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Biomass and mineral element responses of a Serengeti short-grass species to nitrogen supply and defoliation: compensation requires a critical [N]

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Abstract Large mammalian herbivores in grassland ecosystems influence plant growth dynamics in many ways, including the removal of plant biomass and the return of nutrients to the soil. A 10-week growth chamber experiment examined the responses of *Sporobolus kentrophyllus* from the heavily grazed short-grass plains of Serengeti National Park, Tanzania, to simulated grazing and varying nitrogen nutrition. Plants were subjected to two clipping treatments (clipped and unclipped) and five nitrogen levels (weekly applications at levels equivalent to 0, 1, 5, 10, and 40 g N m⁻²), the highest being equivalent to a urine hit. Tiller and stolon production were measured weekly. Total biomass at harvest was partitioned by plant organ and analyzed for nitrogen and mineral element composition. Tiller and stolon production reached a peak at 3–5 weeks in unclipped plants, then declined drastically, but tiller number increased continually in clipped plants; this differential effect was enhanced at higher N levels. Total plant production increased substantially with N supply, was dominated by aboveground production, and was similar in clipped and unclipped plants, except at high nitrogen levels where clipped plants produced more. Much of the standing biomass of unclipped plants was standing dead and stem; most of the standing biomass of clipped plants was live leaf with clipped plants having

significantly more leaf than unclipped plants. However, leaf nitrogen was stimulated by clipping only in plants receiving levels of N application above 1 g N m⁻² which corresponded to a tissue concentration of 2.5% N. Leaf N concentration was lower in unclipped plants and increased with level of N. Aboveground N and mineral concentrations were consistently greater than belowground levels and while clipping commonly promoted aboveground concentrations, it generally diminished those belowground. In general, clipped plants exhibited increased leaf elemental concentrations of K, P, and Mg. Concentrations of B, Ca, K, Mg, and Zn increased with the level of N. No evidence was found that the much greater growth associated with higher N levels diminished the concentration of any other nutrient and that clipping coupled with N fertilization increased the total mineral content available in leaf tissue. The results suggest that plants can (1) compensate for leaf removal, but only when N is above a critical point (tissue [N] 2.8%) and (2) grazing coupled with N fertilization can increase the quality and quantity of tissue available for herbivore removal.

Key words Compensation · Clipping · Fertilization · Nitrogen · *Sporobolus kentrophyllus*

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Introduction

The effect of grazing on compensatory growth, coupled with N fertilization from grazer N inputs in the Serengeti is not completely understood although the interaction clearly plays a vital role in the plant compensatory response (McNaughton 1985, 1990). N fertilization experiments in grassland ecosystems from the arid tropics (Keya 1973; Penning de Vries et al. 1980) to the tundra (Shaver and Chapin 1980; Bazely and Jefferies 1985; Chapin and Shaver 1985; Hik and Jefferies 1990) have documented N limitation of primary productivity. Field and laboratory experiments have also demonstrated the occasional ability of plants to compensate for tissue loss

resulting from herbivory (Oesterheld and McNaughton 1991; Whitham et al. 1991). Compensatory growth (McNaughton 1983; Oesterheld and McNaughton 1988; Whitham et al. 1991), compensatory photosynthesis (Caldwell et al. 1981; Detling and Painter 1983; Wallace et al. 1984; Senock et al. 1991), soil water conservation (Pande and Singh 1981; McNaughton 1983; Archer and Detling 1986), and altered growth form (McNaughton 1983; Oesterheld and McNaughton 1988; Senock et al. 1991) have been demonstrated as responses to defoliation. Plant nutritional status can be an important regulator of their ability to compensate for defoliation (McNaughton et al. 1983; Coughenour et al. 1985ab). Many laboratory N fertilization experiments have either investigated a single treatment and/or nitrogen source interaction with clipping (Coughenour et al. 1985b; McNaughton et al. 1983; Ruess et al. 1983; Ruess and McNaughton 1984; Ruess 1988; Georgiadis et al. 1989) and multiple field studies of African grasslands (O'Connor 1985) provide excellent insights into the interaction but are confounded by the multitude of additional environmental factors that arise in the field. Only one laboratory study has investigated the interaction of multiple nitrogen levels and clipping treatment (Wallace et al. 1985) but did not quantify plant tissue N, an important variable that may determine plant responses to defoliation (McNaughton et al. 1983; Coughenour et al. 1985).

Input of N from grazers, through urination and/or defecation, can affect both plant responses to defoliation and herbivore grazing preference (Day and Detling 1990). The application of urine has been shown to increase leaf N content (Loterio et al. 1966; Joblin and Keogh 1979; Day and Detling 1990), leaf macronutrients (Loterio et al. 1966; Joblin and Keogh 1979), above-ground productivity (Loterio et al. 1966; Joblin and Keogh 1979; Day and Detling 1990), and soil mineral N (Thomas et al. 1986). A single urine hit affects leaf tissue in the area of a circle approximately 1 m² and persists for up to 10 months, with the effect diminishing from the center of the urine hit (Loterio et al. 1966). The diminishing effect generates a gradient of plant responses that may be generated in response to diminishing soil N (Thomas et al. 1986). The effect of a single urine hit therefore is to increase the quality of leaf tissue in the urine patch in which a range of tissue N levels exists.

Comparative studies of terrestrial ecosystems (McNaughton et al. 1988; 1989; 1991) document a close correlation between all significant properties of the herbivore trophic web and net primary productivity, including herbivore biomass, rate of consumption, and secondary productivity. Similar evidence has been presented for diverse aquatic ecosystems (Cyr and Pace 1993) suggesting broad ecological generality. Livestock biomass is also significantly related to primary production, but is about an order of magnitude higher than that of native herbivores, indicating that herbivore abundance is not merely a passive consequence of production-determined carrying capacity (Oesterheld et al. 1992). To the extent that grassland primary productivity

is N limited, recycling can be expected to have positive effects at the producer level that will be reflected at higher trophic levels.

