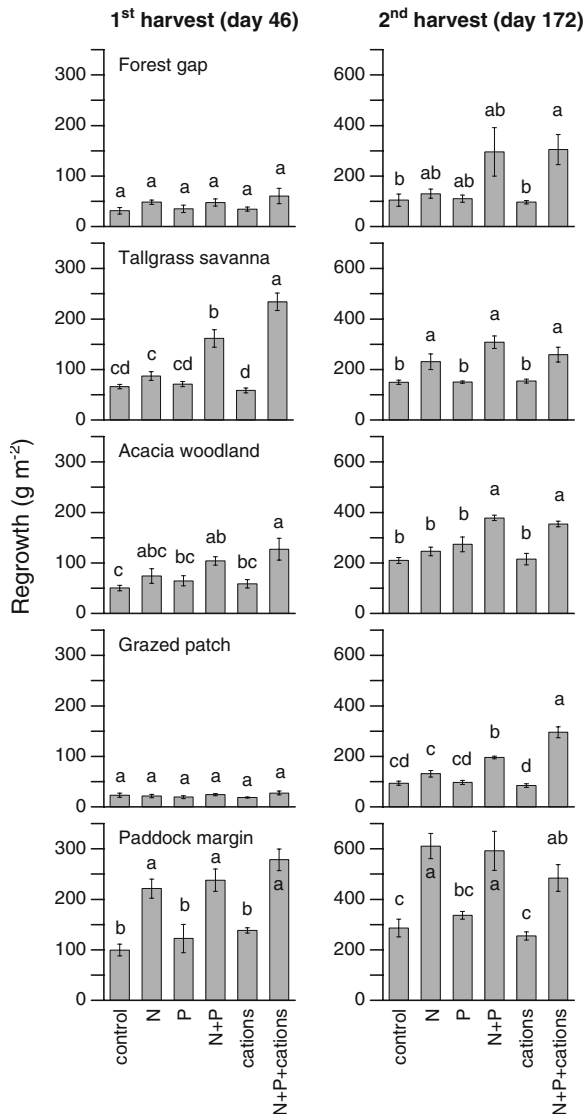


Figure 2. Aboveground biomass production in five different vegetation types as affected by the addition of N, P, cations (= all nutrients other than N and P) and combinations thereof. Bars show mean aboveground biomass harvested after 46 and again after 172 days (\pm SE; $n = 5$). Bars not sharing the same letters are significantly different from each other (Tukey-HSD, $P < 0.05$).

production significantly, and there was no additional effect of adding either P or P + cations. Thus, production in the first period was co-limited by N and P, but in the second period only by N (Figure 2).

In the *Acacia* woodland plot, only the addition of both N and P (that is, N + P and N + P + cations) increased herbaceous biomass production significantly, indicating that growth was co-limited by

these nutrients (Figure 2). Nutrient analyses of aboveground biomass show that only P concentrations increased significantly under full nutrient supply compared to the controls (Table 2). The *Acacia* woodland had the highest N:P ratio in biomass (Table 2).

In the grazed patch, none of the nutrient treatments had any effect upon yield at the first harvest; however, at the second harvest there was a significant increase in aboveground biomass in the N + P treatment, and an even greater increase in the N + P + cations treatment (Figure 2). These results suggest that regrowth was co-limited by N and P, but only at the second harvest. Concentrations of N and K in the biomass were higher in the N + P + cations treatment at the first harvest, whereas N and P concentrations were higher at the second harvest (Table 2).

In the paddock margin plot, N addition significantly increased productivity, but there was no extra effect when P or P + cations were also added; thus, growth was clearly N-limited (Figure 2). The full nutrient addition did increase tissue N concentrations, though the effect was only significant at the first harvest (Table 2).

DISCUSSION

This study was designed to test predictions about long-term effects of fire, herbivory and N_2 -fixation on soil N, N:P-stoichiometry and the type of nutrient limitation (Figure 1). This research was conducted at five sites representative for the studied vegetation types, with only within-site replication. To get a more general impression of the type of nutrient limitation in these savanna vegetation types, we compared our results with data from studies in other tropical savannas and forested areas, which were similar to our sites with regards to the influence of fire, herbivory and N_2 -fixation (Table 3).

The main trends that emerge from these comparisons are generally consistent with our conceptual model (Figure 1B). They include: (1) tallgrass savanna or comparable vegetation types are N-limited or NP-co-limited; (2) areas receiving large amounts of excreta from wild and domestic herbivores (grazing lawns and bomas) are N-limited; (3) vegetation growing in areas of cleared and burned tropical forest is mainly NP-co-limited; (4) mature tropical forests appear to be primarily limited by P (Table 3); (5) N_2 -fixation tends to cause P-limitation (Table 3). This last point was supported by two published studies, but we found co-limitation by N and P in the *Acacia* woodland plot. However, the

Table 2. Nutrient Concentrations and Ratios in Aboveground Biomass at the Five Study Sites

