

Nutrient concentration ratios and co-limitation in South African grasslands

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Summary

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- Assessing plant nutrient limitation is a fundamental part of understanding grassland dynamics. The ratio of concentrations of nitrogen (N) and phosphorus (P) in vegetation has been proposed as an index of the relative limitation of biomass production by N and P, but its utility has not been tested well in grasslands.
- At five sites in Kruger National Park, South Africa, across soil and precipitation contrasts, N and P were added in a factorial design to grass-dominated plots.
- Although the N:P ratio of unfertilized vegetation across all sites (5.8) would have indicated that production was N-limited, aboveground production was consistently co-limited by N and P. Aboveground production was still greater in plots fertilized with N and P than in those fertilized with just N, but the N:P ratio did not exceed standard thresholds for P limitation in N-fertilized vegetation. Comparisons among sites showed little pattern between site N:P ratio and relative responses to N and P.
- When combined with results from other grassland fertilization studies, these data suggest that the N:P ratio of grasses has little ability to predict limitation in upland grasslands. Co-limitation between N and P appears to be much more widespread than would be predicted from simple assumptions of vegetative N:P ratios.

Key words: co-limitation, ecosystem management, grasslands, Kruger National Park, nitrogen (N), phosphorus (P), stoichiometry.

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Introduction

Understanding the dynamics of nutrient-limited grasslands and savannas and informing ecosystem stewards as to best management practices for these ecosystems first require determination of which nutrients are limiting primary production. Nutrient addition experiments are the classic approach to determining which nutrients limit primary production and the relative degrees of limitation at a given site (Chapin *et al.*, 1986; Vitousek & Howarth, 1991). In grasslands, many different nutrients can limit production and often different nutrients co-limit productivity (e.g. Olf & Pöstel, 1994). Yet, fertilization studies can be intensive undertakings and their results can be difficult to interpret when vegetation does not respond to a given nutrient (Rastetter & Shaver, 1992; Aerts & Chapin, 2000). As such,

nitrogen (N) and phosphorus (P) ratios in vegetation have been offered as a simpler index of the limitation of N and P (Penning de Vries *et al.*, 1980; Koerselman & Meuleman, 1996; Tessier & Raynal, 2003; Güsewell, 2004) and have been used to describe broad geographic patterns of nutrient limitation (Reich & Oleksyn, 2004; Wassen *et al.*, 2005).

The use of N:P ratios as an index of nutrient limitation appears to be well rooted in ecological theory and has strong support in the literature. Empirically, N:P ratios have been shown to be effective predictors of nutrient limitation. Most notably, in a review of 40 wetland fertilization studies from northern Europe, vegetation N:P predicted the response of aboveground net primary production to fertilizations (Koerselman & Meuleman, 1996). When plant N:P, expressed as a ratio of mass concentrations, was < 14, N was limiting. When plant N:P was > 16, P was limiting. In

between, N and P were co-limiting. Tessier & Raynal (2003) recently reviewed fertilization studies in upland ecosystems and they supported the use of ratios to predict limitation, stating that 'N:P ratios can be effective predictors of nutrient limitation in upland ecosystems [and] can be used for management and monitoring purposes in considering the nutrient status of upland ecosystems'. Güsewell (2004) proposed a broader range of ratios for co-limitation of plant communities, stating that 'it appears that biomass production is most likely to be enhanced by N fertilization in vegetation with N:P ratio < 10 and by P fertilization in vegetation with N:P ratio > 20, whereas within this range, the effects of fertilization are not unequivocally related to N:P ratios.'

Despite the apparent support for its utility across two recent reviews (Tessier & Raynal, 2003; Güsewell, 2004), the ability of plant N:P to generally predict whether N and/or P is limiting in grasslands should be revisited. First, Tessier & Raynal (2003) largely rely on changes in nutrient concentrations as an index of nutrient limitation, as opposed to increases in biomass or production. Yet, increases in nutrient concentrations with fertilization do not necessarily precede increases in production and their conclusions would not necessarily apply to limitation of production. Secondly, Güsewell (2004) relied on two publications in assessing critical N:P ratios for limitation in upland vegetation. The first was Tessier & Raynal (2003), which suffers from relying on responses of concentrations, not biomass, to fertilization. The second was Penning de Vries *et al.* (1980), which discussed nutrient limitation in Sahelian grasslands. In the paper, the authors suggest critical N:P ratios of 6.6 and 27, but the evidence for these was drawn from unpublished studies and relied on changes in nutrient concentrations, not increases in biomass. Although these suggestions of critical N:P ratios might be suitable for hypothesis generation, they cannot be considered evidence for specific critical ratios of limitation.