Data from the Serengeti ecosystem, Tanzania, indicate that the utilization of grassland vegetation by large mammalian herbivores is significantly influenced by a number of nutrients other than N, particularly those required in large quantities by pregnant and lactating females and growing young (McNaughton 1988; 1990). The distribution of resident ungulates (McNaughton 1988) is associated with forage concentrations of Ca, Mg, Na, and P, and the seasonal movements of migratory herds (McNaughton 1990) are associated with forage levels of those minerals and Cu, N, and Zn. Specific hungers for Na and P (Denton 1984) act as mechanisms governing the pattern of forage utilization by mineral-unsupplemented ungulates at both the vegetation and the plant species level (Chiy and Phillips 1991).

Grazers have manifold effects in grazing ecosystems (Skarpe 1991), including increased rates of nutrient recycling and spatial redistribution due to dung and urine deposition (Afzal and Adams 1992; Russelle 1992). Fire, another pervasive environmental factor in grasslands, also influences nutritional demands of the vegetation (Seastedt et al. 1991), with the degree of response to N fertilization in Kansas tall grasslands decreasing with the interval between burns. Grazers, primary producers, and soil organisms are linked functionally by multi-trophic-level processes, so it is more reasonable to consider their effects as mutually causative than as simple cause and effect (Moore et al. 1991). As Hutchinson (1948) pointed out over 40 years ago, it is more realistic to view nutrient cycling and energy flow as circular causal pathways than as linear chains of cause and effect.

The experiment reported here was designed to determine, under laboratory conditions, the response to defoliation and increasing levels of N supply of a dominant short-grass species from the heavily grazed Serengeti Plains (McNaughton 1985), *Sporobolus kentrophyllus* (McNaughton 1983; Banyikwa et al. 1990) and to determine if a critical concentration of N exists at which growth compensation cannot occur. The levels of N applied were designed to investigate the effect of varying grazer input, and to N saturate plant processes, N at the highest level amounted to urine deposition every week. The combined weekly defoliation and varied fertilization treatments enabled us to examine the influence of loss of N to grazing and the input of N from grazers on the ability of *S. kentrophyllus* to compensate for tissue loss.

Materials and methods

Plant material

S. kentrophyllus, a C₄ graminoid obtained from the Serengeti National Park, Tanzania, was propagated vegetatively in a greenhouse at Syracuse University. Clones were transplanted into polyvinylchloride (PVC) tubes, 10.2 cm in diameter and 54 cm in height. The

growth medium was calcined clay, rinsed with distilled water. The plants were acclimated in a growth chamber for 1 week prior to the start of the experiment. The growth chamber was programmed to duplicate the temperature cycle of the Serengeti, with a 12:12 L:D photoperiod (McNaughton et al. 1983). At upper canopy height, $850 \mu\text{mol m}^{-2} \text{s}^{-1}$ of PAR was supplied by the lights, the maximum achievable in the growth chamber. Because of photon flux and temperature gradients across the chamber, pot position was randomized weekly. Plants were supplied with 60 ml of distilled water daily, except on nitrogen and micronutrient treatment days. To investigate only the effects of N fertilization, all plants received 50 ml of a modified $\frac{1}{2}$ -strength Hoagland's solution (N reduced to 0.019 g N m^{-2}) weekly to provide soil background levels of all other nutrients (deWit 1978). The N levels are presented as g m^{-2} to allow for extrapolation to field conditions.

Treatments

Treatment design was a 5×2 factorial, with five nitrogen levels, and clipped and unclipped plants. There were three replicates per treatment. Nitrogen treatments consisted of equimolar amounts of urea and sodium nitrate supplying N at the following levels: 0, 1, 5, 10 and 40 g m^{-2} [between 1 and 5 g m^{-2} represents background field soil levels of N (deWit 1978, Wallace et al. 1985)]. For clipped plants, lateral growth was pulled taut and clipped to 5 cm to simulate herbivore grazing in the Serengeti (McNaughton 1985). Plants received both N and clipping treatments on the same day weekly. Stolons, in clipped plants only, were pressed into the calcined clay, as would a treading animal. All clipped material was oven dried and weighed weekly. Tiller and stolon number and leaf biomass removed by clipping were determined weekly.

Canopy closure was simulated for unclipped plants with collars of black poster board with white paper on the outside, to prevent reflection of light inside the tube and to reflect light outside the tube (McNaughton 1992). The collar treatment on unclipped plants mimics the closure of the canopy as would occur at an ungrazed site in which changes in light interception and quality alter plant morphology (McNaughton 1992). The collars were raised, according to the growth of the plants, twice weekly. The vertical position of clipped plants was adjusted according to the increasing canopy height of unclipped plants to prevent shading by unclipped pots. Clipped plants maintain a prostrate morphology in which canopy closure does not occur, therefore collars were not raised on clipped pots (McNaughton 1992).

Harvest

All plants were harvested after 10 weeks, the approximate length of the growing season where *S. kentrophyllus* is common (McNaughton 1985). Each plant was separated into green leaf, stem, standing dead, crown, and roots. Each part was oven dried at 60°C for 72 h, weighed, ground, and analyzed by Dumas combustion for nitrogen composition with a Carlo Erba NA1500, and for mineral composition with an Inductively Coupled Plasma (ICP) (Leeman Labs, USA) with protocols described previously (McNaughton 1988).

Statistics for weekly measurements of tiller number and leaf mass removed were performed by General Linear Models for repeated measures in SAS (SAS Institute, Cary, N.C.). Final harvest data was analyzed using standard General Linear Models in SAS. Analysis of the critical concentration curves was performed using a non-linear piecewise breakpoint regression model in Statistica (Statsoft, Tulsa, Okla).

Rationale

The experimental design was chosen to investigate the differences between clipped and unclipped plants, under controlled conditions,

in their responses to N fertilization with the intent of analyzing the magnitude of change between treatments. The fact that all other nutrients were maintained at constant levels was necessary to examine only the effects of clipping and N fertilization. Although the tissue concentrations of elements may not be applicable to field conditions, the magnitude of the difference induced by clipping is relevant to the observed responses in the field. All other components of the design were performed to best mimic field conditions (deWit 1978; McNaughton 1985).