	Forest gap			Tallgrass savanna			Acacia woodland			Grazed patch			Paddock margin		
	Control	N + P + cations	Control	Control	N + P + cations	Control	Control	N + P + cations	Control	Control	N + P + cations	Control	Control	N + P + cations	
Day 46															
N (mg/g)	12.8 ± 0.5 ^{AB}	17.9 ± 0.1 ^{**}	10.9 ± 0.6 ^B	16.7 ± 1.2 ^{**}	14.0 ± 0.8 ^A	16.2 ± 0.8	14.8 ± 1.3 ^A	22.8 ± 0.6 ^{***}	10.2 ± 0.6 ^B	14.8 ± 1.3 ^A	22.8 ± 0.6 ^{***}	10.2 ± 0.6 ^B	14.8 ± 1.3 ^A	22.8 ± 0.6 ^{***}	
P (mg/g)	0.86 ± 0.02 ^C	1.19 ± 0.03 ^{***}	1.21 ± 0.18 ^{BC}	2.04 ± 0.10 ^{**}	0.83 ± 0.04 ^C	1.20 ± 0.07 ^{**}	1.61 ± 0.12 ^{AB}	1.68 ± 0.04	1.89 ± 0.16 ^A	1.61 ± 0.12 ^{AB}	1.68 ± 0.04	1.89 ± 0.16 ^A	1.61 ± 0.12 ^{AB}	1.68 ± 0.04	
Potassium (mg/g)	6.5 ± 0.9 ^C	5.9 ± 0.8	14.1 ± 0.5 ^{AB}	16.0 ± 0.5 [*]	10.4 ± 0.9 ^{BC}	10.1 ± 0.7	13.0 ± 1.4 ^{AB}	17.8 ± 0.3 [*]	17.2 ± 1.4 ^A	13.0 ± 1.4 ^{AB}	17.8 ± 0.3 [*]	17.2 ± 1.4 ^A	13.0 ± 1.4 ^{AB}	17.8 ± 0.3 [*]	
Calcium (mg/g)	3.6 ± 0.4 ^B	3.2 ± 0.5	4.1 ± 0.2 ^B	3.6 ± 0.4	6.5 ± 0.4 ^A	5.2 ± 0.4	4.7 ± 0.2 ^B	4.2 ± 0.3	2.2 ± 0.2 ^C	4.7 ± 0.2 ^B	4.2 ± 0.3	2.2 ± 0.2 ^C	4.7 ± 0.2 ^B	4.2 ± 0.3	
Magnesium (mg/g)	1.9 ± 0.2 ^B	1.4 ± 0.1	2.7 ± 0.0 ^A	2.8 ± 0.2	2.3 ± 0.1 ^A	2.7 ± 0.2	2.7 ± 0.1 ^A	2.8 ± 0.1	1.5 ± 0.1 ^B	2.7 ± 0.1 ^A	2.8 ± 0.1	1.5 ± 0.1 ^B	2.7 ± 0.1 ^A	2.8 ± 0.1	
N:P	15.0 ± 0.8 ^A	15.1 ± 1.0	9.7 ± 1.2 ^B	8.3 ± 0.8	16.9 ± 0.6 ^A	13.6 ± 0.6 ^{**}	9.1 ± 0.1 ^B	13.6 ± 0.4 ^{***}	5.5 ± 0.5 ^C	9.1 ± 0.1 ^B	13.6 ± 0.4 ^{***}	5.5 ± 0.5 ^C	9.1 ± 0.1 ^B	13.6 ± 0.4 ^{***}	
N:K	2.1 ± 0.3 ^A	3.2 ± 0.3 [*]	0.8 ± 0.0 ^{CD}	1.0 ± 0.0 ^{**}	1.4 ± 0.1 ^{AB}	1.5 ± 0.1	1.2 ± 0.1 ^{BC}	1.3 ± 0.0	0.6 ± 0.1 ^D	1.2 ± 0.1 ^{BC}	1.3 ± 0.0	0.6 ± 0.1 ^D	1.2 ± 0.1 ^{BC}	1.3 ± 0.0	
K:P	7.5 ± 1.1 ^B	5.0 ± 0.8	12.5 ± 1.4 ^{AB}	7.9 ± 0.5 [*]	12.8 ± 1.4 ^A	9.2 ± 0.2	8.1 ± 1.0 ^{AB}	10.6 ± 0.2	9.5 ± 1.2 ^{AB}	8.1 ± 1.0 ^{AB}	10.6 ± 0.2	9.5 ± 1.2 ^{AB}	8.1 ± 1.0 ^{AB}	10.6 ± 0.2	
Day 172															
N (mg/g)	10.6 ± 0.4 ^A	10.2 ± 0.8	6.8 ± 0.3 ^C	7.4 ± 0.5	8.8 ± 0.3 ^B	8.4 ± 0.4	9.7 ± 0.3 ^{AB}	12.2 ± 0.3 ^{***}	8.8 ± 0.5 ^B	9.7 ± 0.3 ^{AB}	12.2 ± 0.3 ^{***}	8.8 ± 0.5 ^B	9.7 ± 0.3 ^{AB}	12.2 ± 0.3 ^{***}	
P (mg/g)	0.94 ± 0.13 ^B	0.97 ± 0.23	0.91 ± 0.16 ^{BC}	1.26 ± 0.34	0.53 ± 0.02 ^C	0.96 ± 0.05 ^{***}	0.98 ± 0.07 ^B	1.72 ± 0.04 ^{***}	1.90 ± 0.11 ^A	0.98 ± 0.07 ^B	1.72 ± 0.04 ^{***}	1.90 ± 0.11 ^A	0.98 ± 0.07 ^B	1.72 ± 0.04 ^{***}	
N:P	12.5 ± 2.3 ^{AB}	12.7 ± 2.5	8.6 ± 1.7 ^{BC}	7.7 ± 1.9	16.7 ± 0.5 ^A	8.8 ± 0.5 ^{***}	10.0 ± 0.4 ^{BC}	7.1 ± 0.1 ^{**}	4.8 ± 0.5 ^C	10.0 ± 0.4 ^{BC}	7.1 ± 0.1 ^{**}	4.8 ± 0.5 ^C	10.0 ± 0.4 ^{BC}	7.1 ± 0.1 ^{**}	

Values are means (±SE) of five blocks.