To better understand the patterns of N and P limitation in grasslands, and to test whether ratio thresholds are appropriate for these ecosystems, we test the ability of N:P to predict limitation across five sites in Kruger National Park (KNP), South Africa and then compare those results with those of recently published grassland fertilization studies. At KNP, we fertilized a range of sites that differed in soil parent material (either basalt or granite) and mean annual precipitation. Basalt soils are relatively rich in base cations compared with the granite soils (Venter *et al.*, 2003), but foliar N:P is relatively low for both soil types. Across landscape positions and seasons, Grant & Scholes (2006) found that mean foliar N:P ratios were 6.9 on basalts and 10.5 on granites, with N:P as low as 3 on both soil types. If N:P ratios index nutrient limitation in these ecosystems, we expected that aboveground biomass should primarily respond to N addition. Independent of any specific threshold, vegetation on granite soils, which has higher N:P, should respond more to P addition or be more likely to respond to P alone than that on basalt sites. To test these predictions, we compare average responses of aboveground

biomass to fertilization for all five sites, variation in responses across the sites, and the responses of plots fertilized with a single nutrient with those of plots fertilized with both N and P.

Materials and Methods

Five sites were selected for experimental N and P additions in KNP, which covers approx. 2×10^6 ha in northeastern South Africa, and is largely savanna and grassland with soils generally derived from basalt parent material in the eastern portion of the park and from granites in the west. Precipitation is highest in the southwest and declines eastward and northward. Fire is an important component of most KNP ecosystems, and a diverse contingent of herbivores are present, including megaherbivores. Two sites were selected on granite soils (Pretoriuskop and Letaba) and the other three sites were on basalt soils (Makhohlola, Satara, and Nwashitsumbe) (Table 1). Data for each site on soil texture, water holding capacity, pH, carbon (C) and N concentrations, Bray II available P, and 56-d potential N mineralization were taken from Craine *et al.* (2007).

Each site contained 32 $2 \text{ m} \times 2 \text{ m}$ plots with treatments randomized among plots. Fertilizer was applied in July 2003 and March 2004. Eight plots received no fertilizer, eight received additional N in the form of NH_4NO_3 at a rate of $10 \text{ g m}^{-2} \text{ yr}^{-1}$, eight received additional P in the form of superphosphate ($91.6 \text{ mg g}^{-1} \text{ P}$, 250 mg g^{-1} calcium (Ca) and 132 mg g^{-1} sulphur (S)) at a rate of $5 \text{ g P m}^{-2} \text{ yr}^{-1}$, and eight received additional N and P at the same rates as the single-nutrient addition plots. All vegetation was trimmed to 5 cm at the initiation of the experiment in June 2003 and again after the March 2004 harvest in order to mimic the removal of biomass that generally occurs regularly via fire or grazing in KNP.

In January and March of 2004 and 2005, 0.1 m^2 of biomass was clipped from each plot at a height of 5 cm. Biomass was separated into grass and dicotyledonous biomass, dried at 40°C and then weighed. Very little dead biomass was observed in any of the samples and forb biomass was an average of only 11% of total biomass. Grass biomass for each harvest except March 2004 was then ground and analyzed for C, N, and P concentrations. C and N concentrations were determined via combustion on an elemental analyzer (Carlo-Erba, Milan, Italy). For P concentrations, leaf biomass was first digested in a 10 : 1 : 1 mixture of HNO_3 , HClO_4 , and H_2SO_4 for 60 min at 150°C followed by 20 min at 250°C . Phosphorous concentrations were determined on distilled water extract of the residuum with malachite green colorimetry (Diatloff & Rengel, 2001) on a Biotek Powerwave XS microplate spectrophotometer (Biotek Instruments, Inc., Winooski, VT, USA).