Results

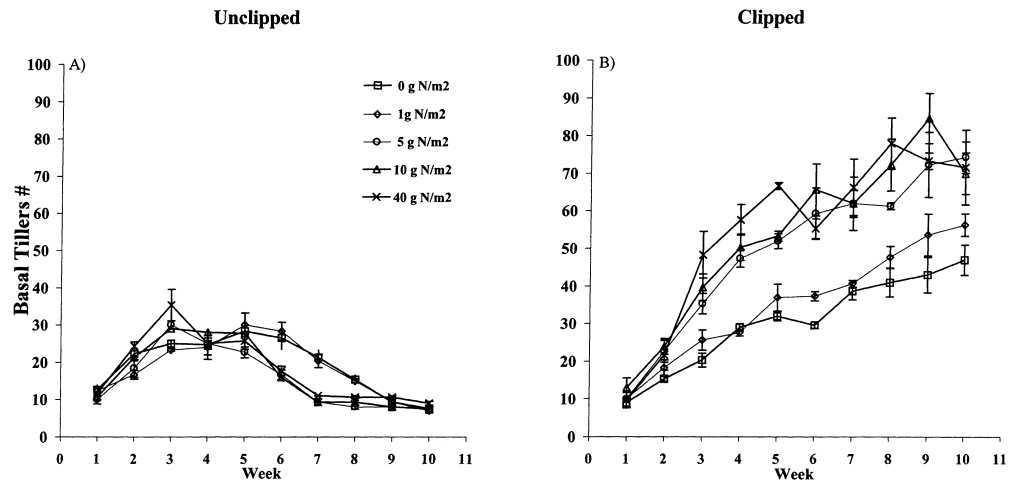
Weekly measurements

Clipping, N level, and week all had significant effects on basal tiller and stolon number which was best addressed by the significant higher-order interactions (Table 1). Clipping increased the number of basal tillers present over time (55% by week 10) while unclipped plants peaked by week 5 and then decreased thereafter (Fig. 1). Nitrogen level increased the number of basal tillers in clipped plants receiving more than 1 g N m^{-2} but had no effect on unclipped plants. The significant clip \times N level interaction was the result of clipped plants producing more tillers over time, and [N] above 1 g N m^{-2} enhanced that effect. Stolon number followed a similar trend as tillers, with final stolon numbers in clipped

Table 1 Repeated-measure ANOVAs for tiller number, stolon number, and green leaf removed by clipping

	df	F-ratio	P-value
Tiller number			
<i>Between subjects</i>			
Unclipped/clipped (U/C)	1	664.23	0.0968
Nitrogen level (N)	4	18.45	0.0000
U/C*N	4	26.81	0.0002
Error (MSE)	20	(83.21)	0.0000
<i>Within subjects</i>			
Time	9	90.79	0.0000
Time*U/C	9	149.45	0.0000
Time*N	36	2.34	0.0002
Time*U/C*N	36	3.74	0.0000
Error (MSE)	180	(24.88)	
Stolon number			
<i>Between subjects</i>			
Unclipped/clipped (U/C)	1	3.04	0.0968
Nitrogen level (N)	4	23.23	0.0000
U/C*N	4	9.41	0.0002
Error (MSE)	20	(11.42)	0.0000
<i>Within subjects</i>			
Time	9	161.01	0.0000
Time*U/C	9	16.72	0.0000
Time*N	36	6.47	0.0002
Time*U/C*N	36	4.02	0.0000
Error (MSE)	180	(2.14)	
Green leaf clipped			
<i>Between subjects</i>			
Nitrogen level	4	78.28	0.0000
Error (MSE)	10	(0.031)	
<i>Within subjects</i>			
Time	10	69.09	0.0000
Time*N	40	5.26	0.0000
Error (MSE)	100	(0.168)	

Fig. 1 Weekly mean \pm SE basal tiller production in unclipped (A) and clipped (B) plants for each nitrogen level applied throughout the 10-week experiment



and unclipped plants of 11.0 and 7.5 respectively ($F_{1,28} = 13.52$; $P < 0.05$).

The amount of green leaf tissue removed increased by 68% between 0 and 40 g N m⁻² plants (Fig. 2). At levels above 1 g N m⁻², productivity increased at a faster rate and at 40 g N m⁻², productivity was maximal, which was reflected in the significant clip \times N level term (Table 1, Fig. 2).

Final harvest

Biomass

Belowground biomass included root and crown tissue below 5 cm. Belowground biomass was 14% lower in clipped plants and the nitrogen level increased belowground biomass at 5 g N m⁻² but decreased it at 40 g N m⁻² (Table 2, Fig. 3A). Root and crown biomass both had the same response when analyzed separately (Table 2, Fig. 3A).

Aboveground biomass was the sum of stem, standing dead, and cumulative leaf mass. Clipping did not affect aboveground biomass but did affect the pattern of allocation to different tissue types (Table 2; Fig. 3B). Nitrogen level increased aboveground biomass with increasing nitrogen addition. The effect of N level was greatest in clipped plants, which was reflected in the significant clip \times N level interaction (Table 2, Fig. 3B).

Clipping reduced stem and standing dead mass by 71 and 77% respectively, and clipping increased green leaf mass by 62%. Nitrogen level increased the amount of stem, standing dead, and leaf tissue. There was a significant clip \times N level interaction (Table 2) for leaf tissue because clipped plants responded by producing more leaf at a given nitrogen level, while unclipped plants produced approximately the same amount of leaf at all N levels (Fig. 3B). Tukey Honest Significant Difference (HSD) means comparisons were performed for aboveground mass to investigate which treatments contributed

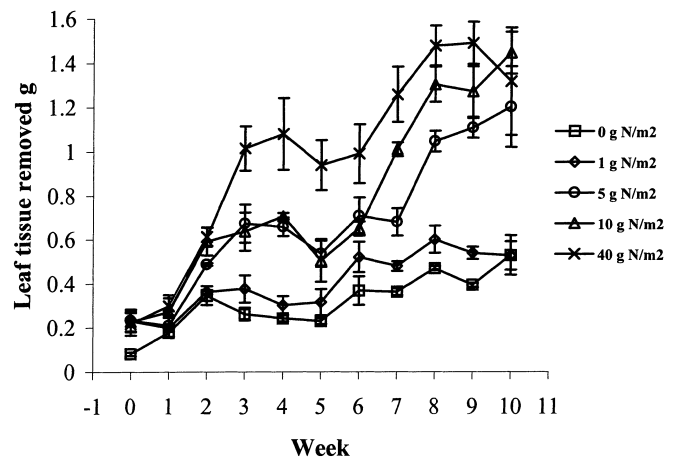


Fig. 2 Weekly mean \pm SE leaf tissue removed (g) in clipped plants for each nitrogen level applied throughout the 10-week experiment

to the significant clip \times N level interaction (Table 2). The comparisons showed that clipped plants at low [N] (0, 1 g N m⁻²) and high [N] (40 g N m⁻²) differed significantly ($P < 0.05$) from all other treatments.