Values of controls not sharing the same letter indicate significant differences between sites (Tukey-HSD, $P < 0.05$).

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ indicate significant differences between control blocks and those receiving N + P + cations.

values for N:P ratios suggest that N₂-fixation by *Acacia* did cause a shift of N:P-stoichiometry in the direction of P-limitation (Table 2).

In all our sites, aboveground production was limited by N or co-limited by N and P, but there was no evidence of primary limitation by any other nutrients (Figure 2). Indeed, the majority of studies on nutrient limitation in savanna ecosystems have assumed that no nutrient other than N and P limits productivity, although without testing this assumption explicitly. It is worth noting that nutrients were not always the factor limiting growth in our experiment; thus, in the first period the nutrient treatments had no effect upon aboveground production in the forest gap or in the grazed patch, presumably because of low water availability. The herbaceous vegetation in the forest gap is likely to have experienced drought because of water uptake by surrounding trees (Bourliere and Hadley 1983), whereas the grazed patch in the southern part of the study area received significantly less rain than the other sites (reflected in a lower soil water content at the start of the experiment; Table 1). Thus, although nutrients have been shown to limit plant production at annual levels of precipitation as low as 200 mm y⁻¹ (Penning de Vries and others 1980), the large seasonal fluctuations in water availability characteristic of savannas may temporally result in water limitation.

Various lines of evidence suggest that our tallgrass site, as well as many other tallgrass savannas, is at the boundary between N-limitation and NP-co-limitation. First, consistent with some other studies (Table 3), production at our tallgrass site was limited by N during the second period, but by both N and P during the first period. Second, in comparable types of vegetation where N alone stimulated production, the joint addition of N and P always caused a further increase in growth (Table 3). These findings demonstrate that besides N availability P availability is also low in tallgrass savanna and comparable vegetation. There are various possible explanations for low levels of P in this habitat: for example, losses of P through fire (although being clearly less than for N and mainly occurring in particulate form; Cook 1994; Laclau and others 2002), or the leaching of P from ancient highly weathered tropical soils (Lambers and others 2008).

At the tallgrass site, the biomass at the first harvest was greater in the N + P + cations treatment than in the N + P treatment. The fact that the N + P + cations treatment increased concentrations of potassium in regrowth but not of calcium and

magnesium (Table 2) suggests that K became limiting when N and P were supplied. Indeed, the losses of K during combustion are usually found to be higher than those of Ca and Mg (Cook 1994; Van de Vijver and others 1999; Laclau and others 2002). The use of the tallgrass site as a cattle pasture between 1954 and 2000 could have contrasting effects upon N and P conditions: on the one hand it could have increased losses of N and P because more nutrients were ingested in this area than were returned in excreta; on the other hand, by reducing the intensity and frequency of fires, grazing could also have reduced N losses relative to those before the area was grazed (Hobbs and others 1991). However, because concentrations of total N and extractable P in the soil of tallgrass sites in the former ranch area were similar to those in an area to the south of our study sites that had never been grazed by cattle (P.G. Cech and others, unpublished data), we conclude that any such effects must have been rather small.

In contrast to the tallgrass pastures, cattle had a large effect upon soils in the vicinity of paddocks, which received high amounts of dung and urine for several decades. Soil N pool, extractable N and extractable P were higher in the paddock margin samples than in the tallgrass savanna samples, the difference being particularly pronounced for extractable P (Table 1). As predicted, biomass production in the paddock margin plot was limited by N (Figure 1), with additional P producing no extra effect. A similar concentration of nutrients by livestock has been reported for a semi-arid savanna in Kenya, with the effects persisting for decades (Augustine 2003).

The grazed patches in our study area were not used continuously by wild herbivores, but were abandoned if the grass grew above a critical height; such an area would then not be grazed again until the accumulated biomass was removed by fire (P.G. Cech, personal observation). This pattern of habitat use by wild herbivores, which has also been observed elsewhere (Archibald and others 2005), might explain why the grazed patch was only moderately enriched compared to tallgrass savanna of the kind from which our grazed patch probably developed (based on similarity in plant species). In the experiment, biomass production in the grazed patch was co-limited by N and P at the second harvest. There is rather little information on nutrient availabilities in grazing lawns (as defined by McNaughton 1984) and in temporarily grazed patches, and we know of no other studies investigating nutrient limitation in such areas. However, McIvor and others (2005) report that the soil in