Fine root productivity was assessed during the 2005 growing season with ingrowth cores (Craine *et al.*, 2002). At each plot during the March 2004 harvest, a 5-cm core to 25 cm depth was removed. Wire mesh was inserted into the hole to delineate the outside edge of the ingrowth core. The hole was

Table 1 Site characteristics for the five sites used in this study

	Site				
	Pretoriuskop	Letaba	Makhohlola	Satara	Nwashitsumbe
Soil type	Granite	Granite	Basalt	Basalt	Basalt
Collection date	27 Mar 04	19 Mar 05	26 Mar 04	1 Apr 05	30 Mar 05
Latitude	-25.1287	-23.7559	-25.2962	-24.4044	-22.7800
Longitude	31.2337	31.4335	31.9140	31.7486	31.2508
MAP (mm)	737	457	620	544	516
Altitude (m)	580	278	247	308	388
WHC	29.15	34.08	49.27	54.70	60.21
LOI	1.37	2.20	6.87	5.78	5.61
Sand content	93.34	71.99	56.62	56.97	66.62
Silt content	5.64	17.51	26.00	26.52	18.00
Clay content	1.02	10.50	17.38	16.51	15.38
Soil pH	6.4	6.6	6.1	6.2	6.9
Soil C (mg g ⁻¹)	3.3	4.76	21.92	18.64	16.76
Soil N (mg g ⁻¹)	0.52	0.76	1.42	1.59	1.67
P Bray II (mg kg ⁻¹)	3.23	11.32	85.43	53.17	39.45
N _{min} (μmol g ⁻¹)	0.63	0.10	2.10	0.43	0.99

Data on mean annual precipitation (MAP) are taken from Zambatis (2003) and other soil data are taken from Craine *et al.* (2007). WHC, soil water holding capacity; LOI, per cent mass lost on ignition; N_{min}, 56-d potential N mineralization.

refilled with sieved soil from the original core and the soil was tamped down by hand to approximate the bulk density of surrounding soil. Cores were harvested a year later during the March 2005 harvest by taking a 4-cm core from inside the mesh. Roots were washed, dried at 40°C and then weighed.

Data on biomass and nutrient concentrations over the four harvests were analyzed with a repeated measures ANOVA in JMP 5.0.1 (SAS Institute, Cary, NC, USA). As we discuss in the results, we compared fertilization responses within sites with additional ANOVAs, one for each site. We also ran individual ANOVAs for each harvest for illustrative purposes associated with graphical representation of differences among harvests in treatments, but consider these results less robust than the repeated measures ANOVA.

In adding two nutrients in factorial, there are two types of responses that would indicate co-limitation. First, biomass could increase when N is added individually as well as when P is added individually. Secondly, biomass could increase only when N and P are added together. If biomass increases significantly when one nutrient is added as well as when both nutrients are added, we consider vegetation to be co-limited when the increase in biomass from adding both nutrients relative to just one nutrient is greater than the increase in biomass from just adding one nutrient.

Results

Average aboveground production for unfertilized plots was highest at the two high-precipitation sites, Pretoriuskop and Makhohlola (362 and 321 g m⁻², respectively; Table 2), and

lowest at the low-precipitation sites, Letaba and Nwashitsumbe (106 and 249 g m⁻²; Table 2). Production was greatest in March 2004, with January 2004 having the lowest biomass (586 g m⁻² vs 118 g m⁻²; Fig. 1), although differences in production among sites varied over time ($P < 0.001$; Table 3, Fig. 1).

The responses of aboveground biomass to fertilization indicate co-limitation between N and P that is consistent across sites and over time (Fig. 1, Table 3). When averaged across sites and over the four harvests, the addition of N or P alone did not lead to significant increases in aboveground biomass, although plots fertilized with N tended to have higher biomass than unfertilized plots (22% greater aboveground biomass). Plots fertilized with N and P had 54% higher biomass than control plots and 31% higher biomass than N-addition plots (Table 2). The effect of fertilization on aboveground biomass did not vary significantly across sites ($P = 0.25$) or harvests ($P > 0.16$; Table 3). Total forb biomass was unresponsive to fertilizer treatment ($P > 0.05$ for all four harvests) and fertilization did not alter fine root productivity in 2004–2005 (Table 2).

Although N and P both limited production, the N:P ratios for unfertilized vegetation were far below established thresholds for co-limitation. For plots that were not fertilized, averaged across all sites and dates, [N] was 8.7 mg g⁻¹ and [P] was 1.8 mg g⁻¹, generating a mean N:P of 5.8 (Table 2).