Nitrogen

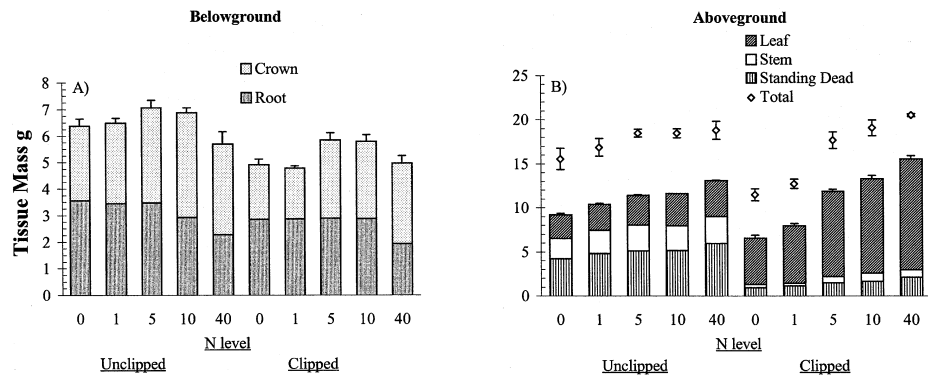
Belowground nitrogen was reduced by 57% in clipped plants and increased with increasing nitrogen level (Table 2, Fig. 4A). Root and crown nitrogen responded similarly to clipping when analyzed separately but root total N did not increase with increasing N level (Table 2). Belowground responses were therefore affected by clipping and N level independently. Belowground and crown [N] showed similar trends to total tissue nitrogen but root [N] increased with increasing N level, which demonstrated the effect of decreasing root biomass with increasing N level (Fig. 3A).

Aboveground nitrogen increased by 33% in clipped plants and increased with N level between 0 and 40 g N m⁻² plants by 86% (Table 2, Fig. 4B). Plant [N] was

Table 2 ANOVAs for Biomass, total N and %N

	Biomass (g)			Total N (mg)		Percent N	
	df	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
<i>Aboveground</i>							
Unclipped/clipped	1	0.088	0.7692	156.341	0.000	75.626	0.000
Nitrogen level	4	57.782	0.0000	489.628	0.000	233.194	0.000
Unclipped/clipped* nitrogen level	4	11.771	0.0000	41.849	0.000	2.718	0.059
Error (MSE)	20	0.701		5.822		0.000043	
<i>Belowground</i>							
Unclipped/clipped	1	18.747	0.000	30.982	0.000	28.278	0.000
Nitrogen level	4	2.367	0.087	15.836	0.000	37.389	0.000
Unclipped/clipped* nitrogen level	4	0.331	0.854	1.798	0.169	2.219	0.103
Error (MSE)	20	0.607		5.38		0.000126	
<i>Root</i>							
Unclipped/clipped	1	7.840	0.011	8.848	0.007	1.730	0.203
Nitrogen level	4	6.772	0.001	3.887	0.171	7.866	0.001
Unclipped/clipped* nitrogen level	4	0.5351	0.711	1.111	0.379	1.181	0.349
Error (MSE)	20	0.191		0.827		0.00026	
<i>Crown</i>							
Unclipped/clipped	1	22.021	0.000	23.833	0.000	26.963	0.000
Nitrogen level	4	6.414	0.002	16.05	0.000	22.178	0.000
Unclipped/clipped* nitrogen level	4	0.535	0.643	1.433	0.259	0.892	0.487
Error (MSE)	20	0.209		3.612		0.0029	
<i>Stem</i>							
Unclipped/clipped	1	362.098	0.000	10.172	0.005	0.088	0.769
Nitrogen level	4	4.635	0.008	3.602	0.023	2.522	0.073
Unclipped/clipped* nitrogen level	4	0.603	0.6649	0.626	0.649	0.255	0.902
Error (MSE)	20	0.092		1.979		0.0161	
<i>Standing Dead</i>							
Unclipped/clipped	1	540.672	0.000	278.148	0.000	107.811	0.000
Nitrogen level	4	10.227	0.000	125.151	0.000	373.497	0.000
Unclipped/clipped* nitrogen level	4	0.375	0.8236	32.408	0.000	9.153	0.000
Error (MSE)	20	0.178		1.029		0.00003	
<i>Leaf</i>							
Unclipped/clipped	1	1316.04	0.000	1085.11	0.000	37.090	0.000
Nitrogen level	4	108.86	0.000	654.255	0.000	175.519	0.000
Unclipped/clipped* nitrogen level	4	9.27	0.000	200.175	0.000	1.460	0.251
Error (MSE)	20	0.179		2.451		0.00006	

Fig. 3 Mean \pm SE tissue mass (g) in belowground (root and crown) (A) and aboveground (standing dead, stem, and cumulative leaf) (B) tissues for unclipped and clipped plants. **B** Diamonds represent mean \pm SE total tissue mass produced



affected similarly (Table 2). Aboveground N increased by a greater fraction in clipped plants than in unclipped plants (Fig. 4B). Stem and standing dead N decreased by 70% in clipped plants and increased with increasing N level (Table 2, Fig. 4B). The N concentration of stem was not affected by either treatment, but standing dead

concentration responded in the same way as total standing dead N. Leaf nitrogen responded to N level as all other tissues responded, but was 61% higher in clipped plants. Clipped plants had a greater N level response than unclipped plants although leaf [N] was lower in clipped plants (Table 2, Fig. 4B). Tukey HSD means

