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Response of Microtus pennsylvanicus to vegetation fertilized with various nutrients, with particular emphasis on sodium and nitrogen concentrations in plant tissues

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Fertilization of 1 × 4 m plots of old-field vegetation in Minnesota, USA, with various compounds resulted in increased plant tissue concentrations of N, P, K, Ca, Mg, Na, and Mn. *Microtrus pennsylvanicus* showed significantly greater activity, estimated by scat counts, on plots fertilized with sodium sulphate. Data also suggested that increased *Microtus* activity in response to elevated plant tissue sodium concentration resulted in greater soil nitrogen availability and higher levels of nitrogen in plant tissues.

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1. Introduction

Plant tissue nitogen (protein) content is a major determinant of food quality for many herbivores (reviews in Mattson 1980, Crawley 1983); many herbivores feed selectively so as to increase their nitrogen intake (e.g. Arnold 1964, Millar and Zwickel 1972, Clutton-Brock 1977, McNeill and Southwood 1978, Onuf 1978, Price 1978, Mattson 1980, Stuebe and Andersen 1984, McNaughton 1985). Herbivores also require other nutrients besides nitrogen, and these other nutrients probably play important roles in diet selection. When multiple constraints are included in foraging models optimal diets may be very different from those that consider only energy, time, or a single nutrient (e.g. Pulliam 1975, Belovsky 1978, Tilman 1982).

Sodium is an essential constituent of mammalian diets (e.g. Church et al. 1971), and the use of salt licks by many large mammals is well documented (Jones and Hanson 1985). Aumann (1965) reported that over a large geographic area *Microtus* density was positively correlated with concentration of sodium in soils (but see Krebs et al. 1970), and Aumann and Emlen (1965) reported greater fecundity for *Microtus* that had unrestricted access to salt (see also Batzli 1986).

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2. Methods

Data were taken on experimental plots created to test the importance of various soil nutrients in limiting primary productivity at the Cedar Creek Natural History Area, Minnesota USA. Four replicates of each of 9 treatments (Tab. 1) were located in a blocked design of 1×4 m plots. Experimental plots were treated annually, beginning in 1982. Nutrients were added in two equal portions applied at the end of May and mid June.

Vegetation on the plots was sampled in 1984 by clip-

Tab. 1. Chemicals added to each treatment. Equal amounts of the two forms of sodium phosphate were added.

Treatment	Nutrient	Amount (gm. m^{-2})
A	NH_4NO_3	40
B	$NaH_2PO_4 \cdot H_2O/Na_2HPO_4$	70
C	K_2SO_4	87
D	$CaCO_3$	75
E	$MgSO_4$	60
F	Na_2SO_4	71
G	Trace metals	60
H	H_2O	1.5 cm $week^{-1}$
I	Control	

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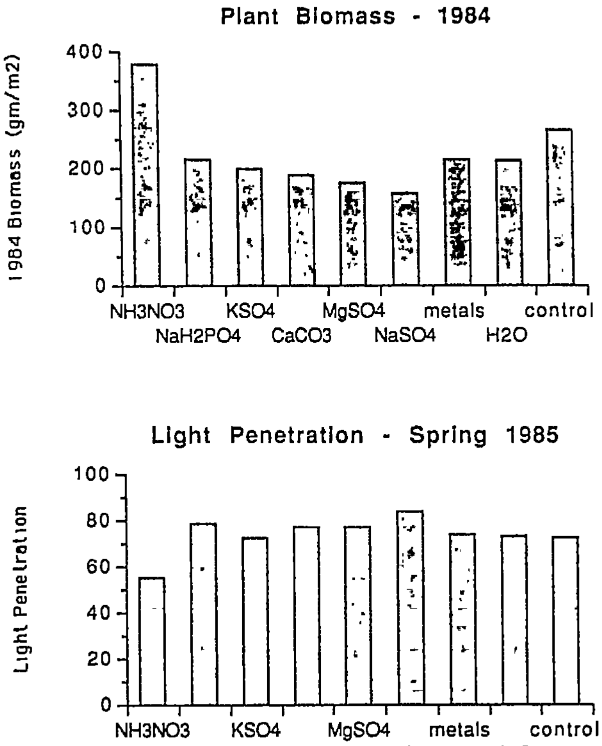

Fig. 1. Plant biomass in July 1984 and light penetration in May 1985.

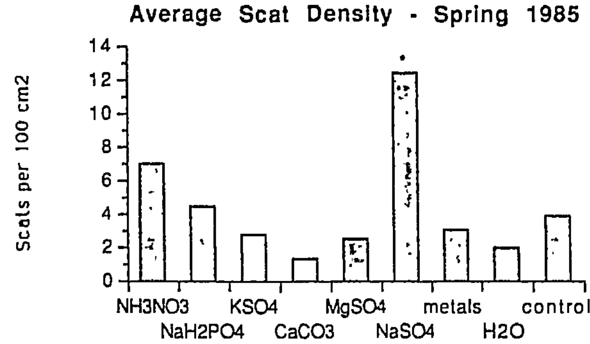

Fig. 2. Mean *Microtus* scat density per 100 cm^2 for the 9 treatments.

ping a 10 × 300 cm strip at ground level. Litter was removed from the samples, which were then dried and weighed. The 1984 plant samples were ground and analyzed for tissue concentrations of 15 elements by inductively coupled plasmospectrophotometry (ICP) in the Research Analytical Laboratory at the University of Minnesota, St Paul, MN. These samples were also analyzed colorimetrically for total nitrogen following a persulfate digestion (Tilman 1983).