Table 3. Types of Nutrient Limitation in Tropical Savanna and Forest Ecosystems

Vegetation type	Location	Nutrient limitation	Study type	N:P ratio	Compares best to	Source
Tropical forests	62 sites worldwide	P	Litter NUE		Forest	(1)
Secondary tropical forest	Amazon, Brazil	P	N:P ratios	28.8	Forest	(2)
Cerrado sensu stricto	Brazil	P	Litter NUE	18	Forest	(3)
Old secondary dry tropical forest	Yucatan, Mexico	P	F		Forest	(4) + (5)
Gap in remnant forest (FG)	Tanzania	NPcations	F	12.5–15.0	TS	
Young secondary dry tropical forest	Yucatan, Mexico	NP	F		FG	(4) + (5)
Succession after removal of mature tropical forest	Amazon, Brazil	N or NP	Modelling		FG	(6)
Herbaceous cover in secondary dry forest	Amazon, Brazil	NP	F	10.2	FG	(7)
Humid tallgrass savanna (TG)	Tanzania	N and NP (NPcations)	F	8.6–9.7	TS	
Mesic savanna, natural pastures	Tanzania	N (NP)	F		TG	(8)
Semi-arid savanna	Tanzania	N (NP)	F	6 ⁴	TG	(9)
<i>Hyparrhenia</i> dominant grassland	Zambia	N (NP)	F		TG	(10)
<i>Hyparrhenia</i> dominant grassland	Kenya	N (NP)	F	8.2	TG	(11)
Sahelian C ₄ grasslands	Mali	N? (NP) ¹	F		TG	(12)
Humid savanna, native pastures	Australia	NP	F	14.8	TG	(13)
Flooded savanna	Venezuela	NP (NPKS)	F		TG	(14)
Secondary savanna	Venezuela	N (NPK)	F		TG	(15)
<i>Trachypogon</i> savanna	Venezuela	N and P (NP, NPK)	F	9.8	TG	(16)
<i>Trachypogon</i> savanna	Venezuela	NP? ²	F	11.0	TG	(17)
Rangeland	South Africa	N (NP)	F		TG	(18)
Humid savanna, secondary <i>Acacia</i> woodland (AW)	Tanzania	NP	F	16.7–16.9	TS	
Semi-arid savanna under <i>Acacia tortilis</i> canopy	Tanzania	P	F	12 ⁴	AW	(9)
Overgrazed Sahelian pasture dominated by legume <i>Zornia</i> sp.	Mali	P ³	F	~20 ³	AW	(12)
Humid savanna, grazed patches (GP)	Tanzania	NP (NPcations)	F	9.1–9.7	TS	
Grazing lawns, humid Guinea savanna	Cameroon	N	N:P ratios	5.8	GP/PM	(19)
Humid savanna, soils derived from bomas (PM)	Tanzania	N	F	4.8–5.5	TS	
Semi-arid savanna, soils derived from bomas	Kenya	N (NP)	F	5.2	PM	(20)

Nutrient limitation: the main limiting nutrient(s) is/are reported, in brackets are nutrient combinations which further increased biomass production compared to the main limiting nutrient(s).

Study type: F, factorial fertilization experiment; Litter NUE, nutrient use efficiencies as calculated from litterfall; N:P ratios, limitation assessed according to relative abundance of N and P in plant tissue; Modelling, model of changes in nutrient pools upon forest clearing based on the dataset from a study site.

N:P ratios: calculated from N and P concentrations in foliage of several tree species in the case of forests, from concentrations in total aboveground herbaceous biomass, or as weighted average from concentrations in aboveground biomass of one or several herbaceous species if they represent at least 75% of total biomass and their relative abundance is known in order to prevent a bias due to the large variation of N:P ratios among plant species (compare Güsewell and Koerselman 2002).

Compares best to: indicates the vegetation types from the conceptual model in Figure 1B to which the respective study site is most similar (based on the influence of herbivory, fire and N₂-fixation). FG: forest gaps; TG: tallgrass savanna; AW: *Acacia* woodland; GP: grazed patches; PM: paddock margin.

¹Effect of N addition alone was not tested, because all plots receiving varying levels of N fertilizer were given a basic P dressing first.

²Neither N nor P addition increased aboveground biomass in cut plots, joint addition of both nutrients was not tested.

³Native vegetation was removed and pure stands of two grass and four legume species were sown, N:P ratios of legume species on control plots averaged 19.9 and the N:P ratio of one grass species was 20.7 (no data available for the second grass species).

⁴N:P ratios were determined from young fully expanded leaves of the dominant grass species.

Source: (TS) this study; see Appendix A for references.

patches of lawn formed by cattle was enriched in P but less so in N. This is consistent with our finding that soil extractable P was enriched more in the grazed patch relative to the tallgrass site than were total and extractable N (Table 1). Data from a recent study of grazing lawns in Cameroon indicate N:P ratios in aboveground biomass around 6 (Verweij and others 2006), which is lower than what we observed in the grazed patch (9–10, Tables 2 and 3). However, those grazing lawns are continuously maintained by herbivores and may thus be expected to be more heavily impacted than our grazed patches.

In the *Acacia* woodland plot, biomass production was co-limited by N and P during both growth periods. Although we did not observe P limitation, as reported for two other savannas with N₂-fixing legumes (Table 3), the N:P ratio in the vegetation of the control plots suggests that growth limitation in our *Acacia* woodland was closer to P-limitation than in our other NP-co-limited sites (Table 2). N₂-fixing plants often have a higher P requirement than other plants (Pate 1986), and several studies have shown N₂-fixation in legumes to be limited by P-availability (Israel 1987; Crews 1993; Perreijn 2002; Binkley and others 2003). N₂-fixing plants increase the overall availability of N, which probably increases the demand and uptake of P by other plants as well. The high P demand of *A. zanzibarica* trees and the increased P demand by other plants may thus have reduced soil P availability, as has already been reported for another woody legume (Binkley 1997). Thus, the high N:P ratio in herbaceous vegetation in the *Acacia* woodland (Table 2) was probably due to the combined effect of increased N and reduced P. N₂-fixation may thus have a stronger effect on N:P-stoichiometry relative to its effect on N-accumulation than was hypothesized in Figure 1. Additionally, the herbaceous cover of the *Acacia* woodland is burnt occasionally (P.G. Cech, personal observation), which may slow down the accumulation of N in the soil.