Fig. 4 Mean \pm SE tissue nitrogen (g) of belowground (root and crown) (A) and aboveground (standing dead, stem, and cumulative leaf) (B) tissues for unclipped and clipped plants. B Diamond represent mean \pm SE total tissue nitrogen

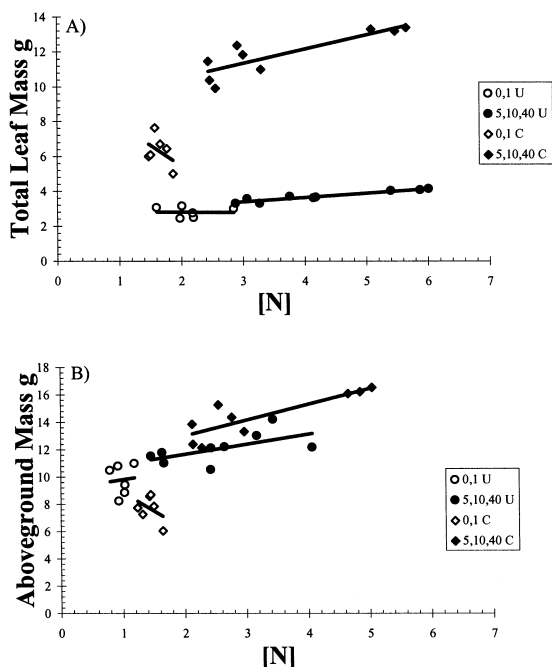
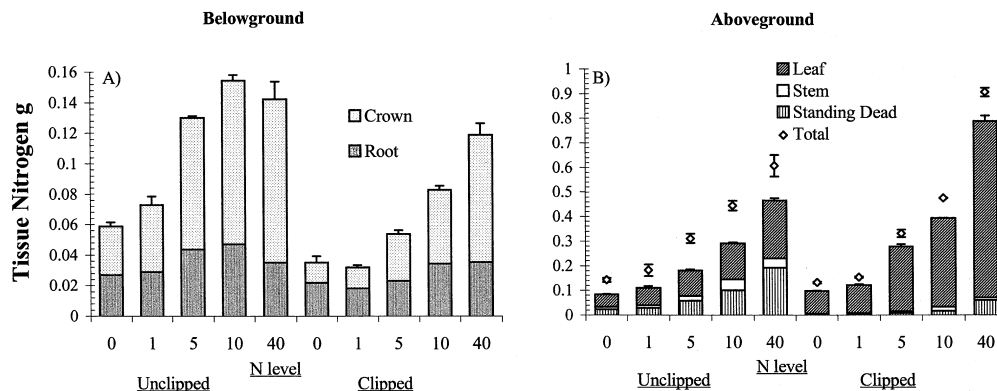


Fig. 5 Regressions of total leaf mass (g) (A) and aboveground mass as a function of tissue N concentration (%N) (B) in clipped (C) (\diamond 0 and 1 g N m⁻², \square 5, 10, and 40 g N m⁻²) and unclipped (U) (\circ 0 and 1 g N m⁻², \bullet 5, 10, and 40 g N m⁻²) plants

comparisons were performed on aboveground nitrogen and showed that 40 g N m⁻² clipped and unclipped plants and 10 g N m⁻² clipped plants are different from all other treatments ($P < 0.05$) and that 10 g N m⁻² unclipped plants contain the same amount of nitrogen as 5 g N m⁻² clipped plants.

A critical N concentration for compensation was evident in clipped plants at levels of N supply below 5 g N m⁻². The N concentrations necessary for aboveground and leaf mass compensation were determined to be 2.53% N and 2.85% N, respectively. The corresponding biomass critical points were 11.76 g and 9.64 g for aboveground and leaf biomass, respectively (Fig. 5A, B). The sum of these two points corresponds to total N values of 0.30 g and 0.27 g of N for aboveground and leaf values, respectively. Unclipped plants

aboveground and leaf biomass maintained a linear response to N level with no significant critical N concentration detected (Fig. 5A, B).

Mineral elements

Both clipping and N level had a wide range of effects on plant mineral concentration (Tables 3, 4), but some of the patterns were extremely consistent. For all nutrients except iron, aboveground concentrations were greater than those belowground, often by substantial margins. Also consistent was the clipping effect: whenever clipping was statistically significant, it was due to higher concentrations in clipped plants. The relationship between nitrogen supply and the levels of other minerals, however, was quite variable, but with three fundamental patterns. For boron, calcium, potassium, magnesium, and zinc, the concentration increased with the level of nitrogen supplied. The concentration of manganese, in contrast, declined with the level of N fertilization. The third pattern was a tendency toward an optimum N level, with maximum concentrations at intermediate levels of N supply, evident for copper, molybdenum, and phosphorus.

There were quite a few higher-order interactions due to variations in mineral content, and the three-way interaction was significant for K, Mg, Mn, P, and Zn. There were two basic patterns, here illustrated by K and P (Fig. 6). K concentrations belowground were higher when plants were clipped and declined with N level; aboveground concentrations, in contrast, varied little with clipping, but increased with the level of N supplied. Similar patterns were evident for B, Ca, Mg, and Zn. The second general pattern, here illustrated with P, was higher concentrations both above- and belowground when clipped, and highest concentrations at intermediate levels of N. A similar pattern was evident for Mn and Cu.