Comparing the biomass of plots fertilized with two nutrients to that of plots fertilized with just one is the equivalent of examining the response of vegetation fertilized with one nutrient to the addition of a second nutrient. Comparing

Table 2 Treatment differences among sites averaged among harvests^a

Site ^b	Treatment	[C] ^{c,d}	[N]	[P]	N:P	AG biomass ^e	2005 BNPP
Pretoriuskop	Control	424.3 ± 2.3 ^b	7.8 ± 0.7 ^b	0.9 ± 0.1 ^c	9.2 ± 0.6 ^b	362.4 ± 63.9 ^b	267.3 ± 35.2 ^a
	N	436.6 ± 1.9 ^a	9.1 ± 1.1 ^a	0.6 ± 0.1 ^c	17.7 ± 1.0 ^a	455.9 ± 70.2 ^{ab}	287.5 ± 68.4 ^a
	P	425.9 ± 2.5 ^b	7.4 ± 0.9 ^b	2.1 ± 0.1 ^a	3.8 ± 0.5 ^d	353.1 ± 62.6 ^b	326.4 ± 90.3 ^a
	NP	427.4 ± 2.7 ^b	10.7 ± 1.5 ^a	1.5 ± 0.1 ^b	7.1 ± 0.8 ^c	488.7 ± 64.7 ^a	278.2 ± 48.8 ^a
Letaba	Control	411.7 ± 2.0 ^a	9.6 ± 0.5 ^c	1.5 ± 0.1 ^a	6.6 ± 0.3 ^c	105.9 ± 17.5 ^c	231.6 ± 27 ^a
	N	410.7 ± 3.3 ^a	15.6 ± 0.7 ^a	1.4 ± 0.1 ^a	11.5 ± 0.4 ^a	134.0 ± 25.3 ^{bc}	219.1 ± 25.4 ^a
	P	405.9 ± 4.6 ^b	10.3 ± 0.5 ^c	1.7 ± 0.1 ^a	6.2 ± 0.3 ^c	168.6 ± 28.3 ^{ab}	326.4 ± 36.6 ^a
	NP	416.4 ± 2.4 ^a	14.1 ± 0.8 ^b	1.7 ± 0.1 ^a	8.7 ± 0.4 ^b	227.4 ± 61.4 ^a	272.0 ± 95.6 ^a
Makhohlola	Control	408.5 ± 3.5 ^{ab}	9.1 ± 0.8 ^b	2.8 ± 0.1 ^a	3.2 ± 0.2 ^b	321.2 ± 43.7 ^b	208.3 ± 32.8 ^a
	N	416.2 ± 2.7 ^a	13.0 ± 1.4 ^a	2.3 ± 0.1 ^b	5.8 ± 0.6 ^a	368.2 ± 57.5 ^b	231.6 ± 73.1 ^a
	P	404.4 ± 3.2 ^b	10.1 ± 1.0 ^b	2.6 ± 0.2 ^a	3.9 ± 0.4 ^b	322.9 ± 51.2 ^b	206.7 ± 50.1 ^a
	NP	410.7 ± 2.5 ^{ab}	13.8 ± 1.4 ^a	2.2 ± 0.1 ^b	6.2 ± 0.6 ^a	514.0 ± 68.8 ^a	223.8 ± 54.8 ^a
Satara	Control	419.4 ± 2.5 ^a	6.9 ± 0.4 ^c	1.8 ± 0.1 ^{ab}	4.4 ± 0.6 ^b	294.2 ± 62.1 ^b	296.9 ± 74 ^a
	N	419.5 ± 2.9 ^a	11.0 ± 0.7 ^a	1.7 ± 0.1 ^{ab}	7.0 ± 0.6 ^a	352.7 ± 56.2 ^{ab}	225.6 ± 49.4 ^a
	P	418.9 ± 3.5 ^a	8.7 ± 0.7 ^{bc}	2.0 ± 0.2 ^a	5.0 ± 0.7 ^b	295.2 ± 48.8 ^{ab}	303.1 ± 55.5 ^a
	NP	419.3 ± 7.4 ^a	10.1 ± 1.0 ^{ab}	1.4 ± 0.1 ^b	7.0 ± 0.4 ^a	532.6 ± 115.6 ^a	267.3 ± 32.8 ^a
Nwashitsumbe	Control	398.4 ± 2.5 ^b	9.9 ± 0.6 ^b	2.3 ± 0.2 ^{ab}	5.1 ± 0.6 ^c	247.8 ± 70.5 ^a	110.4 ± 18.4 ^a
	N	398.4 ± 2.5 ^b	14.1 ± 1.2 ^a	2.1 ± 0.2 ^b	7.5 ± 0.7 ^b	253.2 ± 72.5 ^a	144.5 ± 15.0 ^a
	P	397.7 ± 3.8 ^b	9.6 ± 1.0 ^b	2.5 ± 0.3 ^a	4.5 ± 0.5 ^c	157.7 ± 38.5 ^a	138.3 ± 20.4 ^a
	NP	410.5 ± 2.9 ^a	13.3 ± 1.1 ^a	1.6 ± 0.1 ^c	8.8 ± 0.8 ^a	289.7 ± 89.2 ^a	122.8 ± 13.8 ^a
All sites	Control	412.4 ± 1.4 ^{ab}	8.7 ± 0.3 ^b	1.8 ± 0.1 ^b	5.8 ± 0.3 ^c	266.3 ± 25.3 ^a	222.9 ± 37.5 ^a
	N	416.3 ± 1.7 ^a	12.6 ± 0.5 ^a	1.6 ± 0.1 ^{bc}	9.9 ± 0.5 ^a	312.8 ± 27.4 ^a	221.7 ± 46.3 ^a
	P	410.9 ± 1.8 ^b	9.2 ± 0.4 ^b	2.2 ± 0.1 ^a	4.7 ± 0.2 ^d	258.9 ± 21.8 ^a	260.2 ± 50.6 ^a
	NP	416.9 ± 1.8 ^a	12.5 ± 0.6 ^a	1.7 ± 0.1 ^c	7.6 ± 0.3 ^b	410.5 ± 37.8 ^b	232.8 ± 49.1 ^a