In the forest gap, which was selected as the reference for the other vegetation types, biomass production tended to be co-limited by N and P during the second growth period (Figure 2). Undisturbed tropical forests are thought to be most commonly limited by P because of the advanced weathering of soils (Vitousek 1984), and there is some experimental evidence supporting this view (Table 3; Elser and others 2007), although at the level of individual trees the picture might be more complex with some trees limited by N and others by P (Perreijn 2002). We expected that, starting from P-limited undisturbed forest conditions,

burning would lead in the direction of N-limitation (compare Kauffman and others 1995; Bustamante and others 2006) and hence frequently burned sites (like tall grass savanna) would be N-limited, and less frequently burned sites (like forest gaps) would have an intermediate position between P- and N-limited conditions. The responses of re-growth to nutrient addition in the forest gap were thus in line with our hypothesis that where fire is infrequent the nutrient balance lies in the range of NP-co-limitation. A relatively high total-N:total-P ratio of 19 in the soil of a remnant forest near our forest gaps indicated that the original forest might indeed have been P-limited (P.G. Cech, unpublished data). The shift from P-limitation towards N-limitation following the removal of tropical forest is also supported by other studies (see Table 3).

Our conceptual framework of the effects of fire, herbivory and N₂-fixation on soil N pool and N:P-stoichiometry (Figure 1) was supported by a good match between the hypothesized arrangement of the investigated vegetation types and measured soil N pools and N:P-stoichiometry (represented by the N:P ratio in the aboveground vegetation) (compare Figures 1B and 3). In the case of the *Acacia* woodland and the grazed patch measured N:P ratios deviated from our predictions, we have discussed possible causes for these deviations above.

The data on soil N pools also support the conceptual framework in various ways. First, the site that has probably burnt most frequently—the tallgrass savanna (Frost and Robertson 1987; P.G. Cech, personal observation)—had the lowest soil N pool (Figure 3, Table 1). This result is in line with lower soil N reported for long-term burning experiments (Ojima and others 1994; Fynn and others 2003). Secondly, the localized return of dung and urine by domestic and wild herbivores increased soil N in both the grazed patch and the paddock margin. Enrichment of soil N by domestic herbivores has also been demonstrated in semi-arid savanna (Augustine 2003). Thirdly, the secondary *Acacia* woodland had significantly higher amounts of soil N than the tallgrass savanna, the grazed patch or the paddock margin (Table 1, Figure 3). Several studies report that woody legumes invading grasslands increase total soil N and N availability, these effects being attributed at least partly to their ability to fix atmospheric N₂ (Stock and others 1995; Geesing and others 2000; Archer 2001; Hagos and Smit 2005; Scherer-Lorenzen and others 2007). Finally, the forest gap had the largest soil N pool (Table 1), which may be explained by low fire frequency and possibly by the N input from tree litter (Hudak and others 2003). The correlation

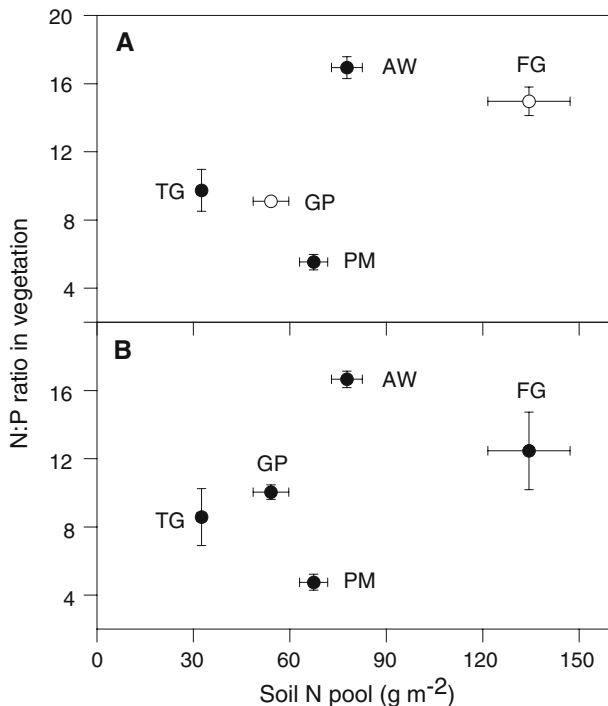


Figure 3. Total soil N in topsoil and N:P ratios in aboveground biomass of five sites in Saadani National Park: forest gap (FG), tallgrass savanna (TG), secondary *Acacia* woodland (AW), grazed patch (GP), paddock margin (PM), at the first harvest (**A**) and the second harvest (**B**). *Open symbols* indicate that N:P ratio in vegetation may not be a good indicator of nutrient limitation, because water was likely the limiting resource. Data are shown as mean per site and *error bars* represent standard errors ($n = 5$).

between soil N and C pools (Table 1) suggests that the loss and accumulation of N is closely bound to the loss and accumulation of organic matter.

The ratio of N:P in the aboveground herbaceous vegetation has been used to assess nutrient limitation in temperate ecosystems (Koerselman and Meuleman 1996; Olde Venterink and others 2003; Güsewell 2004). Koerselman and Meuleman (1996) proposed that at N:P ratios below 14, productivity was limited by N, between 14 and 16 production was co-limited by N and P, and above 16 limited by P. Whether NP-co-limitation can be separated from P-limitation based on N:P ratios is not fully clear (see Olde Venterink and others 2003), but the level of less than about 14 for N-limitation is consistent and supported by other studies in temperate regions (Olde Venterink and others 2003; Güsewell 2004). For a dry savanna, Ludwig and others (2001) reported variation in N:P ratios among growing seasons, and the results of a fertilization experiment did not support the predicted type of limitation based on critical values of N:P from temperate regions. They