In spring 1985 we measured light penetration as an indicator of total plant biomass. Light penetration, measured by taking one light reading above vegetation and a second reading at ground level, was calculated as the proportion of light above vegetation that reached ground level. Light readings were taken on 8 May 1985 with a 1 m long, integrating, cosine-corrected quantum sensor (Li-cor, Inc.). Analyses were done using the average of two sets of light readings for each plot. Light penetration on plots in this and in two other old-fields at Cedar Creek was significantly negatively correlated with added nitrogen (Tilman 1986), and with total biomass (Tilman 1987).

On 7 May 1985 we counted *Microtus pennsylvanicus*

Tab. 2. Mean (S. D.) values for tissue concentrations (ppm) of 8 elements in above-ground plant tissues. Asterisks next to element name indicate significant one-way ANOVA. Asterisks next to numbers in the table indicate treatment values that were significantly different from controls (Dunnett's test). ANOVA and Dunnett's tests of other elements (Ni, Al, Fe, Cu, B, Pb, Cr, and Cd) were not significant. (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

	Treatment								
Element	NH_4NO_3	NaH_2PO_4	K_2SO_4	$CaCO_3$	$MgSO_4$	Na_2SO_4	Metals	H_2O	Control
N**	9208.7*	7602.2	7254.7	7080.8	7319.7	9011.5*	7267.2	7897.5	6770.9
	(821.7)	(1336.7)	(411.1)	(1089.6)	(855.4)	(1042.8)	(735.9)	(390.4)	(703.8)
P***	1150.0	3764.7*	1560.3	1742.0	1760.6	2059.9	1828.8	1994.0	1634.9
	(30.4)	(568.4)	(141.9)	(468.7)	(49.6)	(201.1)	(238.8)	(205.5)	(191.5)
K**	6283.3	9782.0	13168.3	7691.1	9280.8	11818.3	10134.5	9970.3	9450.1
	(672.0)	(980.8)	(1413.2)	(2751.0)	(896.7)	(1316.1)	(1991.0)	(2367.9)	(3607.1)
Ca**	2520.1	3122.8	1703.7	5150.6*	2226.7	3033.5	3705.1	3990.2	2412.0
	(301.2)	(1290.1)	(305.9)	(1747.3)	(678.0)	420.2)	(961.3)	(1431.7)	(380.9)
Mg***	970.3	1248.2	491.4	1112.9	2272.4*	1213.1	1199.4	1522.5*	944.4
	(58.6)	(273.7)	(60.7)	(288.9)	(129.3)	(247.6)	(149 6)	(258.4)	(285.9)
Na***	6.6	443.5*	16.5	24.7	13.1	505.4*	24.2	32.4	17.7
	(2.6)	(109.5)	(10.5)	(19.2)	(8.3)	(226.7)	(14.3)	(20.4)	(18.9)
Mn**	144.2*	39.5	51.9	46.9	62.2	63.4	52.1	51.5	47.8
	(53.2)	(15.6)	(15.9)	(33.6)	(32.2)	(51.8)	(15.5)	(30.9)	(32.3)
Zn	20.5	18.4	18.3	16.2	19.6	30.7*	17.9	22.2	16.3
	(7.6)	(9.5)	(3.8)	(3.4)	(5.9)	(9.5)	(3.0)	(9.7)	(3.6)

scats in seven 10 × 10 cm quadrats in the same relative positions on each plot.

We tested for treatment effects using one-way analysis of variance (ANOVA). To determine which, if any, treatments were significantly different from the controls we used Dunnett's test (Steele and Torrie 1980).

3. Results

There was a significant nutrient treatment effect on total vegetational biomass in 1984 (ANOVA $F = 3.6$, $p = 0.006$) (Fig. 1). Ammonium nitrate (NH_4NO_3) addition produced the largest change in total biomass, however none of the treatments were significantly different from the controls (Dunnett's test). In this field, and in 2 other old-fields in which these nutrient additions were replicated, NH_4NO_3 was the only treatment that produced a consistent increase in plant biomass in three years of nutrient addition (Tilman 1987). Nitrogen is the primary limiting soil resource in old fields at Cedar Creek.

Fig. 2 shows mean *Microtus* scat density for each treatment on 7 May 1985. There was a significant effect of treatment on scat density (ANOVA, $F = 4.36$, $p = 0.002$). Sodium sulphate (Na_2SO_4) was the only treatment for which scat density was significantly different than for controls ($p < 0.05$, Dunnett's test).