^aIncluded are means and standard errors for aboveground biomass (AG biomass; g m⁻²) and belowground productivity (BNPP) 0–20 cm for the 2005 growing season (g m⁻²).

^bPretoriuskop and Letaba were on granite-derived soils, the other three sites on basalt-derived soils.

^cUnits for carbon and nutrient concentrations (mg g⁻¹).

^dData for nutrient concentrations only included three harvests as there were no data for the March 2004 harvest.

^eAboveground biomass data (g m⁻²) were log-transformed before comparisons.

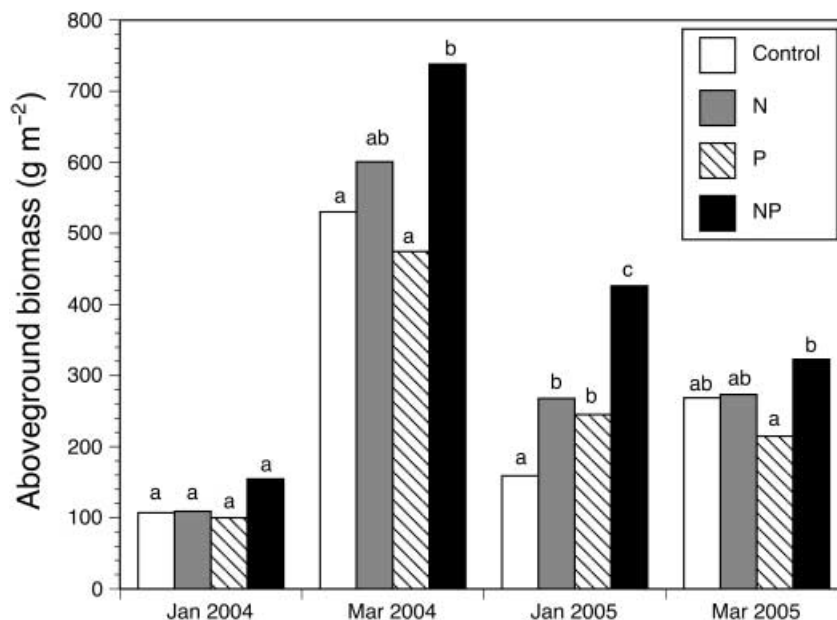


Fig. 1 Aboveground biomass for each treatment at each of four harvests averaged across sites. Differences among treatments in log-transformed aboveground biomass were determined with an ANOVA for each harvest, which is a less robust analysis than the repeated measures ANOVA reported in Table 3.