suggested that the critical N:P values for the boundaries between N-limitation and NP-co-limitation should be at 6 for savanna vegetations, and that between NP-co-limitation and P-limitation at 12. As a possible explanation, they suggested the higher N use efficiency of C₄ grasses compared to C₃ plants. However, it should be noted that N:P ratios reported in the study of Ludwig and others (2001) were measured from young, fully expanded leaves of the dominant grass species and not from above-ground biomass. From our data, we suggest that the critical N:P ratio reflecting the transition from N-limitation to NP-co-limitation lies between 8.6 and 10.0 (compare type of nutrient limitation as determined by fertilization, and N:P ratios of the control plots for the plots not limited by water; Figure 2 and Table 2). If P-limitation and NP-co-limitation can be separated by N:P ratios, the critical boundary value should be higher than 16.9. These ranges come close to the boundaries values of 6.7 and 20.4 given by Penning de Vries and others (1980) for Sahelian grasslands. Considering the data available from literature, we conclude that the critical N:P value indicating N-limitation in tropical savannas is probably less than about 9; this value is less than the below 14 determined for temperate regions, but

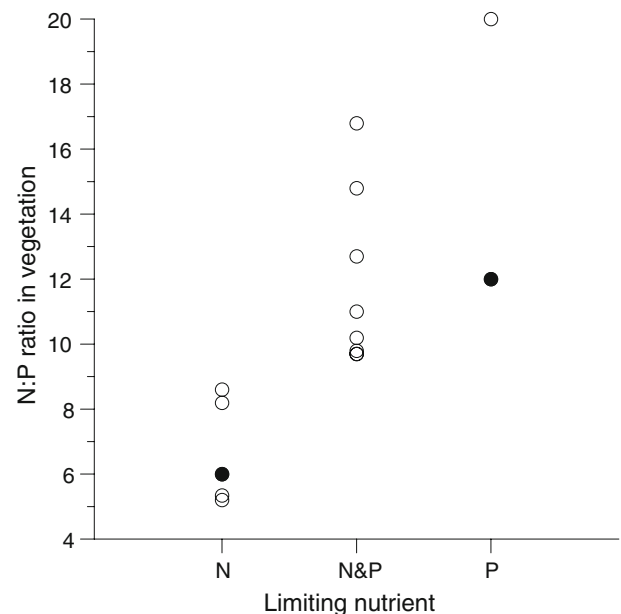


Figure 4. N:P ratios versus type of limitation in aboveground herbaceous vegetation of tropical savannas. N:P ratios are from unfertilized control plots and type of limitation was determined by fertilization experiments (data from Table 3). *Open circles* show data for which N:P ratios represents at least 75% of aboveground herbaceous biomass, *solid circles* show data from Ludwig and others (2001) who measured N:P ratios in young fully expanded leaves of the dominant grass species.

more than the less than 6 mentioned by Ludwig and others (2001) (Figure 4). We agree with Ludwig and others (2001) that the difference compared with temperate ecosystems is probably due to the very high N use efficiency of tropical C₄ grasses (Sage and Percy 1987; Le Roux and Mordelet 1995).