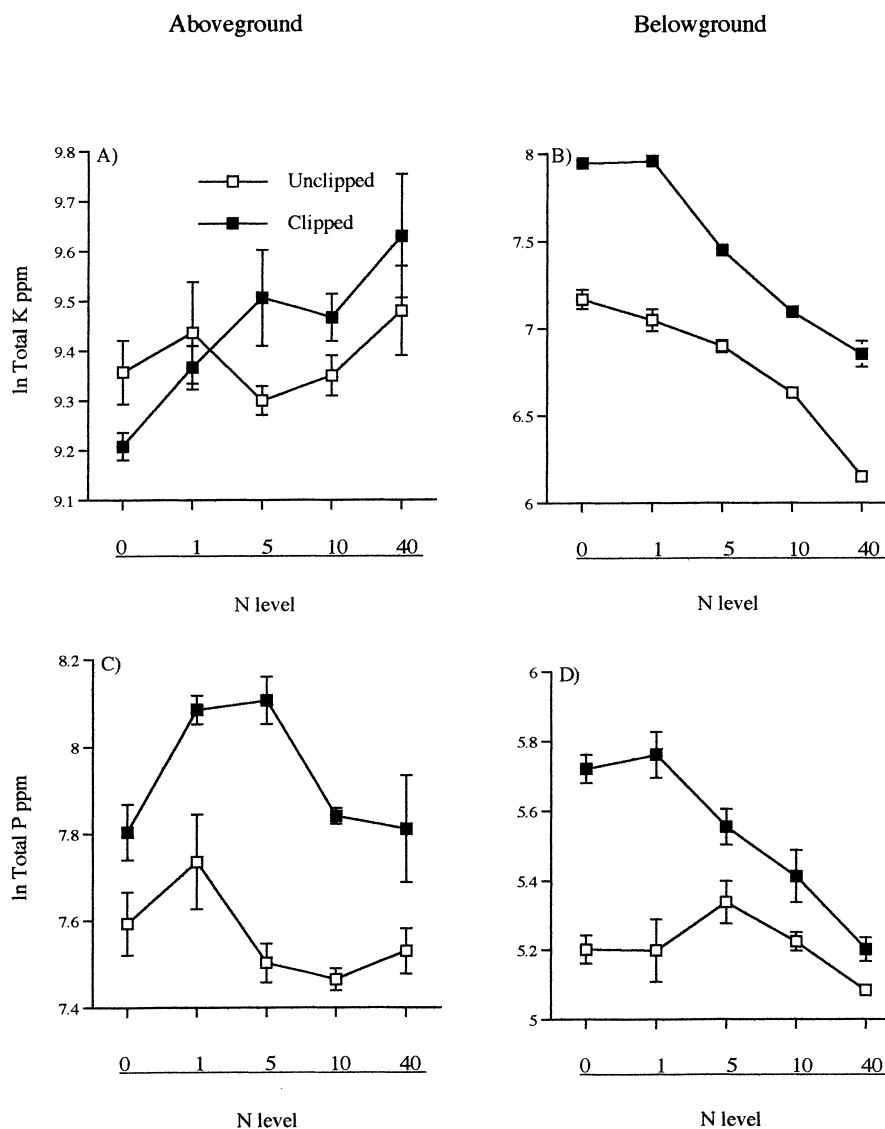
The cumulative mineral content of leaf tissue was significantly affected by both clipping and N level (Table 5). Clipped plants in every case had higher cumulative leaf mineral contents than unclipped plants, with the exception of Fe, for which clipping had no effect.

Table 3 ANOVAs of mineral nutrient concentrations (ppm)

Treatment	df	Boron		Calcium		Copper		Iron		Potassium	
		F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
Unclipped/clipped (U/C)	1	0.087	0.1541	6.89	0.0121	14.576	0.0005	5.042	0.0303	190.924	0.0000
Nitrogen level (N)	4	0.52	0.0000	0.84	0.5067	2.210	0.0851	5.437	0.0014	38.418	0.0000
Aboveground (ABG)/ belowground (BLG)	1	9.084	0.0000	1363.23	0.0000	339.728	0.0000	226.825	0.0000	7554.00	0.0000
U/C*N	4	0.056	0.2620	1.52	0.2155	1.047	0.3950	1.215	0.3196	1.034	0.4017
U/C*ABG/BLG	1	0.14	0.0734	5.77	0.0210	8.263	0.0644	1.028	0.3167	142.427	0.0000
N*ABG/BLG	4	0.044	0.3806	15.81	0.0000	4.948	0.0024	17.171	0.0000	81.156	0.0000
U/C*N*ABG/BLG	4	0.008	0.9346	0.43	0.7887	0.721	0.5826	0.335	0.8526	7.009	0.0002
Error (MSE)	40	0.021		0.0115		0.0697		0.0758		0.0105	
Treatment	df	Magnesium		Manganese		Molybdenum		Phosphorus		Zinc	
		F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
Unclipped/clipped (U/C)	1	100.86	0.0000	64.137	0.0000	10.635	0.0023	154.716	0.0000	186.012	0.0000
Nitrogen level (N)	4	0.00	0.9999	11.420	0.0000	4.15	0.0066	13.617	0.0000	18.439	0.0000
Aboveground (ABG)/ belowground (BLG)	1	4686.91	0.0000	1026.14	0.0000	521.586	0.0000	7469.81	0.0000	107.486	0.0000
U/C*N	4	2.81	0.0378	13.251	0.0000	2.812	0.0379	3.026	0.0286	7.077	0.0002
U/C*ABG/BLG	1	4.67	0.0368	26.447	0.0000	0.2298	0.6342	0.614	0.4379	41.031	0.0000
N*ABG/BLG	4	34.54	0.0000	7.315	0.0002	5.833	0.0008	3.261	0.0209	50.485	0.0000
U/C*N*ABG/BLG	4	2.77	0.0399	2.463	0.0606	4.432	0.0046	5.801	0.0009	2.660	0.0465
Error (MSE)	40	0.0114		0.0314		0.110		0.114		0.0205	

Table 4 Tissue elemental concentrations (ppm) means for whole plant unclipped and clipped, belowground tissues and aboveground tissues, and whole plant by N fertilization level. Means for each group are combined means of all other treatments

Element	Unclipped	Clipped	Belowground	Aboveground	0	1	5	10	40
Boron	3.99	4.11	2.56	5.54	3.31	3.28	3.73	4.87	5.07
Calcium	864.54	903.49	467.81	1300.23	848.21	828.83	843.61	875.88	1 023.57
Copper	8.56	6.09	11.52	3.13	6.86	7.73	6.67	8.46	6.92
Iron	127.24	149.03	199.74	76.53	154.90	120.62	132.30	137.92	144.95
Potassium	6434.96	7282.25	1421.31	12 295.90	6421.09	7061.45	6774.63	6600.17	7435.68
Magnesium	859.16	1084.81	258.70	1685.27	845.01	896.84	923.99	995.92	1 198.17
Manganese	63.41	81.91	29.86	115.46	83.90	93.22	65.60	62.88	67.69
Molybdenum	0.31	0.40	0.09	0.61	0.27	0.34	0.47	0.30	0.38
Phosphorus	1061.46	1531.29	220.42	2372.33	1230.96	1508.87	1398.79	1174.72	1168.53
Zinc	4.37	7.57	4.59	7.35	4.58	4.53	6.44	6.33	7.96