N-fertilized vegetation with vegetation fertilized with both N and P, N:P ratios of N-fertilized vegetation were still lower than would have been expected based on established ratios for P limitation. The addition of N alone increased foliar N

concentrations by 45% on average across sites and dates (Table 2). N fertilization did not alter P concentrations, resulting in significant increases in shoot N:P with N fertilization relative to control plots, although they remained relatively low

Table 3 Results of repeated measures analyses for log-transformed aboveground biomass (AGB) and nutrient concentrations^{a,b,c}

	Log AGB			[N]			[P]		N:P	
	df	F	P	df ^d	F	P	F	P	F	P
Site	4	45.8	< 0.001	4	31.5	< 0.001	71.5	< 0.001	82.7	< 0.001
Treatment	3	9.4	< 0.001	3	65.7	< 0.001	20.7	< 0.001	162.6	< 0.001
Site × treatment	12	1.0	0.25	12	1.8	0.04	8.2	< 0.001	36.3	< 0.001
Harvest	3	24.3	< 0.001	2	147.1	< 0.001	23.4	< 0.001	49.7	< 0.001
Site × harvest	12	22.3	< 0.001	8	51	< 0.001	15.6	< 0.001	46.7	< 0.001
Treatment × harvest	9	0.9	0.16	6	7.1	< 0.001	0.9	0.56	3.0	< 0.001
Site × treatment × harvest	36	2.9	0.20	24	1.7	0.02	1.5	0.10	4.1	< 0.001

^aFor N and P concentrations, no data were collected in the March 2004 harvest.

^bSignificant effects ($P < 0.05$) are in bold.

^cModel r^2 for aboveground biomass, [N], [P] and N:P were 0.64, 0.76, 0.65, and 0.84, respectively.

^dAll nutrient concentration analyses share the same degrees of freedom (df).

(9.9 vs 5.8, respectively with a range of 5.8–17.7 among sites for N-fertilized plots).

Among sites, there was no relationship between the N:P of N-fertilized vegetation and the difference in biomass between co-fertilized plots and N-fertilized plots, whether expressed as a relative response or in terms of absolute biomass response ($P > 0.25$). Fertilization with P did increase P availability to plants as [P] increased by 22% (2.2 vs 1.8 mg g⁻¹; Table 2) and these concentrations were higher than those for plants fertilized with N and P (1.7 mg P g⁻¹). There was no significant change in N concentrations with P addition, leading to a decrease in N:P with P addition (5.8–4.7; Table 2).

Although there was no significant interaction between nutrient treatments and sites (Table 3), the trends among sites in responses to fertilization can be conservatively examined. Examining the patterns of nutrient concentrations across sites, N concentrations were similar among controls of basalt and granites sites (Table 2). Averaged across the three harvests, N concentrations varied by 43%, with the basaltic Satara having the lowest (6.9 mg g⁻¹) and the basaltic Nwashitsumbe having the highest concentration (9.9 mg g⁻¹). P concentrations were consistently higher on basalts than granites (Table 2), with average P concentration varying by 311% across all sites (Table 2). With generally greater P concentrations and similar N concentrations on the basalt sites compared with granite sites, granite sites consistently had higher N:P (Table 2). Yet, there was no general relationship between the average N:P of control plots for each site and the fractional increase in production with N or with N and P, or the marginal fractional increase in production from adding N and P over just N ($P > 0.34$ for all contrasts). If anything, the site with the highest N:P (Pretoriuskop, 9.2), and therefore the site least likely to be limited by N based on nutrient concentration ratios, was the only site that had biomass responses to fertilization that most resembled classical N limitation.

Discussion

Factorial fertilization with N and P in KNP grasslands revealed broad co-limitation of aboveground production across strong soil and precipitation contrasts. Three lines of evidence from this experiment raise questions about the utility of using N:P ratios for predicting nutrient limitation. First, the N:P of unfertilized vegetation, whose production was co-limited by N and P, was well below the relatively high threshold for co-limitation established in northern European wetlands by Koerselman & Meuleman (1996) (N:P < 14) and even below the relaxed threshold suggested by Güsewell (N:P < 10). Secondly, examining the N:P of vegetation fertilized with N, neither sets of thresholds predicted that vegetation would still be limited by P. Thirdly, there was no relationship between site N:P and the degree of limitation by N and P.