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REFERENCES

- Aranibar JN, Otter L, Macko SA, Feral CJW, Epstein HE, Dowty PR, Eckardt F, Shugart HH, Swap RJ. 2004. Nitrogen cycling in the soil-plant system along a precipitation gradient in the Kalahari sands. *Glob Change Biol* 10:359–73.
- Archer S. 1995. Tree-grass dynamics in a *Prosopis*-thornscrub savanna parkland: reconstructing the past and predicting the future. *Ecoscience* 2:83–99.
- Archer S. 2001. Trees in grasslands: biogeochemical consequences of woody plant expansion. In: Schulze E-D, Harrison SP, Heimann M, Holland EA, Lloyd J, Prentice IC, Schimel D, Eds. *Global biogeochemical cycles in the climate system*. San Diego: Academic Press. pp 115–37.
- Archibald S, Bond WJ, Stock WD, Fairbanks DHK. 2005. Shaping the landscape: fire-grazer interactions in an African savanna. *Ecol Appl* 15:96–109.
- Augustine DJ. 2003. Long-term, livestock-mediated redistribution of nitrogen and phosphorus in an East African savanna. *J Appl Ecol* 40:137–49.
- Augustine DJ, McNaughton SJ, Frank DA. 2003. Feedbacks between soil nutrients and large herbivores in a managed savanna ecosystem. *Ecol Appl* 13:1325–37.
- Barger NN, D'Antonio CM, Ghneim T, Brink K, Cuevas E. 2002. Nutrient limitation to primary productivity in a secondary savanna in Venezuela. *Biotropica* 34:493–501.
- Bell RHV. 1982. The effect of soil nutrient availability on community structure in African ecosystems. In: Huntley BJ, Walker BH, Eds. *Ecology of tropical savannas*. Berlin: Springer. pp 192–216.
- Binkley D. 1997. Bioassays of the influence of *Eucalyptus saligna* and *Albizia falcataria* on soil nutrient supply and limitation. *For Ecol Manage* 91:229–34.
- Binkley D, Senock R, Cromack K. 2003. Phosphorus limitation on nitrogen fixation by *Facaltaria* seedlings. *For Ecol Manage* 186:171–6.
- Bond WJ, Woodward FI, Midgley GF. 2005. The global distribution of ecosystems in a world without fire. *New Phytol* 165:525–37.
- Bourliere F, Hadley M. 1983. Present day savannas: an overview. In: Bourliere F, Ed. *Tropical savannas*. Amsterdam: Elsevier. pp 1–18.
- Bray RH, Kurtz LT. 1945. Determination of total, organic and available forms of phosphorus in soils. *Soil Sci* 59:39–45.
- Brockington NR. 1961. Studies of the growth of a *Hyparrhenia*-dominant grassland in Northern Rhodesia. II. Fertilizer responses. III. The effect of fire. *Grass Forage Sci* 16:54–65.
- Brown JR, Archer S. 1989. Woody plant invasion of grasslands: establishment of honey mesquite (*Prosopis glandulosa* var. *glandulosa*) on sites differing in herbaceous biomass and grazing history. *Oecologia* 80:19–26.
- Bustamante MMC, Medina E, Asner GP, Nardoto GB, Garcia-Montiel DC. 2006. Nitrogen cycling in tropical and temperate savannas. *Biogeochemistry* 79:209–37.
- Carter MR. 1993. *Soil sampling and methods of analysis*. Boca Raton: Lewis.
- Cech PG. 2008. Impact of fire, large herbivores and N₂-fixation on nutrient cycling in humid savanna, Tanzania. PhD thesis, Zurich, Switzerland: Federal Institute of Technology (ETH).
- Cook GD. 1994. The fate of nutrients during fires in a tropical savanna. *Aust J Ecol* 19:359–65.
- Cramer MD, Chimphango SBM, Van Cauter A, Waldram MS, Bond W. 2007. Grass competition induces N₂ fixation in some species of African *Acacia*. *J Ecol* 95:1123–33.
- Crews TE. 1993. Phosphorus regulation of nitrogen fixation in a traditional Mexican agroecosystem. *Biogeochemistry* 21:141–66.
- Edwards PJ, Hollis S. 1982. The distribution of excreta on New Forest grassland used by cattle, ponies and deer. *J Appl Ecol* 19:953–64.
- Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol Lett* 10:1135–42.
- Frank DA, Zhang YM. 1997. Ammonia volatilization from a seasonally and spatially variable grazed grassland: Yellowstone National Park. *Biogeochemistry* 36:189–203.
- Frost PGH, Robertson F. 1987. The ecological effects of fire in savannas. In: Walker BH, Ed. *Determinants of tropical savannas*. Oxford: IRL Press. pp 93–140.
- Fynn RWS, Haynes RJ, O'Connor TG. 2003. Burning causes long-term changes in soil organic matter content of a South African grassland. *Soil Biol Biochem* 35:677–87.
- Geesing D, Felker P, Bingham RL. 2000. Influence of mesquite (*Prosopis glandulosa*) on soil nitrogen and carbon development: implications for global carbon sequestration. *J Arid Environ* 46:157–80.
- Güsewell S. 2004. N:P ratios in terrestrial plants: variation and functional significance. *New Phytol* 164:243–66.
- Güsewell S, Koerselman W. 2002. Variation in nitrogen and phosphorus concentrations of wetland plants. *Perspect Plant Ecol Evol Syst* 5:37–61.
- Hagos MG, Smit GN. 2005. Soil enrichment by *Acacia mellifera* subsp. *detinens* on nutrient poor sandy soil in a semi-arid southern African savanna. *J Arid Environ* 61:47–59.
- Hobbs NT, Schimel DS, Owensby CE, Ojima DS. 1991. Fire and grazing in the tallgrass prairie: contingent effects on nitrogen budgets. *Ecology* 72:1374–82.
- Högberg P. 1986. Nitrogen-fixation and nutrient relations in savanna woodland trees (Tanzania). *J Appl Ecol* 23:675–88.