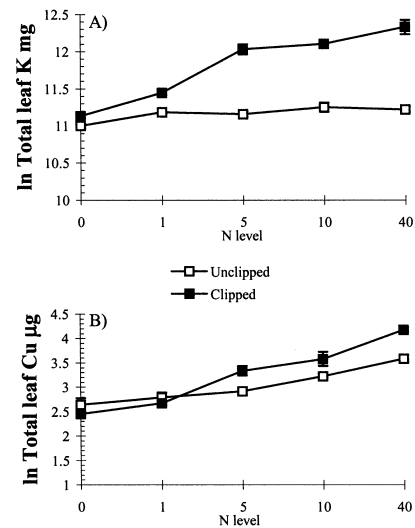
Fig. 6 Mean \pm SE Potassium content (ln ppm) in aboveground (A) and belowground (B) tissues and phosphorus content (ln ppm) in aboveground (C) and belowground (D) tissues for each nitrogen level applied in unclipped and clipped plants

fect. The N level had the effect of increasing the level of all minerals in leaf tissue. The interaction of clipping and N level was significant for all minerals with the exception of B, Fe, and Mn (Table 5). Two trends were observed in the remaining minerals, illustrated here by K and Cu

(Fig. 7A, B). For clipped plants, the N level increased K in leaf tissue with no increase observed in unclipped plants (Fig. 7A). Similar patterns were observed for Ca, Mo, and P. Leaf Cu content increased in both clipped and unclipped plants but the increase was greater in

Table 5 ANOVAs of leaf tissue total mineral nutrient content (μmg)

Treatment	df	Boron		Calcium		Copper		Iron		Potassium	
		F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
Unclipped/clipped	1	13.34	0.0016	123.76	0.0000	34.24	0.0000	1.19	0.2876	208.93	0.0000
Nitrogen level (N level)	4	10.84	0.0001	39.14	0.0000	75.53	0.0000	7.74	0.0006	35.48	0.0000
Unclipped/clipped * N level	4	4.10	0.1369	11.91	0.0000	13.51	0.0000	0.32	0.8631	26.00	0.0000
Error (MSE)	20	152.663		2 156 669		18.52		189 412		21 262E ³	
Treatment	df	Magnesium		Manganese		Molybdenum		Phosphorus		Zinc	
		F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
Unclipped/clipped	1	239.13	0.0000	125.37	0.0000	20.89	0.0002	665.92	0.0000	1 456.92	0.0000
Nitrogen level (N level)	4	56.35	0.0000	3.26	0.0324	3.05	0.0410	51.19	0.0000	278.26	0.0000
Unclipped/clipped * N level	4	20.45	0.0000	0.88	0.4948	3.30	0.0314	38.68	0.0000	112.08	0.0000
Error (MSE)	20	3 848 495		20 910.7		7.776		3 768 678		56.537	

**Fig. 7** Total mean \pm SE leaf mineral element content for K (mg) (A) and Cu (μg) (B) in clipped and unclipped plants

clipped plants above 1 g N m^{-2} (Fig. 7B). A similar pattern was evident for Mg and Zn.

Discussion

A variety of species, particularly grasses, can compensate for the loss of leaf tissue (McNaughton 1983; Oesterheld and McNaughton 1988; Whitham et al. 1991). By treating plants weekly with simulated herbivory events coupled with varying N fertilization levels, both plant growth and nutritional responses to leaf removal were examined. This study simulated field conditions by (1) bracketing levels of nitrogen fertilization that are relevant in grazed ecosystems (Lotero et al. 1966; deWit 1978; Day and Detling 1990) and (2) using a herbivory regime that occurs during a 10-week growing season of *S. kentrophyllus* (McNaughton 1985). The experimental conditions enabled us to determine that a critical tissue [N] is required for compensation to occur and that clipping dramatically alters morphological and biomass variables and, when coupled with N fertilization, enhances the quality and quantity of tissue available for herbivore consumption. The results establish an important link between the cycling of nitrogen from plant to grazer and suggests that N cycling can be accelerated by grazers through reasonable inputs of N and the reduction of standing dead. Additionally, selection for grazer-beneficial morphological and physiological traits of *S. kentrophyllus*, such as increased leaf tissue quality and quantity, could only occur when N inputs from grazers are above critical levels. Clipping profoundly affected the morphological components of yield, stolon and tiller productivity, and also increased the amount of green leaf tissue while decreasing the amount of non-photosynthetic tissue (stem and standing dead). Nitrogen addition predictably increased both biomass and N variables. The

results demonstrated that productivity, in *S. kentrophyllus*, is N limited, but only in clipped plants. The main factor that generated this result was that unclipped and clipped plants had strikingly differential allocation of biomass and nitrogen to plant tissue types. The differential allocation can be attributed primarily to morphological changes resulting from canopy closure in unclipped plants (Knapp and Seastedt 1986; McNaughton 1992). A greater proportion of N was allocated to leaf tissue when *S. kentrophyllus* was clipped, without a significant effect on total biomass above the level of 1 g N m⁻² but, below that, shoot and crown N and biomass decreased. The altered allocation of biomass and nitrogen could potentially affect future growth. With decreased storage in root and crown in 0 and 1 g N m⁻² clipped plants, a reduction in growth for the next growing season could occur (Vinton and Hartnett 1992). There was no indication that clipping or N level had detrimental effects on any other mineral element. In general, clipping increased aboveground concentrations with minimal changes in response to increasing N level. The increase in both N and other mineral elements could potentially support increased photosynthetic rates, a well-documented compensatory response in grasses (Caldwell et al. 1981; Detling and Painter 1983; Wallace et al. 1984; Senock et al. 1991). Given that when found in the Serengeti *S. kentrophyllus* is the dominant grass in the short-grass plains (McNaughton 1985) and that no reductions in any mineral element were observed in response to clipping, it would be expected that *S. kentrophyllus* would successfully compete under the conditions in which grazing intensity is severe and coupled with sufficient but nominal N inputs from grazers. In a pot study performed in the field using *S. kentrophyllus*, Georgiadis et al. (1989) found that whole-plant productivity was stimulated by N fertilization at a level equivalent to the 1 g N m⁻² in this experiment and by clipping to 6 cm only twice during the experiment. The other significant factor contributing to increased productivity was the interval at which water was supplied (Georgiadis et al. 1989). The results of this field-performed pot experiment support the productivity results of our experiment which utilized a more frequent clipping regime and a wider range of N fertilization levels.