Although the observed limitation patterns at KNP would benefit from independent verification, there are multiple reasons suggesting that they are not misleading. First, although N fertilization did not significantly increase production, had the trend for increased biomass been significant, the general response to nutrient treatments should still be considered co-limitation more than serial limitation. The increases in production from adding N and P relative to just N were much larger than the nonsignificant increase from just adding N. Secondly, although the P fertilizer used in this experiment also contained Ca and S, changes in P concentrations with different treatments more parsimoniously support co-limitation with P than with the other two elements. When P was added alone, there was no biomass response and P concentrations increased (Table 2). When P and N were added together, biomass was greater than that of N-fertilized vegetation, but P concentrations did not increase above control levels (Table 2). Had it been Ca or S that was causing the increase in biomass with N, P, which is actively acquired by plants, would have continued to have been supplied in excess and accumulated in tissues just as occurred in P-fertilized plots. Lastly, as there was no difference

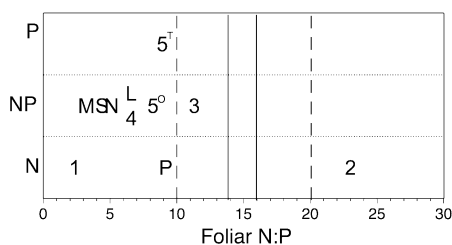


Fig. 2 Nutrient limitation and distribution of observed nitrogen:phosphorus (N:P) ratios for unfertilized vegetation for this study (M, Makhohhola; S, Satara; N, Nwashitsumbe; L, Letaba; P, Pretoriuskop), and other published studies in grasslands that had included both biomass and N:P data (1, D'Antonio & Mack, 2006; 2, Barger *et al.*, 2002; 3, Mamolos *et al.*, 2005; 4, Niinemets & Kull, 2005; 5, Ludwig *et al.*, 2001, with superscript O referring to vegetation in open grasslands and T referring to herbaceous vegetation under trees). Letaba and Niinemets & Kull (2005) data are offset because they had similar N:P ratios. The position on the x-axis indicates the N:P of unfertilized vegetation. Solid vertical lines are thresholds from Koerselman & Meuleman (1996); dashed vertical lines are thresholds from Güsewell (2004). Inclusion in N, NP, and P rows indicates limitation by N, N and P, or P, respectively.

in belowground production in 2005, the differences in aboveground productivity probably reflect Net Primary Productivity (NPP) and do not appear to be explained by a shift in production from belowground to aboveground.

The inability of N:P ratios to explain fertilization responses to N and P addition is not just limited to South African savannas (Fig. 2). For example, P limitation can occur at relatively low plant N:P. Exotic C_4 grasslands in Hawaii had foliar N:P of 2.3 and were shown to be primarily limited by N, which itself supports the use of the thresholds (D'Antonio & Mack, 2006). Yet, after N fertilization, biomass in the D'Antonio & Mack study increased with additional P, but foliar N:P of the vegetation fertilized with N was only 4.3. Such a low N:P would generally be considered to indicate N limitation. Similarly, N limitation can occur at relatively high N:P in grasslands. In a Venezuelan savanna, aboveground grass production was primarily limited by N, yet grass foliar N:P was 23 (Barger *et al.*, 2002). Co-limitation between N and P at low N:P can be found above the lower range of 10 suggested by Güsewell, from 11.3 in a Greek grassland (Mamolos *et al.*, 2005) to 10.8 in grasses of an Amazonian secondary forest (Davidson *et al.*, 2004). Yet, just like at KNP, co-limitation occurs below this lower threshold: 6.6 in both a calcareous European grassland and a semi-arid Australian grassland (Tupper, 1978; Niinemets & Kull, 2005) and 8.2 for a Tanzanian savanna (Ludwig *et al.*, 2001).