There was a significant treatment effect on light pene tration on 8 may 1985 (ANOVA, $F = 3.29$, $p = 0.010$) (Fig. 1), however none of the treatment means were significantly different from the control. Light penetration was highest on Na_2SO_4 plots, and lowest on NH_4NO_3 plots. Increased light penetration on Na_2SO_4 plots reflected grazing and disturbance on those plots during the previous winter. In spring 1985 much of the ground on those plots had been disturbed. and most living and dead standing vegetation had been removed.

There was not a significant rank correlation between plant biomass in 1984 and scat density in 1985, suggesting that differential *Microtus* activity did not simply reflect differences in plant biomass.

Tab. 2 lists results of ICP and nitrogen analyses. Concentrations of elements that were added to plots were commonly greater in plant tissues on those plots (e.g. Na, P, K, Ca, Mg). Relative to controls, Na concentration was significantly higher on plots that received NaH_2PO_4 and on plots that received Na_2SO_4 (Dunnett's test). Relative to controls, nitrogen concentration was significantly higher on plots that received NH_4NO_3 and on plots that received Na_2SO_4 (Dunnett's test).

Of all the elements that were measured, only Na ($F = 28.7$, $p < 0.001$), P ($F = 13.2$, $p = 0.001$), and Cr ($F = 9.8$, $p = 0.004$) tissue concentrations were significantly correlated with average scat density in a stepwise multiple regression. When only those elements for which there was a significant treatment effect (one-way ANOVA) were loaded in a stepwise multiple regression, using either forward or backward procedures, only Na and P were significantly correlated with average scat density (scats $= 0.018 \times Na - 0.003\,P + 8.03$, $r = 0.62$, $n = 36$, $p < 0.0001$).

4. Discussion

Our data show a significant response by *Microtus* to vegetation fertilized with Na_2SO_4. Both the scat counts (Fig. 2) and the degree of grazing and disturbance during the winter, evidenced by increased light penetration in spring 1985, indicate selective use of Na_2SO_4-enriched plots by *Microtus*.

Data for plant biomass and light penetration in 1984 indicate that the higher level of *Microtus* activity on Na_2SO_4 plots was not due to differences in food quantity or cover. The most obvious difference between Na_2SO_4 plots and all other treatments except the sodium phosphate plots is the tissue concentration of sodium, which was more than an order of magnitude greater than on control plots. The lack of a *Microtus* response to the $MgSO_4$ and the K_2SO_4 treatments, which added SO_4^{2-} at levels equivalent to those of the Na_2SO_4 treatment, suggests that SO_4^{2-} did not cause their response to the Na_2SO_4 treatment. However, comparable logic might suggest that Na is not the cause, either, since there was no response to the sodium phosphate treatment. Our regression analysis of the dependence of *Microtus* activity on plant tissue chemistry may offer a resolution to this problem. Regression analyses susggest that *Microtus* showed a negative response to phosphorous. This was most apparent for plots treated with NaH_2PO_4. Tissue concentrations of sodium were significantly elevated on these plots (Tab. 2), but average scat density was only slightly higher on these plots than on controls. We do not know, however, why voles might avoid plants with high tissue phosphorous levels.

We have found significant behavioral and population responses by *Microtus* (Inouye and Huntly, unpubl., Huntly and Inouye, unpubl.), pocket gophers *Geomys bursarius* (Tilman 1983, Inouye and Huntly, unpubl., Huntly and Inouye, unpubl.), and grasshoppers (unpublished data) to plots fertilized with NH_4NO_3 in this field and in 3 other old-fields at Cedar Creek. Scat densities (Fig. 2) suggest a slight response of *Microtus* to plots treated with NH_4NO_3; those plots showed the second highest scat density. However, it was only on Na_2SO_4 plots that scat densities were significantly greater than on control plots, and tissue nitrogen concentration was not significantly correlated with scat density in multiple regressions. We cannot rule out the possibility that *Microtus* were responding to a combination of elevated sodium and nitrogen on Na_2SO_4 plots (Tab. 2), however we know of no direct mechanism whereby sodium or sulphate addition might result in elevated plant tissue nitrogen.

The high concentration of nitrogen in plants from plots treated with Na_2SO_4 is probably an indirect effect resulting from preferential use of these plots by *Microtus*. Greater deposition of urine and feces probably increased the amount of nitrogen available to plants on these plots, which in turn resulted in higher nitrogen concentrations in plant tissues.

If *Microtus* activity resulted in greater nitrogen availability on plots treated with Na_2SO_4, then one might expect plant biomass to be greater on those plots as well. Although there have been consistent increases in plant biomass on plots to which we have added nitrogen (NH_4NO_3), there has not been a plant biomass response on Na_2SO_4 plots. This is probably a direct result of the consumption of vegetation on these plots by *Microtus*. While the total area in this field treated with NH_4NO_3 was much larger than that treated with Na_2SO_4 (two nitrogen gradient experiments were adjacent to the experimental plots described here), it is interesting to note that the most obvious impact of *Microtus* on vegetation resulted from a response to tissue sodium and not to nitrogen, which limits both primary productivity (Tilman 1982, 1987) and *Microtus* density (Huntly and Inouye, unpubl., Huntly and Inouye, in press).

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