- Holdo RM, Holt RD, Coughenour MB, Ritchie ME. 2007. Plant productivity and soil nitrogen as a function of grazing, migration and fire in an African savanna. *J Ecol* 95:115–28.
- Hudak AT. 1999. Rangeland mismanagement in South Africa: failure to apply ecological knowledge. *Hum Ecol* 27:55–78.
- Hudak AT, Wessman CA, Seastedt TR. 2003. Woody overstorey effects on soil carbon and nitrogen pools in South African savanna. *Aust Ecol* 28:173–81.
- Hunt J. 1982. Dilute hydrochloric acid extraction of plant material for routine cation analysis. *Commun Soil Sci Plan* 13:49–55.
- Huntley BJ. 1982. Southern African savannas. In: Huntley BJ, Walker BH, Eds. *Ecology of tropical savannas*. Berlin: Springer. pp 101–19.
- Israel DW. 1987. Investigation of the role of phosphorus in symbiotic dinitrogen fixation. *Plant Physiol* 84:835–40.
- Jewell PL, Kauferle D, Gusewell S, Berry NR, Kreuzer M, Edwards PJ. 2007. Redistribution of phosphorus by mountain pasture in cattle on a traditional the Alps. *Agr Ecosyst Environ* 122:377–86.
- Kauffman JB, Cummings DL, Ward DE, Babbitt R. 1995. Fire in the Brazilian Amazon: 1. Biomass, nutrient pools, and losses in slashed primary forests. *Oecologia* 104:397–408.
- Klötzli F. 1980. Analysis of species oscillations in tropical grasslands in Tanzania due to management and weather conditions. *Phytocoenologia* 8:13–33.
- Koerselman W, Meuleman AFM. 1996. The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. *J Appl Ecol* 33:1441–50.
- Laclau JP, Sama-Poumba W, Nzila JD, Bouillet JP, Ranger J. 2002. Biomass and nutrient dynamics in a littoral savanna subjected to annual fires in Congo. *Acta Oecol* 23:41–50.
- Lambers H, Raven JA, Shaver GR, Smith SE. 2008. Plant nutrient-acquisition strategies change with soil age. *Trends Ecol Evol* 23:95–103.
- Lamoot I, Callebaut J, Degezelle T, Demeulnaere E, Laquière J, Vandenberghe C, Hoffmann M. 2004. Eliminative behaviour of free-ranging horses: do they show latrine behaviour or do they defecate where they graze? *Appl Anim Behav Sci* 86:105–21.
- Le Roux X, Mordelet P. 1995. Leaf and canopy CO₂ assimilation in a West African humid savanna during the early growing season. *J Trop Ecol* 11:529–45.
- Ludwig F, de Kroon H, Prins HHT, Berendse F. 2001. Effects of nutrients and shade on tree-grass interactions in an East African savanna. *J Veg Sci* 12:579–88.
- McIvor JG, McIntyre S, Saeli I, Hodgkinson JJ. 2005. Patch dynamics in grazed subtropical native pastures in south-east Queensland. *Aust Ecol* 30:445–64.
- McNaughton SJ. 1984. Grazing lawns: animals in herds, plant form and coevolution. *Am Nat* 124:863–86.
- McNaughton SJ. 1988. Mineral nutrition and spatial concentrations of African ungulates. *Nature* 334:343–5.
- McNaughton SJ. 1990. Mineral nutrition and seasonal movements of African migratory ungulates. *Nature* 345:613–5.
- Medina E. 1987. Nutrients: requirements, conservation and cycles in the herbaceous layer. In: Walker BH, Ed. *Determinants of tropical savannas*. Oxford: IRL Press. pp 39–65.
- Norman MJT. 1966. Katherine research station 1956–64: a review of published work. Melbourne: CSIRO.
- Ojima DS, Schimel DS, Parton WJ, Owensby CE. 1994. Long-term and short-term effects of fire on nitrogen cycling in tallgrass prairie. *Biogeochemistry* 24:67–84.
- Olde Venterink H, Wassen MJ, Verkroost AWM, de Ruiter PC. 2003. Species richness-productivity patterns differ between N-, P-, and K-limited wetlands. *Ecology* 84:2191–9.
- Pate JS. 1986. Economy of symbiotic nitrogen fixation. In: Givinish TJ, Ed. *On the economy of plant form and function*. Cambridge: Cambridge University Press. pp 299–325.
- de Penning Vries FWT, Krul JM, Van Keulen H. 1980. Productivity of Sahelian rangelands in relation to the availability of nitrogen and phosphorus from the soil. In: Rosswall T, Ed. *Nitrogen cycling in West African ecosystems*. Stockholm: Royal Swedish Academy of Sciences. pp 95–113.
- Perreijn K. 2002. Symbiotic nitrogen fixation by leguminous trees in tropical rain forest in Guyana. *Tropenbos-Guyana Series* 11. Wageningen: Tropenbos.
- Ruess RW, McNaughton SJ. 1988. Ammonia volatilization and the effects of large grazing mammals on nutrient loss from East African grasslands. *Oecologia* 77:382–6.
- Sage RF, Percy RW. 1987. The nitrogen use efficiency of C₃ and C₄ plants. 2. Leaf nitrogen effects on the gas-exchange characteristics of *Chenopodium album* (L.) and *Amaranthus retroflexus* (L.). *Plant Physiol* 84:959–63.
- Sanchez PA. 1976. *Properties and management of soils in the tropics*. New York: Wiley.
- Sankaran M, Hanan NP, Scholes RJ, Ratnam J, Augustine DJ, Cade BS, Gignoux J, Higgins SI, Le Roux X, Ludwig F, Ardo J, Banyikwa F, Bronn A, Bucini G, Caylor KK, Coughenour MB, Diouf A, Ekaya W, Feral CJ, February EC, Frost PGH, Hiernaux P, Hrabar H, Metzger KL, Prins HHT, Ringrose S, Sea W, Tews J, Worden J, Zambatis N. 2005. Determinants of woody cover in African savannas. *Nature* 438:846–9.
- Scherer-Lorenzen M, Olde Venterink H, Buschmann H. 2007. Nitrogen enrichment and plant invasions: the importance of nitrogen fixing plants and anthropogenic eutrophication. In: Nentwig W, Ed. *Biological invasions, Ecological studies*. Berlin: Springer. pp 163–80.
- Snyman HA. 2002. Short-term response of rangeland botanical composition and productivity to fertilization (N and P) in a semi-arid climate of South Africa. *J Arid Environ* 50:167–83.
- Stock WD, Wienand KT, Baker AC. 1995. Impacts of invading N₂-fixing *Acacia* species on patterns of nutrient cycling in 2 Cape ecosystems: evidence from soil incubation studies and ¹⁵N natural abundance values. *Oecologia* 101:375–82.
- Tobler MW, Cochard R, Edwards PJ. 2003. The impact of cattle ranching on large-scale vegetation patterns in a coastal savanna in Tanzania. *J Appl Ecol* 40:430–44.
- Treydte AC, Edwards PJ, Suter W. 2005. Shifts in native ungulate communities on a former cattle ranch in Tanzania. *Afr J Ecol* 43:302–11.
- Van de Vijver CADM, Poot P, Prins HHT. 1999. Causes of increased nutrient concentrations in post-fire regrowth in an East African savanna. *Plant Soil* 214:173–85.
- Verweij RJT, Verrelst J, Loth PE, Heitkonig IMA, Brunsting AMH. 2006. Grazing lawns contribute to the subsistence of mesoherbivores on dystrophic savannas. *Oikos* 114:108–16.
- Vitousek PM. 1984. Litterfall, nutrient cycling, and nutrient limitation in tropical forests. *Ecology* 65:285–98.
- Weinmann H. 1938. Effect of fertiliser treatment on Transvaal highveld. *S Afr J Sci* 35:246–9.