The results of this study and previously published ones are insufficient to reject the utility of N:P in predicting limitation in grasslands, yet there is currently more evidence that suggests that N:P ratios will not predict limitation in grasslands than there is evidence that suggests that it will. It is possible that there is a lower threshold for N limitation at the KNP sites than currently suggested thresholds, but even unfertilized plots with

an N:P of 3.2 at Makhohhola, which is close to the lower bound of N:P seen in terrestrial vegetation (Sterner & Elser, 2002), were co-limited by N and P. Instead this research suggests that there might be no threshold that consistently separates N limitation and co-limitation by N and P in grasslands.

Examining the patterns of biomass response to factorial fertilization at KNP suggests that co-limitation might have been caused by the ratio of supplies being equivalent to the ratios demanded by plants for optimal growth. When production responds to the addition of two nutrients individually, plants are probably facing tradeoffs in how they allocate biomass or a common limiting nutrient (Bloom *et al.*, 1985; Gleeson & Tilman, 1992). For example, nutrients might differ in availability between two layers of soil, creating tradeoffs in where biomass is allocated in the soil and which nutrients are acquired. When either nutrient is added, productivity increases as resources are allocated away from acquisition of the one co-limiting nutrient to acquisition of the other, leading to greater total resource acquisition and productivity. Alternatively, when productivity only responds to the addition of two nutrients when they are added together, (a) the two nutrients are probably supplied to unfertilized vegetation at the same ratio as demanded by plants and (b) acquisition costs for the two resources are not separate. Without tradeoffs, adding one of the two limiting resources only leads to accumulation of the added resource as both need to be added to stimulate production. At KNP, with biomass primarily responding to the addition of N and P together rather than individually, it is more likely that across the five sites N and P were generally supplied in similar ratios to those required by plants, implying that the plants must have a low optimal N:P.

The low N:P of KNP grasses supports the idea that optimal biomass N:P might not be well constrained among species (Townsend *et al.*, 2007), with species differing widely in their N:P ratio when growth is optimized. For KNP grasses to have a low optimal N:P, they must have either low N requirements or high P requirements compared with other plants. Grasses with the C_4 photosynthetic pathway could have lower N requirements than C_3 grasses, lowering optimal N:P. The consistency over time in low N:P reduces the probability that P concentrations are high as a result of storage. High P concentrations also are unlikely to be attributable to P-containing metabolites with secondary function such as defensive chemicals or osmolytes (Lambers *et al.*, 1998). Although similar classes of compounds that contain N can be responsible for causing high N:P, compounds with secondary functions rarely contain P and are unlikely to increase P concentrations. Yet, grasses could still have a high P requirement as their cell walls can be covered with sheets of mineral that contain P and other elements such as calcium and potassium (McManus *et al.*, 1977). Whether the cell wall mineralization explains the high requirement for P (and low N:P) of many grasses, or whether the low N:P can be explained by greater amounts of compounds with primary functions such as RNA, DNA, ATP,

or phospholipids (e.g. Chapin *et al.*, 1982) remains to be seen. More controlled experiments that identify optimal N:P supply and demand ratios are needed as well as better understanding of how N and P are partitioned among cellular components in unfertilized vegetation such that N:P can be as low as 3.

In addition to understanding the mechanisms that lead to variation in plant optimal N:P, it is uncertain how the ratio of supplies of N and P could consistently be similar to the demands of plants. All other things equal it seems that the odds of the ratios of supplies and demand matching one another would be low. Although some of the matching could be a result of the ratios of supplies promoting species with similar ratios of demand, there are probably additional mechanisms that are constraining the ratio of supplies. For example, variation in P availability might be associated with compensatory N fixation, which would narrow the range of supplies to plants. When P is limiting, N might be more likely to be lost from the ecosystem through denitrification or leaching (Hall & Matson, 2003; Davidson *et al.*, 2004), which would also constrain the ratios of supplies to plants.

Theoretical considerations aside, the presence of co-limitation between N and P at KNP complicates decisions on management strategies. For example, N deposition from upwind power plants is a concern in the park (Dentener *et al.*, 2006). With vegetation co-limited by N and P, N deposition might not directly increase aboveground production. Yet, grass N concentrations might still increase with N deposition, which could benefit herbivores. Longer time-scales of analysis would help determine if plant species composition would be altered by increases in nutrient availability, while experiments that included woody vegetation would provide a more complete picture of the response of the ecosystems of KNP.

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