Compensation for loss of leaf tissue did occur, but only at N fertilization levels above 1 g N m⁻². Analysis of the aboveground biomass and leaf biomass as a function of %N indicated that a critical tissue concentration of 2.5% N must exist for compensation of leaf loss in *S. kentrophyllus*. At 2.5% N, full compensation occurred and at the highest level of N fertilization (40 g N m⁻² corresponding to a tissue concentration of 4.5% N) over-compensation was observed. Compensation and over-compensation also occurred when the co-dominant C₄ sedge *Kyllinga nervosa* was clipped to 5 cm (McNaughton et al. 1983). At tissue [N] below 2.5%, compensation did not occur and a decline in leaf and total biomass was observed. The piecewise regression

model derived biomass by %N regression breaking points in clipped plants corresponding to a total plant N content of 0.3 g N, which equated to a clipped plant fertilized with 5 g N m⁻². This suggests that N is limiting only below the level of N required for sustainable growth (between 1 and 5 g N m⁻²), which is not above that observed in field soils (deWit 1978).

Day and Detling (1990), in a field experiment, found that aboveground productivity and [N] increased in natural and simulated urine patches and bison preferentially grazed urine patches. Jaramillo and Detling (1992) also demonstrated that cattle preferentially graze simulated urine patches with the preference persisting for at least 2 months. In an agricultural field, plants on experimental urine patches had increased N and mineral element concentrations and urine patches were preferentially grazed by sheep (Joblin and Keogh 1979) and urine patches were discriminated from control patches within 24 h of treatment (Keogh 1973). In our experiment, total biomass productivity was only enhanced by grazing when the level of N was approximately what a grazer would contribute through urination. Yield to grazers in terms of leaf productivity was significantly greater in clipped plants above the fertilization level of 1 g N m⁻². This was the result of two factors: (1) tiller production was stimulated in the absence of canopy closure and increased with N level, and (2) leaf mass increased with clipping and N level. The nutritional quality of tissue available to herbivores increased with clipping and the addition of N increased the levels of several important herbivore mineral nutrients (Ca, Cu, Mg, P, and Zn) and under no circumstance did clipping significantly reduce any leaf mineral nutrient. Clipping altered plant morphology and plant quality synergistically by increasing both quantity and quality of leaf tissue available for herbivore removal and given the area, gradient, and duration of effect of a single urine hit, the observed results can be applied to plant responses to either a recent or past urine hit.

Estimates of growing season aboveground primary productivity (aPn) from this data set, for both clipped and unclipped plants, correspond well with values obtained from the site in which the *S. kentrophyllus* clone used in this experiment was obtained. McNaughton (1985) found growing-season mean aPns for ungrazed and grazed sites of 1.89 ($s^2 = 1.14$, range 0.47–4.04) and 3.78 ($s^2 = 4.88$, range 0.94–10.36) g m⁻² day, respectively. The growing season aPn values calculated in this experiment for clipped 0, 1, 5, 10, and 40 g N m⁻² plants were 1.17, 1.40, 2.18, 2.44, and 2.86 g m⁻² day⁻¹, respectively. Ungrazed aPn values estimated in this experiment for unclipped 0, 1, 5, 10, and 40 g N m⁻² plants were 1.90, 2.14, 2.45, 2.56, and 2.74 g N m⁻² day⁻¹, respectively. The values in this experiment fall well within the range observed in the Serengeti, further supporting the validity of the experimental design.

When plants are not grazed, N level had a minimal effect on productivity. Therefore N was less limiting when tissue was not being removed on a weekly basis.

This is supported by lack of a critical [N] in unclipped plants. In a grazed system, nitrogen can be recycled faster than in an ungrazed system by going from plant to grazer and then back to plant by urination and/or defecation. Heavily grazed site, in which *S. kentrophyllus* occurs, have soils with rapid rates of urea hydrolysis (McNaughton et al. 1997) and reduced rates of ammonia volatilization in field-applied urea experiments (Ruess and McNaughton 1988). Rapid urea hydrolysis and decreased volatilization coupled with increased grazer inputs can potentially increase available plant N. Ruess (1988) performed an experiment that investigated the response of N uptake to clipping in *S. kentrophyllus* and found that clipped plants increase their uptake rates of both nitrate and ammonium and allocated a larger proportion of N to shoots. Although urea uptake was stimulated by clipping, the rate was significantly less than that of nitrate and ammonium. This result coupled with the above results relating to soil properties suggest a possible mechanism linking the input of grazer N to increased plant N. Additionally, ungrazed systems have more N tied up in standing dead and stem. The N in standing dead and stem must then become litter which must then be recycled by either microbial means or fire with associated N loss by volatilization (Isichei and Sanford 1980). N tied up in detritus limits primary productivity because microbial processing has slower recycling and increased losses due to immobilization (Knapp and Seastedt 1986).

This experiment demonstrated that there is a nitrogen level switch-over point at which clipping adversely affects total plant biomass, with aboveground and leaf tissue concentration playing critical functions. In terms of green leaf biomass produced, clipping had a greater affect than N fertilization, but the quality of the tissue produced was profoundly affected by N fertilization. In a system in which large mammalian herbivores persistently remove leaf tissue and urinate where they graze, it is possible for compensation to occur that increases the nutritional quality and quantity of leaf tissue in a mutualistic manner for both the plant and herbivore.

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